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Title: Direct ingestion, trophic transfer, and physiological effects of microplastics in the early life stages of *Centropristis striata,* a commercially and recreationally valuable fishery species.

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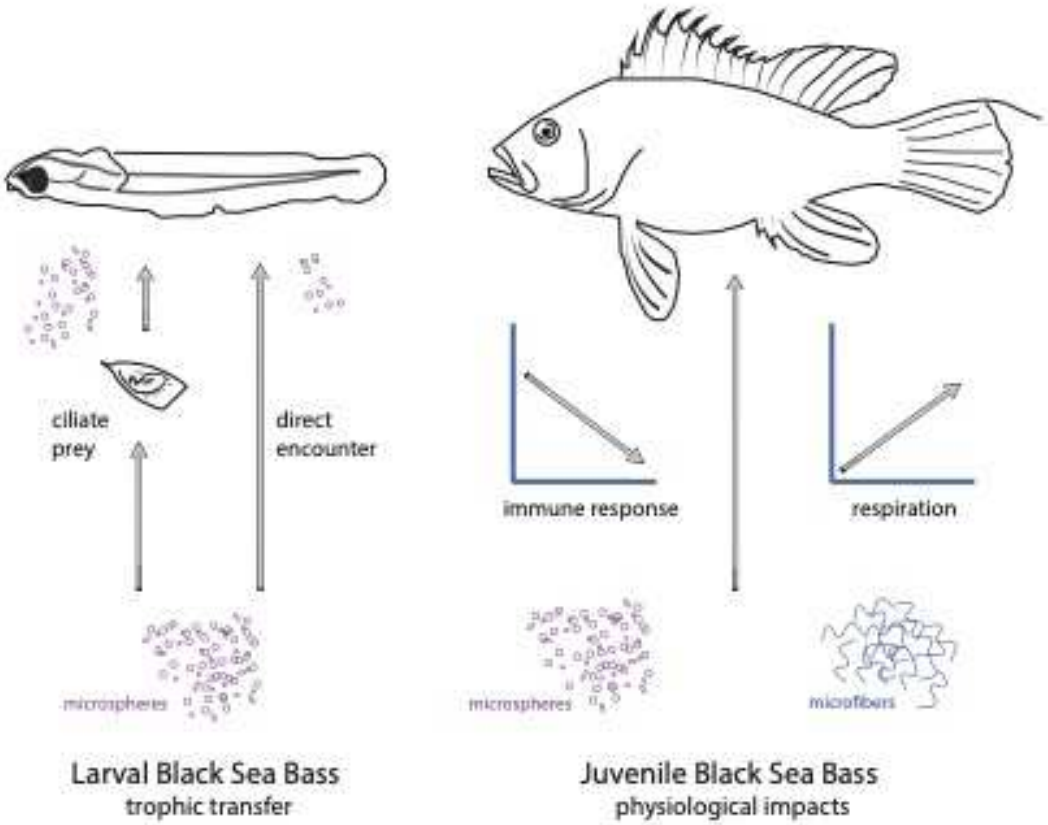
ABSTRACT:

Microplastics are ubiquitous in marine and estuarine ecosystems, and thus there is increasing concern regarding exposure and potential effects in commercial species. To address this knowledge gap, we investigated the effects of microplastics on larval and early juvenile life stages of the Black Sea Bass (*Centropristis striata*), a North American fishery. Larvae (13-14 days post hatch, dph) were exposed to 1.0 x 10^4, 1.0 x 10^5, and 1.0 x 10^6 particles L^{-1} of low-density polyethylene (LDPE) microspheres (10-20 µm) directly in seawater and via trophic transfer from microzooplankton prey (tintinnid ciliates, *Favella* spp.). We also compared the ingestion of virgin and chemically-treated microspheres incubated with either phenanthrene, a polycyclic aromatic hydrocarbon, or 2,4-di-tert-butylphenol (2,4-DTBP), a plastic additive. Larval fish did not discriminate between virgin or chemically-treated microspheres. However, larvae did ingest higher numbers of microspheres through ingestion of microzooplankton prey than directly from the seawater. Early juveniles (50-60 dph) were directly exposed to the virgin and chemically-treated LDPE microspheres, as well as virgin LDPE microfibers for 96 h to determine physiological effects (i.e., oxygen consumption and immune response). There was a

significant positive relationship between oxygen consumption and increasing microfiber concentration, as well as a significant negative relationship between immune response and increasing virgin microsphere concentration. This first assessment of microplastic pollution effects in the early life stages of a commercial finfish species demonstrates that trophic transfer from microzooplankton can be a significant route of microplastic exposure to larval stages of *C. striata*, and that multi-day exposure to some microplastics in early juveniles can result in physiological stress.

Key words: microspheres, microfibers, concentration-response, contaminated prey, commercial fishery, North America, Black Sea Bass, respiration, immune response

Graphical Abstract:



INTRODUCTION

The demand for plastic has steadily increased over the last half century, driving the current global annual plastic production to 335 million metric tons (Geyer et al. 2017; PlasticsEurope 2017), and with developed nations such as the United States leading in the production of plastic waste (Borelle et al. 2020). Plastic ingestion has been documented in over 220 species of marine organisms (Lusher et al. 2017, 2020), including finfish (Lusher et al. 2017, Savoca et al. 2020).

Microplastics (synthetic particles ranging between 1 μm – 5 mm in size; Brander et al. 2020) of both primary and secondary sources are ubiquitous and persistent in the aquatic environment (Barnes et al. 2009; Eriksen et al. 2014). The effect of microplastics on commercial fisheries is of growing concern due to the potential impact of exposure on populations, as well as possible human health risks of consuming microplastic-contaminated seafood (Santillo et al. 2017; Karami et al. 2018). There is limited information about the effects in commercial fish species, particularly those native to North America (Baechler et al. 2020, Granek et al. 2020). Field studies involving commercial fisheries primarily report presence or absence of microplastics (Foekema et al. 2013; Lusher et al. 2013; Bessa et al. 2018; Liboiron et al. 2018) and laboratory studies often use the same few non-commercial freshwater species, e.g. Zebrafish, Fathead Minnows, Japanese Medaka (reviewed in Jacob et al. 2020), and species sensitivity can vary widely (e.g. Besseling et al. 2019), thus it is important to gather data on responses to emerging contaminants, such as microplastics, across a wider range of species (Granek et al. 2020). To provide a greater understanding of how species outside of these typical models, such as commercial finfish, may be affected by microplastic pollution, we used the Black Sea Bass (*Centropristis striata*) as the focal species for our experiments.

C. striata, a commercially and recreationally valuable fishery along the Atlantic coast of North America, is a widely distributed temperate reef fish with a range from the Gulf of Maine to the Gulf of Mexico (Able and Hales 1997). This species feeds opportunistically upon a variety of prey items and thus accidental ingestion of microplastics from the water column as the fish mistakes plastic for prey is a potential concern (Sedberry 1988; Devriese et al. 2015). *C. striata* utilize nursery habitats in estuaries and coastal waters that are notably impacted by anthropogenic activities, during their early life stages (Beck et al. 2001; Rabalais 2015; Vendel et al. 2017). Interspecific variation in microplastics ingestion is likely due to the species-specific feeding strategies and abundance of plastics in their surrounding environment (Lusher et al. 2013, de Ruijter et al. 2020). Also of importance, microplastics prevalence is pronounced in coastal zones due to their proximity to terrestrial inputs and tidal processes that cause accumulation and fragmentation (Weinstein et al. 2016; Gray et al. 2018).

The potential risks of direct microplastic ingestion during early life stages of fishes likely arise from a combination of physical stress and chemical exposure (Jacob et al. 2020, Pannetier et al. 2020). An additional exposure route includes ingestion of microplastics and associated pollutants via trophic transfer from contaminated prey items (Nelms et al. 2018), documented in both natural systems and in artificial laboratory food webs (Carbery et al. 2018; Welden et al. 2018). Notably, both Athey et al. (2020) and Hasegawa and Nakoaka (2021) demonstrated that fish obtain more microplastics from prey (ciliates and mysid shrimp, respectively) than they do directly from the water. To what degree commercial finfish are affected by the trophic transfer of microplastics and associated pollutants remains unknown and the mechanisms poorly understood, particularly under environmentally relevant conditions.

Additionally, due to their ubiquity and high surface area to volume ratio, microplastics have the potential to serve as transport vectors not only for plastic additives but also for hydrophobic persistent organic pollutants (Rios et al. 2007; Bakir et al. 2014; Gallo et al. 2018). Chemicals commonly associated with microplastics are adsorbed hydrophobic aqueous pollutants (DDT, PAHs, PCBs) (Ziccardi et al. 2016). It has been suggested that the transfer of chemicals adsorbed to microplastics from the environment is not a significant means of exposure when compared to other exposure pathways (e.g., through the environment or prey) (Koelmans et al. 2016). However, plastic additives, added at high concentrations during manufacturing, may be a greater concern because of their potential for endocrine disruption at low concentrations (Brander 2013; Brander et al. 2016; Franzellitti et al. 2019; Bucci et al. 2021).

Given these knowledge gaps, we sought to address the impacts of microplastics of different morphologies with and without associated chemicals in two early life stages of an estuarine commercial fishery species. Our objectives were 1. to assess ingestion directly from the water compared to trophic transfer in larvae, and 2. to investigate whether physiological responses were perturbed by microplastic exposure in young juveniles, by measuring respiration and immunity. To accomplish the first objective, we used a model food chain with single-celled microzooplankton (tintinnid ciliates; *Favella* spp.) and larval *C. striata*, and exposed larvae to microspheres with and without associated chemicals. Ciliates are important food sources for larval fish, including *C. striata*, in marine and freshwater habitats (Zingel et al. 2019) and may serve as significant vectors of microplastics to enter food webs via trophic transfer (Athey et al. 2020). For the second objective, we conducted exposures to microplastics of two morphologies (sphere and fiber) with and without associated chemicals in early juvenile stage *C. striata* and assessed two physiological endpoints: oxygen consumption and gross immune response. Three

124 microplastic concentrations were used for both objectives to provide the type of dose-
125 concentration data necessary for risk assessment. Microplastic-associated chemicals used were
126 the common environmental pollutant phenanthrene, and a frequently used UV stabilizer - 2,4-di-
127 tert-butylphenol (2,4-DTBP) (Black et al. 1983; Samanta et al. 2002; McConville et al. 2018,
128 Rani et al. 2015). To the best of our knowledge, this is the first set of laboratory microplastic and
129 microfiber exposures conducted with early life stages of an estuarine commercial finfish species
130 native to North America.
131
132 **METHODS**
133 *Contamination mitigation*
134 All glassware used in the laboratory feeding experiments was rinsed with deionized (DI)
135 water, soaked in a nitric acid solution (10% v/v) for 24 h prior, and soaked in DI water for 24 h
136 prior to experimentation. The glassware was then baked at 450°C for 4 h and rinsed with either
137 dichloromethane (DCM) or acetone (ultrapure grade) to prevent additional contamination.
138 Equipment (e.g., glass pipettes, dip nets, etc.) was designated to specific treatment groups to
139 ensure no cross-contamination between virgin, phenanthrene-treated, and 2,4-DTBP-treated
140 microspheres. Beakers were covered with foil (larvae) or lids (juveniles) during exposures to
141 prevent contamination from plastics in the air.
142 *Microsphere and microfiber stock preparation*
143 Given polyolefins such as polyethylene are frequently documented in the water column
144 due to their extensive use in fishing gear and single-use plastic products (Jambeck et al. 2015;
145 Reisser et al. 2015; Conkle et al. 2018; Pozo et al. 2019), we selected low-density polyethylene
146 (LDPE) microspheres for both the larval and juvenile exposures, and PE microfibers for use only

147 in the juvenile experiments. LDPE microspheres (10-20 µm in diameter; Grant Industries, NJ,
148 USA) were used for the larval and early juvenile laboratory feeding experiments. Microspheres
149 were rinsed with methanol for 6 d and then dried in a fume hood at ambient temperature. To
150 ensure proper dispersion of the microspheres in aqueous media, a 0.01% (v/v) solution of the
151 non-ionic surfactant Tween20 (Fisher Scientific, Pittsburgh, PA, USA) was prepared in 100 mL
152 Milli-Q ultrapure (MQ) water, stirred at ambient temperature for 30 min, and heated to 100 °C
153 for 5 min in a water bath (Athey et al. 2020). The methanol-rinsed LDPE microspheres were
154 resuspended in 0.01% Tween20 solution and vortexed in a glass bottle. Stock LDPE microsphere
155 solutions were prepared by adding MQ water to the Tween20-microsphere mixture, to yield
156 stocks of 1.0×10^4, 1.0×10^5, 1.0×10^6 particles per L^{-1}. A hemocytometer was used to confirm
157 microsphere concentrations. PE microfibers (700 µm in length, 10-15 µm diameter;
158 MiniFIBERS, Inc., Johnson City, TN, USA) were resuspended in 0.01% Tween20 solution at 30
159 mg in 15 mL (Cole 2016). The stock solution was created by adding MQ water (85 mL) to the
160 Tween20-microfiber mixture and vortexed in a glass bottle to break up fiber clumps. The
161 microfibers were not solvent rinsed and small clumps were visible, making it difficult to validate
162 the exact number of microfibers mg^{-1}. As a result, the microfiber stock solutions and
163 experimental concentrations are expressed in mass of microplastics L^{-1} rather than fiber count L^{-1}.
164
165 *Phenanthrene and 2,4-di-tert-buytlphenol (2,4-DTBP) loading*
166 LDPE microspheres were stirred in a mixture of toluene: hexane (1:1 v/v) containing
167 phenanthrene (>99.5% purity) or methanol containing 2,4-DTBP (>99% purity) (Sigma-Aldrich,
168 St. Louis, MO, USA) for 6 d at ambient temperature. The resulting slurry was filtered through a
169 glass fiber filter (Whatman #1820-021, retention: 1.6 µm) (Sigma-Aldrich, St. Louis, MO, USA)

before being washed four times with hexane and dried at ambient temperature for 24 h. The concentrations of phenanthrene (1.9 μg g^{-1}) and 2,4-DTPB (12 μg g^{-1}) sorbed on the microspheres were selected to reflect environmental or additive concentrations, respectively, of these compounds (Rani et al. 2015; Peng et al. 2019). Sorption was confirmed by extraction and subsequent gas chromatography and flame-ionization detection (GC/FID) analysis (see Supplemental 1 for details). Fibers were not treated with chemicals.

LARVAL EXPOSURES

Larval C. striata maintenance

C. striata broodstock were maintained at the UNC-Wilmington Aquaculture Facility, Wrightsville Beach, NC according to the methodology described by Watanabe (2011) and Watanabe et al. (2021) and in accordance with UNCW IACUC Protocol #A1819-009. Approximately 1000 *C. striata* larvae were obtained at 12 dph (days post hatch) and stocked in 18 L rearing containers of artificial seawater (ASW, 30 ppt) at a density of 30 larvae L^{-1} in a temperature-controlled room (16°C). ASW was prepared using Instant Ocean (Middleton, Wisconsin, USA) and DI water until the appropriate salinity was reached. Larvae were fed nutritionally enriched rotifers (10 rotifers mL^{-1}) twice daily during the acclimation period. Salinity (29.40 ± 1.96 ppt), temperature (17.25 ± 0.14°C), dissolved oxygen (8.29 ± 0.93mg L^{-1}), ammonia (0.00 ± 0.00 ppm), and pH (7.35 ± 0.04) were monitored daily during the acclimation and experimental periods (see Supplemental 2).

Culturing of tintinnid ciliates

Tintinnid ciliates (*Favella* spp.) were cultured based on previous methodology described in Athey et al. (2020). Ciliate cultures were maintained in 200 mL batches of filtered seawater in

193 a temperature-controlled incubator (14-16°C, 30 ppt) and sub-cultured every 3 – 4 d. The ciliates
194 were fed phytoplankton (*Heterocapsa triquetra, Isochrysis galbana, and Mantoniella squamata*)
195 every 3 – 4 d. The phytoplankton cultures were maintained in 40 – 1000 mL batches of filtered
196 seawater supplemented with f/2 media and Guillard's vitamins. The phytoplankton were
197 maintained in an illuminated incubator with 50-100 µmol photons m^{-2} s^{-1} on a 14:10 day:night
198 cycle at 14–16 °C and were sub-cultured every 1–2 wks.
199 *Experimental design of larval exposures*
200 The purpose of this feeding experiment was to assess microplastic ingestion in cultured
201 *C. striata* larvae (13-14 dph) exposed to virgin and chemically-treated LDPE microspheres
202 through direct ingestion and trophic transfer. For the direct ingestion and trophic transfer feeding
203 experiments we used virgin, phenanthrene-treated, and 2,4-DTBP-treated LDPE microspheres
204 (10-20 µm) at three concentrations (1.0×10^4, 1.0×10^5, and 1.0×10^6 particles L^{-1}). The lowest
205 concentration was 10,000 particles / L, or 10 particles / mL, an approximation of an
206 environmentally relevant level of small microplastic particles recently recommended for use in
207 experiments by Bucci et al. (2019). It is difficult at this time to verify how accurate this
208 approximation is, as many field surveys do not account for plastics smaller than 300 microns
209 (Brander et al. 2020). For the trophic transfer groups, *C. striata* larvae were exposed to rinsed
210 ciliates that were previously exposed to virgin or chemically treated microspheres at the three
211 concentrations (see below). In total, there were 18 experimental groups (exposed to microplastics
212 directly and via trophic transfer) and 3 control groups (not exposed to microplastics) with 4
213 replicates for each group
214 *Larval direct exposure*

215 Immediately prior to the feeding experiment, glass treatment beakers were filled with 250
216 mL ASW (16 °C, 30 ppt) into which virgin and chemically microspheres (1.5 x 10^5 beads mL^{-1}
217 stock) were added volumetrically: 16.7 µL, 167 µL and 1.67 mL to achieve the low, medium,
218 and high concentration replicates of 1.0 x 10^4, 1.0 x 10^5, and 1.0 x 10^6 particles L^{-1} respectively.
219 These concentrations are on the low end of those typically used in exposures with larval and
220 juvenile fish (reviewed in Jacob et al. 2020). Given that most field measurements focus on larger
221 plastic size fractions (Brander et al. 2020), an accurate estimate of 10-20 µm LDPE found in
222 estuarine waters was not available at the onset of our experiments. A glass pipette was used to
223 gently stir each replicate to disperse the microspheres evenly. No microspheres were added to the
224 control group. Black sea bass larvae were starved 3 h prior to experimentation before transferring
225 10 individuals into each experimental replicate. After the 2 h microplastics exposure in foil-
226 covered beakers, 3 larvae from each of the direct ingestion replicates were sampled to obtain
227 microsphere ingestion counts. The larvae were rinsed in MQ water to remove any microspheres
228 adhered to the skin, sacrificed on ice, rinsed in phosphate buffer saline (PBS), and preserved in
229 glutaraldehyde (2.5% v/v) to prevent degradation until microscopic analysis (Oozeki and Hirano
230 1988).
231 *Larval trophic transfer exposure*
232 Ciliate cultures were starved 24 h prior to experimentation, pooled into a 2 L glass
233 container, gently reverse filtered using a 40 µm nylon mesh cell strainer, and reconstituted to 2 L
234 with ASW. This washing process was repeated several times to remove algal prey cells and
235 culture debris. Three 1 mL subsamples of the final ciliate pool were counted using a Sedgewick-
236 Rafter counting chamber to determine ciliates mL^{-1}.

For each trophic transfer replicate, washed ciliates were volumetrically added from the pooled container to a glass beaker to achieve a concentration of 15 ciliates mL^{-1} in 100 mL ASW (16 °C, 30 ppt). Three 1 mL samples were collected and preserved in Lugol's iodine (20 µL) and glutaraldehyde (20 µL, 2.5% v/v) and stored at 4°C for later counting to confirm the starting ciliate density. Then, chemically treated or virgin microspheres were added to these beakers to achieve the high, medium, and low concentration replicates as described above before stirring with a glass pipette to disperse the microspheres and ciliates evenly. Ciliates were allowed to feed on microspheres for 1 h. One set of ciliate controls were not fed microplastics and were not fed to C. *striata* larvae. The other set of ciliate controls were not fed microplastics but *were* fed to *C. striata* larvae for trophic transfer experiments.

Following 1 h exposure, ciliates in each beaker were reverse filtered through a 40 µm nylon mesh cell strainer from 100 mL to 20 mL and reconstituted to 100 mL with ASW. This was repeated twice to remove any extraneous microspheres. Three 1 mL ciliate samples were taken to enumerate the number of ingested microspheres per ciliate and the number of ciliates per mL following the 1 h exposure. The final volume of each beaker was increased from 100 mL to 250 mL before transferring 10 *C. striata* larvae that were allowed to feed on ciliates for 2 h, after which 3 larvae from each replicate, including ciliate control, were sampled to obtain microsphere ingestion counts. Larvae were sacrificed and preserved as described above in the previous section.

Microsphere quantification

Each of the 1 mL samples collected after the 1 h ciliate feeding period were centrifuged for 15 s and pipetted into a glass depression slide, and viewed using a polarized light microscope (ZEISS Axioskop, Oberkochen, Germany) to quantify the total number of ciliates in 1 mL and

260 determine microspheres ingested per ciliate. The preserved *C. striata* larvae were whole mounted
261 on a microscope slide and also analyzed using polarized light microscopy (ZEISS Axioskop,
262 Oberkochen, Germany). A first-order phase plate was used to provide additional contrast
263 between the microspheres and the soft tissues of the gut. The number of microspheres within the
264 gut of each larva were obtained to determine total microplastic consumption across all treatment
265 groups.
266
267 **JUVENILE EXPOSURES**
268 *Juvenile C. striata maintenance*
269 *C. striata* juveniles were maintained at the UNC-Wilmington Aquaculture Facility in
270 Wrightsville Beach, NC according to the methodology described by Watanabe (2011) and
271 Watanabe et al. (2021) in accordance with IACUC Protocol #A1819-009. Approximately 1000
272 *C. striata* juveniles (50-60 dph, each approx. 0.75 g) were temporarily stocked in aerated 10 L
273 glass aquaria with full-strength high-quality seawater (HQSW, 20-22°C, 30-34 ppt). HQSW was
274 obtained from the Center of Marine Science's Seawater Systems: raw seawater from the
275 Intracoastal Waterway is processed through a series of filters (60 µm, 10 µm, 1 µm). Juveniles
276 were fed a commercially prepared diet (Otohime, Reed Mariculture Inc., Campbell, CA) twice
277 daily *ad libitum*. Salinity (ppt), temperature (°C), dissolved oxygen (mg L^{-1}), ammonia (ppm),
278 and pH were monitored daily, and 50% water changes in the holding tanks were conducted daily
279 (Supplemental 3).
280 *Experimental design of juvenile exposures*
281 The purpose of this experiment was to measure the rates of oxygen consumption and
282 immune response in early juvenile *C. striata* following a 4-d direct exposure to virgin

283 microspheres, chemically-treated microspheres (phenanthrene or 2,4-DTBP), and virgin
284 microfibers. We used virgin, phenanthrene-treated, and 2,4-DTBP-treated LDPE microspheres
285 (10-20 μm) and virgin LDPE microfibers (700 μm in length) at three concentrations (1.0×10^4 ,
286 1.0×10^5, and 1.0×10^6 microplastic particles L^{-1}). In total, there were 12 experimental groups
287 and 1 control group each with 4 replicates.
288 *Juvenile direct exposure*
289 For each treatment, 8 *C. striata* juveniles were removed from the stock tanks and placed
290 in 3 L glass containers filled with aerated HQSW (20-22 °C, 30-34 ppt). The 8 juveniles in each
291 experimental unit were of similar sizes (~0.75 g) to avoid cannibalism which has been observed
292 during nursery rearing (Watanabe 2011). The fish were initially starved 24 h prior to the first
293 addition of microspheres. Virgin and chemically-treated microspheres were added to each
294 replicate volumetrically: 5.0 mL, 0.50 mL, and 0.05 mL of microspheres were added from the
295 100 mL stock solutions (6×10^5 mL^{-1} stock) to achieve the low (10,000 particles L^{-1}), medium
296 (100,000 particles L^{-1}), and high (1,000,000 particles L^{-1}) microplastic treatments, respectively.
297 These are the same concentrations used for larval *C. striata*. Complete water changes of the *C.*
298 *striata* juvenile tanks were conducted after each 24 h period, followed by microplastic addition
299 to maintain the same level of exposure. Subsets of juveniles were randomly selected for endpoint
300 analyses (immune response assay and respiration analysis) following the 4-d exposure to virgin
301 and chemically treated microspheres.
302 *Respiration analysis*
303 Using methodology adapted from Watts et al. (2014), closed-system respiration
304 chambers (RC400 Respiration Cell, Strathkelvin Instruments, Motherwell, Scotland, UK) were
305 used in conjunction with oxygen electrodes and a six-channel oxygen meter (SI130

306 Microcathode Oxygen Electrode; SI929 6-Channel Oxygen Meter, Strathkelvin Instruments,
307 Motherwell, Scotland, UK) to measure oxygen concentration. The respiration analysis was
308 designed to measure rates of oxygen consumption in a subset of juvenile *C. striata* after the 4-d
309 microplastic exposure.

310 Oxygen electrodes were calibrated daily in both oxygen-saturated water and oxygen-free
311 water (by addition of sodium sulfite). Each respiration chamber was fitted with a stir bar below a
312 grated bottom to insure mixing, filled with fully saturated HQSW, and spatially arranged to
313 prevent any interaction between fish that could affect the respiration rates. The temperature (°C)
314 and salinity (ppt) of the HQSW along with the atmospheric pressure (mmHg), were measured to
315 calculate the oxygen saturation of the water. Oxygen concentration data were collected for a
316 minimum of 30 min prior to experimentation to determine background oxygen concentration.

317 Two fish per replicate were placed in each chamber and oxygen consumption recorded
318 continuously for a total of 20 min (10 min of acclimation to the chambers and 10 min of
319 recording to be used in analysis). Following the data collection period, fish were removed from
320 the chambers and euthanized via lethal concentration of MS-222 (described below in *Immune*
321 *Response Assay*). The water was discarded, the chamber was rinsed, and refilled with fully
322 saturated HQSW prior to every subsequent respiration trial.

323 Oxygen concentration (μmol L^{-1}) was analyzed via Strathkelvin SI929 Software
324 (Strathkelvin Instruments, Motherwell, Scotland, UK). The background O_2 levels were recorded
325 in chambers with no fish and then subtracted from the measured O_2 concentrations for each
326 experimental replicate. The rate of oxygen consumption (μmol hr^{-1}) of *C. striata* juveniles from
327 exposed and control treatments was calculated over the 10 min period after acclimation for each

328 replicate. Oxygen consumption calculations were normalized to the body mass of the fish
329 (approximately 0.75 g per individual).
330 *Immune response assay*
331 The immune response assay, a proxy for stress, was measured at the end of the 4-d
332 microplastic exposure experiment using 3 juvenile *C. striata* per replicate. The assay was
333 performed as described by DeCourten et al. (2020) and adapted from Breckels and Neff (2013).
334 Phytohemagglutinin (PHA) is a novel antigen known to induce a cell-mediated response of T-
335 cell proliferation and localized swelling at the site of injection (Ardia and Clotfelter 2006). As a
336 result, injection of PHA can provide an assessment of immune function though a localized
337 swelling response. The caudal peduncle of *C. striata* was selected as the injection site because it
338 is a measurable location with limited variability (Ardia and Clotfelter 2006; Clotfelter et al.
339 2007). Two fish from each experimental and control replicate were randomly assigned to receive
340 a subcutaneous injection of 2 µg PHA (Sigma-Aldrich, St. Louis, Missouri, USA) in 1 µL of
341 phosphate buffered saline (PBS) using a 10 µl 26-gauge syringe with a beveled tip (Hamilton
342 Company, Reno, NV, USA). The third fish of the same replicate was assigned to receive a
343 control injection of only 2 µl of PBS. Juveniles were first anaesthetized with a sublethal dosage
344 of tricaine methanesulfonate (MS-222, 0.25 g L^{-1}) (Sigma-Aldrich, St. Louis, Missouri, USA) for
345 approximately 90 s. The caudal peduncle width was measured three times with a manual caliper
346 before a subcutaneous injection of either PHA or PBS was administered to that site for each fish.
347 The post-injection fish were placed in isolation chambers (20-22 °C, 30-34 ppt) to recover for 24
348 h without food, after which they were euthanized with a lethal concentration of MS-222 (1.25 g
349 L^{-1}). The average of three caudal peduncle measurements was taken and the immune response of

each juvenile was determined as the difference in swelling between pre-injection and post-injection caudal peduncle widths.

Hurricane Florence impact statement

As a result of severe building damage caused by Hurricane Florence at UNC-Wilmington in September 2018 all frozen samples from these experiments were lost when the back-up generator failed due to severe flooding. Therefore, we were unable to confirm ingestion / quantify the number of microspheres and microfibers within juvenile gut or gill tissues. Ingestion was however confirmed in larvae. Due to funding constraints and our use of a non-model fish species, we could not spawn more fish to repeat these experiments within the timeframe of the project. These results therefore provide a baseline study for understanding of how juvenile *C. striata* may be physiologically impaired after direct exposure to microplastics.

Statistical analyses

A generalized linear model (GLM + Poisson distribution) was used to analyze the average number of microspheres ingested per ciliate across virgin, phenanthrene-treated, or 2,4-DTBP-treated microspheres. The same approach was also used to analyze the number of microspheres ingested per *C. striata* larva, and to compare the number of microspheres ingested directly from the water or via trophic transfer from prey. A GLM (+ normal distribution) was used to compare the effects of virgin microfibers and virgin, phenanthrene-treated, and 2,4-DTBP-treated microspheres on juvenile *C. striata* oxygen consumption. Immune response measurements were analyzed in a similar manner to compare caudal peduncle measurements across treatment groups. In the case of both respiration and immune response, treatment responses were normalized by subtracting the mean control responses. We represent the range of

control data as a shaded area in each graph. To estimate the potential effect of low replication within the GLM prior to line-fitting, a leave-one-out analysis was conducted to determine the marginal effect of having even fewer data points. In all cases, the average effect on the slope of the line was < 1%, indicating that the data were sufficient to fit the regression (Simberloff 1978). Regressions were also fit to determine the relationship between increasing concentration of microplastics and either immune response or respiration. We calculated the 95% confidence interval around the regression line, using the point at which the lower bound of the confidence interval is >0 to be the point of departure, or the point at which the effect is greater than zero (Montgomery et al. 2021). All statistical analyses were performed in JMP Pro 14. Regressions were fit in lieu of using categorical comparisons (e.g. Anova with post-hoc comparison) based on recommendations from Cottingham et al. (2005) and implementing curve-fitting approaches similar to those published in Brander et al. (2016), Goff et al. (2017), and Mundy et al. (2020). All model parameters are reported in Supplemental tables 4A-4E.

RESULTS AND DISCUSSION

Ciliate LDPE microsphere ingestion

Ciliates (*Favella* spp.) ingested virgin, phenanthrene-treated, and 2,4-DTBP-treated LDPE microspheres at the three microplastic densities (1.0×10^4, 1.0×10^5, and 1.0×10^6 microspheres L^{-1}) following a 1 h direct exposure (Table 1). As might be expected, ciliate ingestion of microspheres increased with microsphere concentration (Figure 1, GLM (Poisson), microsphere concentration effect: $P < 0.0001$). However, there was no effect of chemical treatment on the average number of microspheres ingested per ciliate (Figure 1, GLM + Poisson

395 distribution, chemical effect: $P < 0.9999$). No microspheres were detected in the unfed control
396 ciliates.

397 The data show that *Favella* spp. readily ingested the LDPE microspheres but did not
398 ingest a greater number of virgin or chemically treated microspheres. Similar results were
399 reported by Athey et al. (2020) in which *Favella* spp. did not differentiate between virgin and
400 DDT-treated microspheres, even though the amount of DDT (2.15×10 ng^6 g^{-1}) sorbed onto the
401 microspheres exceeded environmentally relevant concentrations. Tintinnid ciliates have a
402 preferred prey size range of 5 – 25 μm, indicating the organisms will reliably ingest objects –
403 natural or synthetic – within the appropriate size range (Echevarria et al. 2014). Although
404 microzooplankton are selective feeders that can use chemical as well as physical cues to feed
405 upon prey (Griniene et al. 2016), the *Favella spp.* used in this study did not demonstrate a
406 difference in ingestion of the 10-20 μm phenanthrene and 2,4-DTBP microspheres.

407 *Larval C. striata: direct ingestion and trophic transfer of LDPE microspheres*

408 Black sea bass larvae ingested virgin, phenanthrene-treated, and 2,4-DTBP-treated
409 LDPE microspheres of three microplastic densities (1.0×10^4, 1.0×10^5, and 1.0×10^6
410 microspheres L^{-1}) following a 2 h exposure to microspheres directly in the water and via trophic
411 transfer from prey (Table 1). Larvae that fed upon microplastic-containing ciliates ingested
412 significantly more microspheres than larvae directly exposed to microplastics in the water
413 (Figure 2, A, GLM (Poisson), direct ingestion vs. trophic transfer effect: $P = 0.0168$). There was
414 no effect of chemical treatment on the total number of microspheres ingested by *C. striata* larvae
415 via trophic transfer from prey (Figure 2, B, GLM (Poisson), chemical effect: $P = 0.3722$). *C.*
416 *striata* initially appeared to ingest a greater number of virgin microspheres directly from the
417 water at the highest concentration (Figure 2, C, GLM (Poisson), microsphere effect: $P = 0.6824$),

418 but this result was not significant. Significantly more microspheres across all treatments were
419 ingested at the highest microplastic density (Figure 2, A-C, GLM (Poisson), microsphere effect:
420 $P < 0.0001$). No microspheres were detected in the control larvae and limited ingestion occurred
421 at the low and medium microplastic concentrations (Table 1).

422 Larval *C. striata* did not ingest a greater number of the virgin microspheres compared to
423 either of the chemically treated microspheres when directly available in the water or through the
424 ciliate prey. The olfactory system is important for discriminating odors that mediate feeding and
425 social behaviors in larval fish (Firestein 2001), but the sensitivity of olfaction is not well
426 established for many species of marine finfish larvae (Lara 2008). The olfactory system becomes
427 more developed as fishes transition into juvenile and adult life stages, so it is plausible that *C.*
428 *striata* larvae were unable to discriminate against or are indifferent to the chemically treated
429 microspheres via olfaction.

430 Larval fish are visual predators (Voesenek et al. 2018), which is consistent with our
431 finding that *C. striata* larvae potentially ingest more microspheres via contaminated prey items
432 (i.e., tintinnid ciliates) than directly from the water. For the highest concentration of
433 microspheres, ciliates ingested an average of 2 microspheres per individual (Table 1), but high
434 concentrations of larval fish (direct ingestion) contained less than 1 microsphere per individual
435 across all concentrations. However, slightly greater than 2 microspheres per individual fish were
436 observed in the highest trophic transfer concentration treatments. At 15 ciliates mL^{-1}, each
437 trophic transfer treatment beaker had a microplastic exposure of approximately 3×10^3
438 microspheres L^{-1}, which is an order of magnitude lower than the lowest direct ingestion exposure
439 treatment (1×10^4 microspheres L^{-1}) in which only one microsphere was ingested among 12
440 specimens (Table 1). Microplastic-containing zooplankton in the natural environmental may

441 pose significant risk of exposure to juvenile salmon (Desforges et al. 2015), indicating that
442 trophic transfer of plastics is an important consideration for estuarine and coastal food webs.
443 Athey et al. (2020) recently demonstrated increased ingestion of microplastics through
444 microzooplankton prey by larvae of the estuarine model species *Menidia menidia* and here we
445 show that common microzooplankton such as ciliates also have the potential to serve as
446 significant vectors of microplastics in commercially valuable fishes.
447 *Early juvenile C. striata: physiological responses following LDPE microsphere and microfiber*
448 *exposures*
449 Only juvenile *C. striata* exposed to virgin microfibers exhibited a significant increase in
450 oxygen consumption with increasing plastic concentration (Figure 3, GLM (Normal), P =
451 0.0352), indicating the microfibers had a more pronounced effect on the respiratory system in
452 comparison to microspheres. Based on the 95% CI we estimate that juveniles began to respond to
453 microfibers at a concentration of 2.7 x 10^5 per L^{-1}. Respiratory distress (measured in terms of
454 increased oxygen consumption) is a likely physiological response to a microplastic exposure,
455 considering the potential for microsphere and microfiber uptake via the gills (Watts et al. 2016).
456 Recently, increased mucus production in the gills was observed in maturing *O. latipes* following
457 a 10-week dietary exposure to 10 µm polystyrene microplastics (Zhu et al. 2020). Given that the
458 gills are extremely sensitive to toxicants and the presence of foreign substances (Wang et al.
459 2013), respiratory distress and increased oxygen consumption may occur when a foreign
460 substance (i.e., microplastics) interferes with normal gill function (Van Cauwenberghe et al.
461 2015). It is possible that this toxicity is dependent on the shape of the microplastic, and that
462 microfibers may have become entrapped in the gills of the exposed juvenile *C. striata,* although

463 it is important to mention that another recent study in finfish found little impact on fish gills from
464 microplastic exposure (Batel et al. 2018).

465 Only juveniles exposed to increasing concentrations of virgin microspheres for 96-h had
466 a significant decrease in normalized caudal peduncle widths (Figure 4, GLM (Normal), P =
467 0.0049), with no effect observed with chemically treated microspheres. The adaptive immune
468 response (T cell-mediated) works to identify foreign substances, proliferate in the infected area,
469 and remove the substance (Janeway 2001). A smaller caudal peduncle indicates less T cell
470 proliferation and a potentially suppressed immune response. This relationship is most evident at
471 higher concentrations of microspheres, and calculations based on the 95% CI estimate that an
472 effect was measurable at a concentration of 3.23×10^5 per L^{-1} and above. The presence of
473 ingested or inhaled microplastics alone may be enough to elicit an inflammatory response within
474 the organism (Wright and Kelly 2017). The apparent lack of response to the other treatments is
475 difficult to explain because potentially toxic additives and monomers are used to manufacture
476 plastics (Avio et al. 2017), however, it is possible that unlike the larval *C. striata*, juveniles (50-
477 60 dph) were able to differentiate between virgin and chemically treated microspheres, hence
478 avoiding the latter. This was not possible to determine following exposures due to hurricane-
479 related sample loss, as explained in the Methods.

480 The effects of microplastics on finfish are diverse and variability in experimental design
481 can make it difficult to compare across studies. Laboratory studies investigating the trophic
482 transfer of virgin and chemically treated microspheres from prey to finfish report different
483 physiological endpoints, some with significant latent impacts at high concentrations (e.g.,
484 reduced growth two weeks post-exposure in larval Inland Silversides (*Menidia beryllina*; Athey
485 et al. 2020) and others showing no effect. A study in Zebrafish indicated that microplastic-

associated pollutants ingested from prey (*Artemia* nauplii) potentially desorb in fish intestines (Batel et al. 2016). However, no altered behavior was observed in Krefft's Frillgobies (*Bathygobius krefftii*) exposed via trophic transfer (Tosetto et al. 2017) and no effect on hepatic CYP1A levels was found in Zebrafish exposed to microplastics with sorbed benzo(*k*)fluoranthene trophically via *Daphnia magna* and *Chironomus riparius* (Hanslik et al. 2020).

The physiological effects of microplastics in non-commercial finfish include decreased lipid metabolism and oxidative and hepatic stress in adult Zebrafish (*D. rerio)* (Lu et al. 2016), decreased growth and body condition of juvenile forage fish (*Acanthochromis polyacanthus*) (Critchell and Hoogenboom 2018), decreased body length and mass in juvenile Glassfish (*Ambassis dussumieri*) (Naidoo and Glassom 2019), reduced predatory performance in juvenile Common Goby (*Pomatoschistus microps*) (de Sa et al. 2015), and endocrine disruption in adult Japanese Medaka (*Oryzias latipes*) (Rochman et al. 2014). It is therefore apparent that concern is warranted and additional research is necessary, especially in commercial species. Even in the limited studies on commercial species, microplastic exposure can result in weakened feeding behaviors and reduced energy reserves in juvenile Korean Rockfish (*Sebastes schlegelii*) (Yin et al. 2018) and pathological alterations to intestinal epithelium in juvenile European sea bass (*Dicentrarchus labrax*) (Peda et al. 2016), although minimal effects were observed in European Sea Bass larvae (*D. labrax)* (Mazurais et al. 2015) and juvenile Gilt-head Seabream (*Sparus aurata*) (Jovanovic et al. 2018). Additional experiments are needed to resolve the interaction of microplastics across different morphologies and polymer types, with a focus on frequently detected fibers (Ross et al. 2021), as well as there being a need for a better understanding of the role of olfaction and particle selection across early life stages in fishes.

CONCLUSIONS

This study provides the first assessment of the effects of microplastic exposure in early life stages of the commercially and recreationally important fish species (*C. striata*). We found that direct ingestion of LDPE microspheres by larval *C. striata* was only detected at high levels of exposure with no difference between virgin and chemically treated microspheres. Importantly, *C. striata* larvae ingested significantly more microspheres via trophic transfer from microzooplankton prey (*Favella* spp.), indicating that ingestion via prey should be further evaluated in future assessments. Juvenile *C. striata* are susceptible to physiological impairment (i.e., increased oxygen consumption and altered immune response) following 96-h exposure to some but not all microplastic treatments, additional research in this area is clearly needed.

In the present study, chemically treating microspheres with a plastic additive and a PAH did not have a significant effect on ingestion, oxygen consumption, or immune response of early juvenile *C. striata*. However, the presence of microfibers resulted in significantly increased oxygen consumption in early juvenile *C. striata* compared to the presence of microspheres (virgin or chemically treated). This information is important considering the growing body of literature suggesting that microfibers are the most prevalent type of microplastic ingested by wild-caught marine organisms and may present the greatest risk to the respiratory system in aquatic animals (Lusher et al. 2013; Mishra et al. 2019).

This study aimed to address several critical knowledge gaps, particularly through using a commercial marine finfish species at early life stages, evaluating relatively low microplastic concentrations in a concentration-response design, and plastic additive-treated microplastics.

Data such as those produced here can be used to inform future risk assessments, especially considering that studies measuring responses across microplastic concentrations are currently limited in commercial fishery species (Granek et al. 2020). Future research is necessary to fully understand how commercial finfish will be affected by microplastics across shapes, sizes, and polymer types (e.g. Rochman et al. 2019, Cunningham et al. in review) and the role of microplastics as one of a suite of multiple stressors (e.g., overharvest, ocean warming, and hypoxia; Baechler et al. 2019), but there are unique challenges associated with using commercial finfish in the laboratory (e.g., complex life histories, feeding strategies, nutrient requirements, and intensive husbandry; Watanabe et al. 2019). With over 88% of global fisheries production and aquaculture being utilized for human consumption (FAO 2018), it is imperative to determine if the trophic transfer of microplastics and associated pollutants and additives present a potential risk of exposure to humans by way of seafood consumption.

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Table 1. Average number (± SEM) of virgin (untreated), phenanthrene-treated, and 2,4-DTBP-treated microspheres ingested by ciliates and *C. striata* larvae across three microplastic densities.[a]

	Virgin			**Phenanthrene**			**2,4-DTBP**		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Ciliate – Direct Ingestion	0	0.25 ± 0.06	2.04 ± 0.06	0.03 ± 0.02	0.21 ± 0.05	1.84 ± 0.33	0.01 ± 0.01	0.24 ± 0.06	2.34 ± 0.46
Larvae – Direct Ingestion	0.08 ± 0.08	0	0.91 ± 0.56	0	0.25 ± 0.25	0.33 ± 0.33	0	0	0.32 ± 0.22
Larvae – Trophic Transfer	0	0.42 ± 0.19	2.18 ± 1.70	0	0	2.17 ± 0.87	0	0.42 ± 0.26	2.08 ± 1.28

[a] Ciliate data reflects the average number of microspheres that were ingested by ciliates in three 1 mL samples for each of the 4 replicates. The larval data refers to the average number of microspheres ingested by 3 individual larvae for each of the 4 replicates – either directly from the water or via trophic transfer from prey.

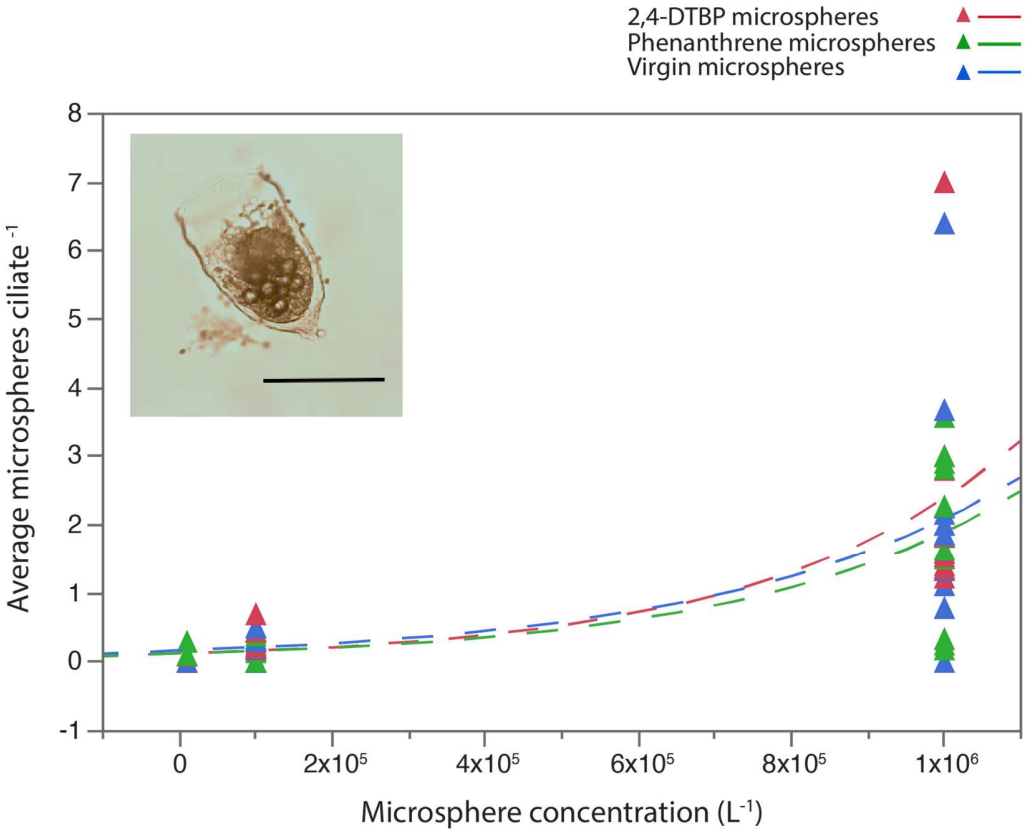

Figure 1. Average number of microspheres internalized per ciliate, the red line and red (▲) represent the 2,4-DTBP treatment, green line and green (▲) represent the phenanthrene microsphere treatment, and blue line and (▲) represent the virgin microsphere treatment. The micrograph scale bar is 100 microns. Solid lines are a significant fit, dotted lines are not significant. GLM (Poisson), $\alpha = 0.05$

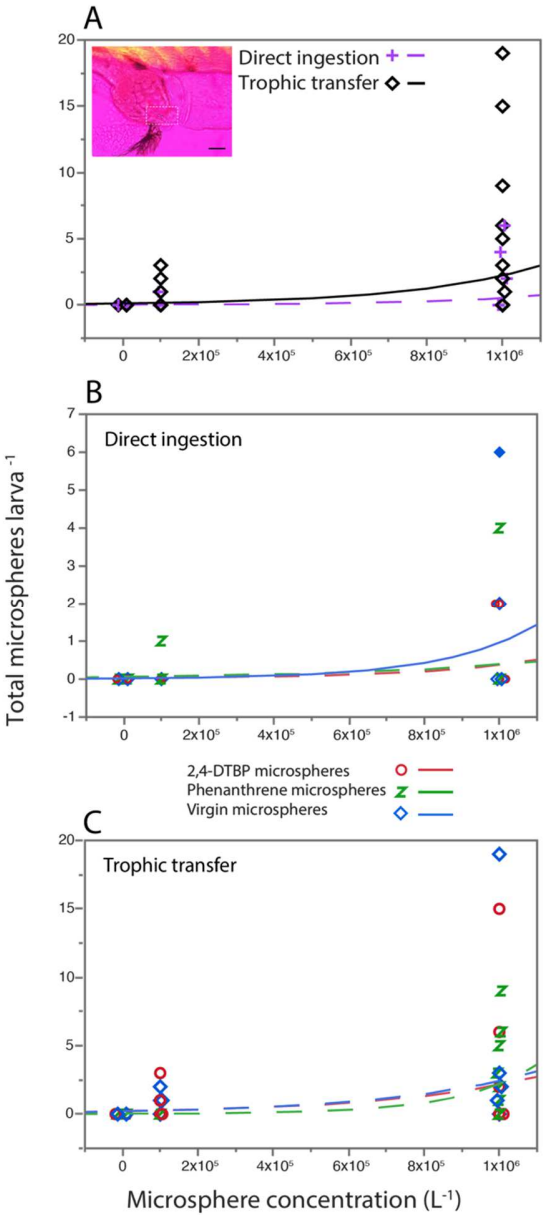

Figure 2. (A) Trophic transfer and direct microsphere ingestion by C. striata larvae, the purple line and purple (+) represent the direct ingestion treatments and black line and black (◇) represent the trophic transfer treatments. The micrograph scale bar is 100 microns. (B) Microspheres ingested by C. striata larvae via trophic transfer from prey, the red line and red (○) represent the 2,4-DTBP treatment, green line and green (z) represent the phenanthrene microsphere treatment, and blue line and (◇) represent the virgin microsphere treatment. (C) Microsphere ingestion by *C. striata* larvae directly from the water, same colors and symbols as

(B). Solid lines are a significant fit, dotted lines are not significant. All analyses used GLM (Poisson), $\alpha = 0.05$

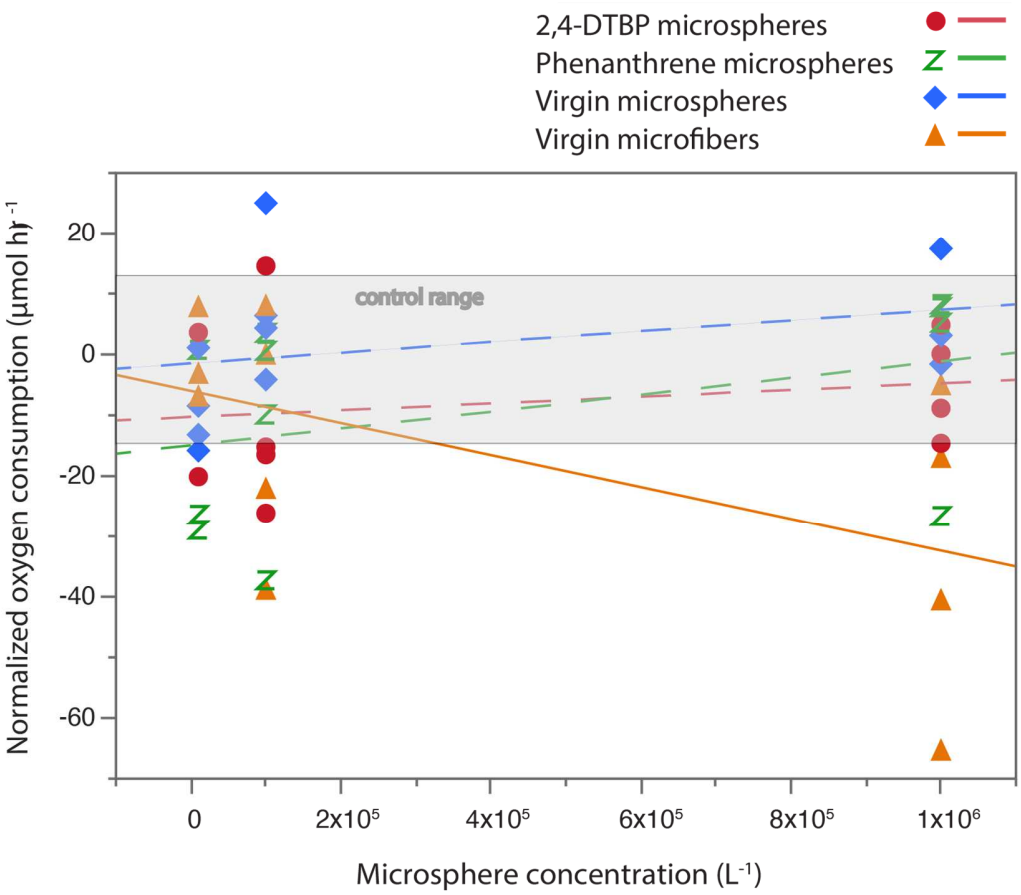

Figure 3. Oxygen depletion in juvenile *C. striata* following a direct 96-h exposure to microplastics, the red line and red (●) represent the 2,4-DTBP treatment, green line and green (z) represent the phenanthrene microsphere treatment, blue line and (◆) represent the virgin microsphere treatment, and the orange line and orange (▲) represent the virgin microfiber treatment. Data from exposure treatments were standardized by subtracting the mean oxygen depletion in the control treatment (not exposed to microplastics), hence control data are not included in the regression. The shaded box (centered on zero) represents the range of control values. GLM (Poisson), $\alpha = 0.05$

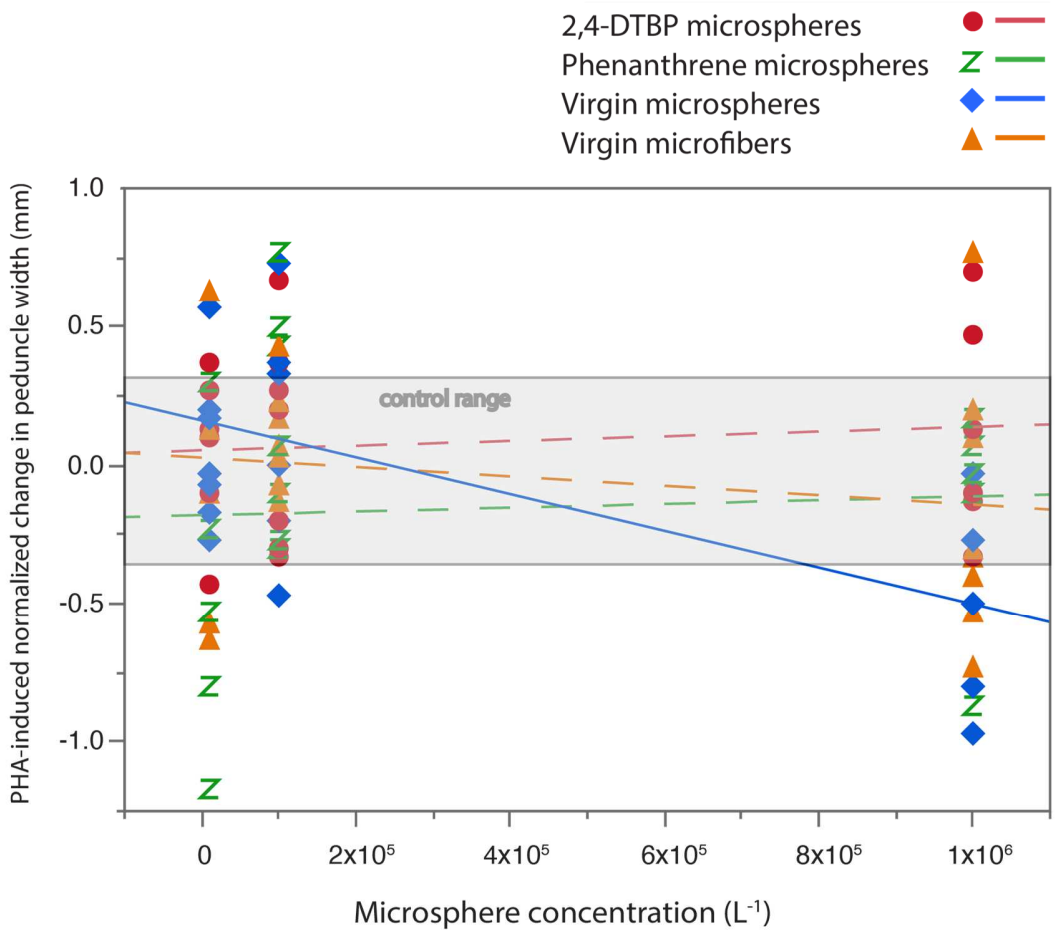

Figure 4. PHA-induced change, normalized by saline-injected control, in peduncle width (proxy for immune response) in juvenile *C. striata* following a direct 96-h exposure to microplastics, the red line and red (●) represent the 2,4-DTBP treatment, green line and green (**z**) represent the phenanthrene microsphere treatment, blue line and (◆) represent the virgin microsphere treatment, and the orange line and orange (▲) represent the virgin microfiber treatment. Data from exposure treatments were standardized by subtracting the mean response in the control treatment (not exposed to microplastics), hence control data are not included in the regression. The shaded box (centered on zero) represents the range of caudal peduncle swelling for the control animals. Solid lines are a significant fit, dotted lines are not significant. GLM (Poisson), $\alpha = 0.05$

References

Able KW, Hales LS. 1997. Movements of juvenile Black sea bass *Centropristis striata* (Linnaeus) in a southern new jersey estuary. *J Exp Mar Bio Ecol*. **213**(2):153-167.

Andrady AL, Neal MA. 2009. Applications and societal benefits of plastics. *Philos Trans R Soc Lond B Biol Sci*. **364**(1526):1977-1984.

Athey SN, Albotra SD, Gordon CA, Monteleone B, Seaton P, Taylor AR, Brander SM. 2020. Trophic transfer of microplastics in an estuarine model and the effects of a sorbed legacy pollutant. *Limnol Oceanogr: Lett*. **5**: 154-162. Doi: 10.1002/lol2.10130

[ASMFC] Atlantic States Marine Fisheries Commission [Internet]. 2018. Arlington (VA): ASMFC; [cited 2019 January 10]. Available from: http://www.asmfc.org/species/black-sea-bass

Ardia DR, Clotfelter ED. 2006. The novel application of an immunological technique reveals the immunosuppressive effect of phytoestrogens in *Betta splendens*. *J Fish Biol*. **68**:144-149.

Avio CG, Gorbi S, Regoli F. 2017. Plastics and microplastics in the oceans: From emerging pollutants to emerged threat. *Mar Environ Res*. **128**:2-11.

Baechler BR, Stienbarger CD, Horn DA, Joseph J, Taylor AR, Granek EF, Brander SM. 2019. Microplastic occurrence and effects in commercially harvested North American finfish and shellfish: Current knowledge and future directions. *Limnol Oceanogr: Lett.* 5: 113-136 doi:10.1002/lol2.10122

Bakir A, Rowland SJ, Thompson RC. 2014. Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. *Environ Pollut*. **185**:16-23.

Barnes DKA, Galgani F, Thompson RC, Barlaz M. 2009. Accumulation and fragmentation of plastic debris in global environments. *Philos Trans R Soc Lond B Biol Sci*. **364**(1526):1985-1998.

Batel A, Linti F, Scherer M, Erdinger L, Braunbeck T. 2016. Transfer of benzo[a]pyrene from microplastics to *Artemia nauplii* and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. *Environ Toxicol Chem*. **35**(7):1656-1666.

Batel A, Borchert F, Reinwald H, Erdinger L, Braunbeck T. 2018. Microplastic accumulation patterns and transfer of benzo[a]pyrene to adult zebrafish (*Danio rerio*) gills and zebrafish embryos. *Environmental Pollution* **235**: 918–930.

Beck MW, Heck KL, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ et al. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience*. **51**(8):633-641.

Bessa F, Barria P, Neto JM, Frias JPGL, Otero V, Sobral P, Marques JC. 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. *Mar Pollut Bull*. **128**:575-584.

Besseling E, Redondo-Hasselerharm P, Foekema EM, Koelmans AA. 2019. Quantifying ecological risks of aquatic micro- and nanoplastic. *Crit Rev Env Sci Tech*. **49**:1, 32-80.
Black JA, Birge WJ, Westerman AG, Francis PC. 1983. Comparative aquatic toxicology of aromatic hydrocarbons. *Fundam Appl Toxicol*. **3**(5):353-358.
Borrelle SB, Ringma J, Lavender Law K, Monnahan CC, Lebreton L, McGivern A, Murphy E, et al. 2020. Predicted Growth in Plastic Waste Exceeds Efforts to Mitigate Plastic Pollution." *Science* **369** (6510): 1515.
Brander SM, Renick VC, Foley MM, Steele C, Woo M, Lusher A, Carr S, Helm P, Box C, Cherniak S. 2020. Sampling and Quality Assurance and Quality Control: A Guide for Scientists Investigating the Occurrence of Microplastics Across Matrices. *Applied Spectroscopy* **74** (9): 1099–1125.
Brander SM, Jeffries KM, Cole BJ, DeCourten BM, White JW, Hasenbein S, Fangue NA, Connon RE. 2016. Transcriptomic changes underlie altered egg protein production and reduced fecundity in an estuarine model fish exposed to bifenthrin. Aquatic Toxicology 174, 247–260.
Brander SM. 2013. Thinking outside the box: Assessing endocrine disruption in aquatic life. In: Ahuja S, editor. Monitoring Water Quality: Pollution Assessment, Analysis, and Remediation. Waltham (MA): Elsevier. p. 103-147.
Breckels RD, Neff BD. 2013. The effects of elevated temperature on the sexual traits, immunology and survivorship of a tropical ectotherm. *J Exp Biol*. **216**(14):2658-2664.
Browne MA, Dissanayake A, Galloway TS, Lowe DM, Thompson RC. 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environ Sci Technol*. **42**(13):5026-5031.
Bucci K, Tulio M, Rochman CM. 2019. What Is Known and Unknown about the Effects of Plastic Pollution: A Meta-Analysis and Systematic Review. *Ecol Apps* **30** (2): e02044.
Bucci K, Bikker J, Stevack K, Watson-Leung T, Rochman C. 2021. Impacts to Larval Fathead Minnows Vary between Preconsumer and Environmental Microplastics. *Env Tox Chem*. https://doi.org/10.1002/etc.5036
Carbery M, O'Connor W, Thavamani P. 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. *Environ Int*. **115**:400-409.
Clotfelter ED, Ardia DR, McGraw KJ. 2007. Red fish, blue fish: Trade-offs between pigmentation and immunity in *Betta splendens*. *Behav Ecol*. **18**(6):1139-1145.
Cole M. 2016. A novel method for preparing microplastic fibers. *Sci Rep*. 6.
Cole M, Lindeque P, Fileman E, Halsband C, Goodhead R, Moger J, Galloway TS. 2013. Microplastic ingestion by zooplankton. *Environ Sci Technol*. **47**(12):6646-6655.
Collignon A, Hecq JH, Galgani F, Voisin P, Collard F, Goffart A. 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. *Mar Pollut Bull*. **64**(4):861-864.
Conkle JL, Del Valle CDB, Turner JW. 2018. Are we underestimating microplastic contamination in aquatic environments? *Environ Manage*. **61**(1):1-8.
Cottingham KL, Lennon JT, Brown BL. 2005. Knowing when to draw the line: designing more informative ecological experiments. Frontiers in Ecology and the Environment **3**: 145–152.
Critchell K, Hoogenboom MO. 2018. Effects of microplastic exposure on the body condition and behaviour of planktivorous reef fish (*Acanthochromis polyacanthus*). *PLoS One*. **13**(3).

de Ruijter VN, Redondo-Hasselerharm PE, Gouin T, Koelmans AA. 2020. Quality Criteria for Microplastic Effect Studies in the Context of Risk Assessment: A Critical Review. *Environ. Sci. Technol.* **54**:11692–11705.

de Sa LC, Luis LG, Guilhermino L. 2015. Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. *Environ Pollut.* **196**:359-362.

DeCourten BM, Forbes JP, Roark HK, Burns NP, Major KM, White JW, Li J, Mehinto AC, Connon RE, Brander SM. 2020. Multigenerational and Transgenerational Effects of Environmentally Relevant Concentrations of Endocrine Disruptors in an Estuarine Fish Model. *Environmental Science & Technology* **54** (21): 13849–60.

Desforges JPW, Galbraith M, Ross PS. 2015. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. *Arch Environ Contam Toxicol*. **69**(3):320-330.

Devriese LI, van der Meulen MD, Maes T, Bekaert K, Paul-Pont I, Frere L, Robbens J, Vethaak AD. 2015. Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. *Mar Pollut Bull*. **98**(1-2):179-187.

Echevarria ML, Wolfe GV, Strom SL, Taylor AR. 2014. Connecting alveolate cell biology with trophic ecology in the marine plankton using the ciliate *Favella* as a model. *FEMS Microbiol Ecol*. **90**(1):18-38.

Eriksen M, Lebreton LCM, Carson HS, Thiel M, Moore CJ, Borerro JC, Galgani F, Ryan PG, 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**(12).

Firestein S. 2001. How the olfactory system makes sense of scents. *Nature*. **413**(6852):211-218.

Foekema EM, De Gruijter C, Mergia MT, van Franeker JA, Murk AJ, Koelmans AA. 2013. Plastic in North Sea fish. *Environ Sci Technol*. **47**(15):8818-8824.

[FAO] Food and Agriculture Organization of the United Nations. 2018. The State of World Fisheries and Aquaculture 2018 - meeting the sustainable development goals [Internet]. Rome, Italy: FAO; [cited 2019 10 January]. Available from: http://www.fao.org/3/i9540en/i9540en.pdf

Franzellitti S, Canesi L, Auguste M, Wathsala RHGR, Fabbri E. 2019. Microplastic exposure and effects in aquatic organisms: A physiological perspective. *Environ Toxicol Pharmacol*. **68**:37-51.

Gall SC, Thompson RC. 2015. The impact of debris on marine life. *Mar Pollut Bull*. **92**(1-2):170-179.

Gallo F, Fossi C, Weber R, Santillo D, Sousa J, Ingram I, Nadal A, Romano D. 2018. Marine litter plastics and microplastics and their toxic chemicals components: The need for urgent preventive measures. *Environ Sci Eur*. **30**.

Geyer R, Jambeck JR, Law KL. 2017. Production, use, and fate of all plastics ever made. *Sci Adv*. **3**(7).

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. Aquatic Toxicology 186, 96–104.

Gray AD, Wertz H, Leads RR, Weinstein JE. 2018. Microplastic in two South Carolina estuaries: Occurrence, distribution, and composition. *Mar Pollut Bull*. **128**:223-233.

Griniene E, Sulcius S, Kuosa H. 2016. Size-selective microzooplankton grazing on the phytoplankton in the Curonian Lagoon (SE Baltic Sea). *Oceanologia*. **58**(4):292-301.
Hanslik, L., Sommer, C., Huppertsberg, S., Dittmar, S., Knepper, T.P., Braunbeck, T., 2020. Microplastic-associated trophic transfer of benzo(k)fluoranthene in a limnic food web: Effects in two freshwater invertebrates (Daphnia magna, Chironomus riparius) and zebrafish (Danio rerio). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 237, 108849.
Hasegawa, T., Nakaoka, M., 2021. Trophic transfer of microplastics from mysids to fish greatly exceeds direct ingestion from the water column. Environmental Pollution 273, 116468.
Hermabessiere L, Dehaut A, Paul-Pont I, Lacroix C, Jezequel R, Soudant P, Duflos G. 2017. Occurrence and effects of plastic additives on marine environments and organisms: A review. *Chemosphere*. **182**:781-793.
Jacob H, Besson M, Swarzenski PW, Lecchini D, Metian, M. 2020. Effects of Virgin Micro-and Nanoplastics on Fish: Trends, Meta-Analysis, and Perspectives. *Environmental Science & Technology* **54** (8): 4733–45.
Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, Narayan R, Law KL. 2015. Plastic waste inputs from land into the ocean. *Science*. **347**(6223):768-771.
Janeway CA. 2001. How the immune system protects the host from infection. Microbes Infect. **3**(13):1167-1171.
Jovanovic B, Gokdag K, Guven O, Emre Y, Whitley EM, Kideys AE. 2018. Virgin microplastics are not causing imminent harm to fish after dietary exposure. *Mar Pollut Bull*. **130**:123-131.
Karami A, Golieskardi A, Choo CK, Larat V, Karbalaei S, Salamatinia B. 2018. Microplastic and mesoplastic contamination in canned sardines and sprats. *Sci Total Environ*. **612**:1380-1386.
Koelmans AA, Bakir A, Burton GA, Janssen CR. 2016. Microplastic as a vector for chemicals in the aquatic environment: Critical review and model-supported reinterpretation of empirical studies. *Environ Sci Technol*. **50**(7):3315-3326.
Koelmans AA, Besseling E, Foekema EM. 2014. Leaching of plastic additives to marine organisms. *Environ Pollut*. **187**:49-54.
Lara MR. 2008. Development of the nasal olfactory organs in the larvae, settlement-stages and some adults of 14 species of Caribbean reef fishes (*Labridae, Scaridae, Pomacentridae*). *Mar Biol*. **154**(1):51-64.
Liboiron F, Ammendolia J, Saturno J, Melvin J, Zahara A, Richard N, Liboiron M. 2018. A zero percent plastic ingestion rate by silver hake (*Merluccius bilinearis*) from the south coast of Newfoundland, Canada. *Mar Pollut Bull*. **131**:267-275.
Lu Y, Zhang Y, Deng Y, Jiang W, Zhao Y, Geng J, Ding L, Ren H. 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. *Environ Sci Technol*. **50**(7):4054-4060.
Lusher AL, Munno K, Hermabessiere L, Carr S. Isolation and Extraction of Microplastics from Environmental Samples: An Evaluation of Practical Approaches and Recommendations for Further Harmonization. *Applied Spectroscopy* **74** (9): 1049–65.
Lusher AL, Hollman P, Mendoza-Hill J. 2017. Microplastics in fisheries and aquaculture status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO Fisheries and Aquaculture Technical Paper. **615**:1-126,IV,V.

Lusher AL, McHugh M, Thompson RC. 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar Pollut Bull*. 67(1-2):94-99.

Mazurais D, Ernande B, Quazuguel P, Severe A, Huelvan C, Madec L, Mouchel O, Soudant P, Robbens J, Huvet A et al. 2015. Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (*Dicentrarchus labrax*) larvae. *Mar Environ Res*. **112**:78-85.

McConville MM, Roberts JP, Boulais M, Woodall B, Butler JD, Redman AD, Parkerton TF, Arnold WR, Guyomarch J, LeFloch S et al. 2018. The sensitivity of a deep-sea fish species (*Anoplopoma fimbria*) to oil-associated aromatic compounds, dispersant, and Alaskan North Slope crude oil. *Environ Toxicol Chem*. **37**(8):2210-2221.

Mishra S, Rath CC, Das AP. 2019. Marine microfiber pollution: A review on present status and future challenges. *Mar Pollut Bull*. **140**:188-197.

Montgomery, DC, Peck EA, Vining GG. 2021. Introduction to linear regression analysis. John Wiley & Sons.

Mundy PC, Carte MF, Brander SM, Hung T-C, Fangue N, Connon RE. 2020. Bifenthrin exposure causes hyperactivity in early larval stages of an endangered fish species at concentrations that occur during their hatching season. Aquatic Toxicology 228, 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.

Nelms SE, Galloway TS, Godley BJ, Jarvis DS, Lindeque PK. 2018. Investigating microplastic trophic transfer in marine top predators. *Environ Pollut*. **238**:999-1007.

Oozeki Y, Hirano R. 1988. Effects of glutaraldehyde fixation on the body size of red sea bream (*Pagrus major*) larvae. *Aquaculture*. **71**(3):265-269.

Pannetier P, Morin B, Le Bihanic F, Dubreil L, Clérandeau C, Chouvellon F, Van Arkel K, Danion M, Cachot J. Environmental Samples of Microplastics Induce Significant Toxic Effects in Fish Larvae. *Environment International* **134**: 105047.

Peda C, Caccamo L, Fossi MC, Gai F, Andaloro F, Genovese L, Perdichizzi A, Romeo T, Maricchiolo G. 2016. Intestinal alterations in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) exposed to microplastics: Preliminary results. *Environ Pollut*. **212**:251-256.

Peng X, Sun X, Yu M, Fu W, Chen H, Chen J. 2019. Chronic exposure to environmental concentrations of phenanthrene impairs zebrafish reproduction. *Ecotox Environ Safe*. 182.

Pozo K, Gomez V, Torres M, Vera L, Nuñez D, Oyarzún P, Mendoza G, et al. 2019. Presence and Characterization of Microplastics in Fish of Commercial Importance from the Biobío Region in Central Chile. *Marine Pollution Bulletin* **140**: 315–19.

Rabalais NN. 2015. Human impacts on fisheries across the land-sea interface. *Proc Natl Acad Sci U S A*. **112**(26):7892-7893.

Rani M, Shim WJ, Han GM, Jang M, Al-Odaini NA, Song YK, Hong SH. 2015. Qualitative analysis of additives in plastic marine debris and its new products. *Arch Environ Contam Toxicol*. **69**(3):352-366.

Reisser J, Slat B, Noble K, du Plessis K, Epp M, Proietti M, de Sonneville J, Becker T, Pattiaratchi C. 2015. The vertical distribution of buoyant plastics at sea: An observational study in the North Atlantic gyre. *Biogeosciences*. **12**(4):1249-1256.

Rios LM, Moore C, Jones PR. 2007. Persistent organic pollutants carried by synthetic polymers in the ocean environment. *Mar Pollut Bull*. **54**(8):1230-1237.
Rochman CM, Kurobe T, Flores I, Teh SJ. 2014. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. *Sci Total Environ*. **493**:656-661.
Samanta SK, Singh OV, Jain RK. 2002. Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. *Trends Biotechnol*. **20**(6):243-248.
Santillo D, Miller K, Johnston P. 2017. Microplastics as contaminants in commercially important seafood species. *Integr Environ Assess Manag*. **13**(3):516-521.
Sedberry GR. 1988. Food and feeding of Black sea bass *Centropristis striata* in live bottom habitats in the South Atlantic Bight Atlantic Ocean. *J Elisha Mitchell Sci Soc*. **104**(2):35-50.
Setala O, Fleming-Lehtinen V, Lehtiniemi M. 2014. Ingestion and transfer of microplastics in the planktonic food web. *Environ Pollut*. **185**:77-83.
Simberloff, D. 1978. Use of Rarefaction and Related Methods in Ecology. in *Biological Data in Water Pollution Assessment: Quantitative and Statistical Analyses*, ed. K. Dickson, J. Cairns, and R. Livingston (West Conshohocken, PA: ASTM International), 150-165.
Tosetto L, Williamson JE, Brown C. 2017. Trophic transfer of microplastics does not affect fish personality. *Anim Behav*. **123**:159-167.
Van Cauwenberghe L, Devriese L, Galgani F, Robbens J, Janssen CR. 2015. Microplastics in sediments: A review of techniques, occurrence and effects. *Mar Environ Res*. **111**:5-17.
Vendel AL, Bessa F, Alves VEN, Amorim ALA, Patricio J, Palma ART. 2017. Widespread microplastic ingestion by fish assemblages in tropical estuaries subjected to anthropogenic pressures. *Mar Pollut Bull*. **117**(1-2):448-455.
Voesenek CJ, Muijres FT, van Leeuwen JL. 2018. Biomechanics of swimming in developing larval fish. *J Exp Biol*. **221**(1).
Wang H, Liang Y, Li S, Chang J. 2013. Acute toxicity, respiratory reaction, and sensitivity of three cyprinid fish species caused by exposure to four heavy metals. *PLoS One*. **8**(6).
Wardrop P, Shimeta J, Nugegoda D, Morrison PD, Miranda A, Tang M, Clarke BO. 2016. Chemical pollutants sorbed to ingested microbeads from personal care productsaccumulate in fish. *Environ Sci Technol*. **50**(7):4037-4044.
Watanabe, W.O. 2011. Species profile: Black Sea Bass. Southern Regional Aquaculture Center. Texas A & M University. SRAC Publication No. 7207.
Watanabe WO, Alam MS, Carroll PM, Daniels HV, Hinshaw JM. 2019. Marine Finfish Aquaculture. In: Lucas JS, Southgate PC, Tucker CS, editors. Aquaculture: Farming Aquatic Animals and Plants. 3rd Edition. Hoboken (NJ): Wiley-Blackwell. p. 437-481.
Watanabe, WO, Carroll, PM, Alam, MS, Dumas, CF, Gabel, JE, Davis, TM, and Bentley, CD. 2021. The status of black sea bass, *Centropristis striata*, as a commercially ready species for U.S. marine aquaculture. Journal World Aquaculture Society. https://doi.org/10.1111/jwas.12803
Watts AJR, Lewis C, Goodhead RM, Beckett SJ, Moger J, Tyler CR, Galloway TS. 2014. Uptake and retention of microplastics by the shore crab *Carcinus maenas*. *Environ Sci Technol*. **48**(15):8823-8830.
Watts AJR, Urbina MA, Goodhead R, Moger J, Lewis C, Galloway TS. 2016. Effect of microplastic on the gills of the shore crab *Carcinus maenas*. *Environ Sci Technol*. **50**(10):5364-5369.

Weinstein JE, Crocker BK, Gray AD. 2016. From macroplastic to microplastic: Degradation of high-density polyethylene, polypropylene, and polystyrene in a salt marsh habitat. *Environ Toxicol Chem*. **35**(7):1632-1640.

Welden NA, Abylkhani B, Howarth LM. 2018. The effects of trophic transfer and environmental factors on microplastic uptake by plaice, *Pleuronectes plastessa*, and spider crab, *Maja squinado*. *Environ Pollut*. **239**:351-358.

Wright SL, Kelly FJ. 2017. Plastic and human health: A micro issue? *Environ Sci Technol.* **51**(12):6634-6647.

Yin L, Chen B, Xia B, Shi X, Qu K. 2018. Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (*Sebastes schlegelii*). *J Hazard Mater*. **360**:97-105.

Yonkos LT, Friedel EA, Perez-Reyes AC, Ghosal S, Arthur CD. 2014. Microplastics in four estuarine rivers in the Chesapeake Bay, USA. *Environ Sci Technol*. **48**(24):14195-14202.

Ziccardi LM, Edgington A, Hentz K, Kulacki KJ, Driscoll SK. 2016. Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: A state-of-the-science review. *Environ Sci Technol*. **35**(7):1667-1676.

Zingel P, Agasild H, Karus K, Buholce L, Nõges T. 2019. Importance of ciliates as food for fish larvae in a shallow sea bay and a large shallow lake. *Eur J Protistol*. **67**:59-70.

Zhu M, Chernick M, Rittschof D, Hinton DE. 2020. Chronic dietary exposure to polystyrene microplastics in maturing Japanese medaka (*Oryzias latipes*). *Aquat Toxicol*. **220**: 105396.