SUPPLEMENTAL INFORMATION

# TROPHIC TRANSFER OF MICROPLASTICS IN AN ESTUARINE FOOD CHAIN AND THE EFFECTS OF A SORBED LEGACY POLLUTANT

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**Culturing Ciliates, Phytoplankton and Silversides**

*Favella* spp. were obtained from Dr. Susanne Strom (Shannon Point Marine Laboratory, Anacortes, WA, USA) and maintained in 200 mL batches at 16°C at 50-80 µmol photons $m^{-2}$ $s^{-1}$ with a 12:12 light cycle in 30 ppt filtered seawater supplemented with a trace metal solution (Strom et al. 2007). Ciliates were fed a mixture of phytoplankton, including *Heterocapsa triquerta*, *Isochrysis galbana*, and *Mantoniella squamata* every 3 d and split into fresh media every week. Phytoplankton were maintained as 40 mL batch cultures in filtered seawater (30 ppt) supplemented with f/2 nutrients and Guillard's vitamins.

Silversides were obtained from Aquatic BioSystems (Fort Collins, Colorado, USA) and reared to maturity in a recirculating system (22 ºC, 15 ppt) at the UNCW Center for Marine Science. Adult silversides were spawned according to protocols described in Middaugh et al. (1987), Brander et al. (2016), and DeCourten and Brander (2017). Water quality was monitored daily during the incubation period (average conditions were 7.8±0.05 pH; 16.4±0.26 °C; 6.07±0.48 mg $O_2$ $L^{-1}$; 0.05±0.13 ppm $NH_{3/4}$) and 50% water changes (artificial seawater – ASW, Instant Ocean, Blacksburg, VA) were completed after 4 d. Hatched larvae were fed a powdered diet of B.P. American (Ocean Star International Inc. (O.S.I.), Snowville, Utah, USA) twice daily while being acclimated over 4 d to conditions favored by *Favella*. Temperature of the incubator was decreased approximately 1°C each day and salinity increased approximately 4 ppt per day to final levels of 16°C and 30 ppt by day 4. All experiments were performed in accordance with UNCW IACUC protocols 1410 and 1415-024.

**Preliminary experiments**

Independent groups of larvae (n=5) were allowed to prey on either fluorescent or non-fluorescent LDPE microspheres (Cospheric, Santa Barbara, CA, USA) for 2 h before sampling,

fixation and microscopy examination. It was determined that both fluorescent and non-fluorescent microspheres were taken up by the larval fish.

DDT-treated microspheres were incubated in filtered seawater. Water samples (1 mL) were taken before and after adding microspheres and then every 30 min for 2h, extracted using 1 mL of chloroform and analyzed using gas chromatography mass spectrometry (GCMS) to evaluate background and levels of DDT leaching from microplastics throughout the feeding period. Samples were analyzed using a Varian Saturn 2000 ion trap GCMS system. The temperature program started at 180°C, ramped up to 270°C at 15 degrees $min^{-1}$, and held at 270°C for an additional 12 min. All solvents used were $GC^2$ grade. The limit of quantification (LOQ) for DDT was 50 ng $L^{-1}$.

**Microsphere Preparation**

Microspheres treated with 4,4'-dichlorobiphenyltrichloromethane (4,4-DDT) were prepared by incubating 1 g of LDPE microspheres (0.92 g $mL^{-1}$) per 1 mL of a solution of methanol and 4,4-DDT (5 mg $mL^{-1}$) for one week at 32°C, after which particles were filtered, washed with methanol and dried at room temperature. DDT concentration was determined using high-pressure liquid chromatography (HPLC) ($2.15 \times 10^6$ ng $g^{-1}$). All glassware used was washed in deionized (DI) water and soaked overnight in 10% nitric acid prior to experimentation. The glassware used in microsphere preparation were cleaned using the method above, but also rinsed with dichloromethane (DCM) and baked in a muffle furnace at degrees 450°C for 4 hours to prevent organic contamination. A 0.01% surfactant solution was created using 1µL of Tween20 (Fisher Scientific, Pittsburgh, PA, USA) in 10 mL of ultra-pure water and mixed for 30 min. 5.5 mL of the solution was added to 11 centrifuge tubes (0.5 mL to each tube), which were placed on

a hot plate and incubated at 100 – 103°C for 5 min. 1 mg of untreated microspheres was added to each tube. The tubes were then vortexed until the microspheres were evenly dispersed.

**Favella spp. Microplastic Ingestion**

Prior to the addition of microspheres, cells were counted under a Leica DME microscope (Wetzler, Germany) using a Sedgewick-Rafter chamber. After addition of plastics, the mixture was stirred gently using a glass pipette to ensure equal distribution of microspheres. Three 1 mL subsamples were then taken from each treatment beaker to determine ciliate density and to validate microsphere concentration. After 1 h, ciliates were observed under the microscope to check their health and degree of microsphere ingestion and then reverse filtered through a 40 µm cell strainer twice in order to remove non-ingested microspheres.

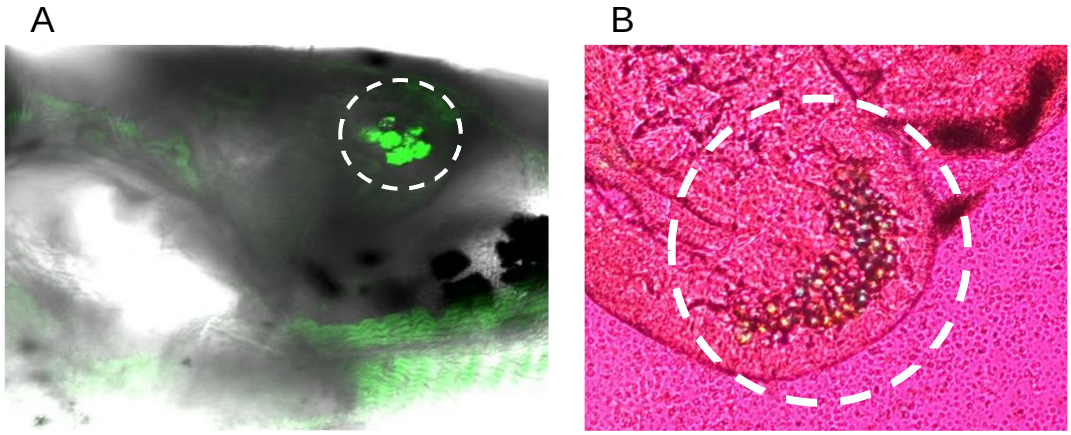


**Figure S1.** (A) Image of a larval silverside with ingested fluorescent microspheres viewed using a confocal microscope. Flourescent microspheres are visible in the stomach region indicated by white dotted circle (B) Larval silverside viewed using polarizing light microscopy (PLM) under a ZEISS Axio microscope to count ingested microplastic particles (10-20µm). White dotted circle indicates ingested plastic particles in gastrointestinal tract visualized as yellow-blue with first order plate.