



The effects of microplastic on freshwater *Hydra attenuata* feeding, morphology & reproduction[☆]

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ABSTRACT

Microplastic pollution has been a growing concern in the aquatic environment for several years. The abundance of microplastics in the environment has invariably led them to interact with a variety of different aquatic species. The small size of microplastics may make them bioavailable to a great range of species however, the impact this may have is not fully understood. Much of the research on microplastic pollution has focused on the marine environment and species with little research undertaken in freshwater. Here we examine the effect of microplastics on the freshwater cnidarian, *Hydra attenuata*. This study also describes the development and use of a bioassay to investigate the impact of microplastic on freshwater organisms. *Hydra attenuata* play a vital role in the planktonic make up of slow moving freshwater bodies which they inhabit and are sensitive environmental indicators. *Hydra attenuata* were exposed to polyethylene flakes (<400 μm) extracted from facewash at different concentrations (Control, 0.01, 0.02, 0.04, 0.08 g mL^{-1}). The ecologically relevant endpoint of feeding was measured by determining the amount of prey consumed (*Artemia salina*) after 30 and 60 min. The amount of microplastics ingested was also recorded at 30 min and 60 min. After which *Hydra attenuata* were transferred to clean media and observed after 3, 24, 48 & 96 h with changes in their morphology and reproduction (Hydranth numbers) recorded. The results of this study show that *Hydra attenuata* are capable of ingesting microplastics, with several individuals completely filling their gastric cavities. Significant reductions in feeding rates were observed after 30 min in 0.02 & 0.08 g mL^{-1} and after 60 min in 0.04 & 0.08 g mL^{-1} exposures. Exposure to the microplastics caused significant changes to the morphology of *Hydra attenuata*, however these changes were non-lethal. This study demonstrates that freshwater *Hydra attenuata* is capable of ingesting microplastics and that microplastic can significantly impact the feeding of freshwater organisms.

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1. Introduction

Plastic pollution in the environment has been well studied for a number of decades (Azzarello and Van Vleet, 1987; Pruter, 1987; Derraik, 2002). The impact of larger plastic material on birds (Azzarello and Van Vleet, 1987), marine mammals (Laist, 1997) and turtles (Tomás et al., 2002) has been given considerable attention however, in recent years the issue of smaller plastic material known as microplastics has been gaining increasing attention (Andrady, 2011). Microplastics are pieces of plastic <5 mm (Arthur et al., 2009) and have been found in sediments (Browne et al., 2011;

Eriksen et al., 2013), aquatic water bodies (Collignon et al., 2012; Lechner et al., 2014; Free et al., 2014) and ingested by a range of species with varying feeding strategies and habitats (Lusher et al., 2016; Welden and Cowie, 2016a). The study of microplastic pollution has primarily focused on the marine environment with comparatively little research conducted on the freshwater environment, however research indicates that microplastic pollution of the freshwater environment may be as prevalent, as reviewed by (Eerkes-Medrano et al., 2015).

Sources of microplastic in the freshwater environment include treated effluent from wastewater treatment plants (WWTP), with one plant in Scotland estimated to release up to 65 million microplastics into the freshwater/brackish environment everyday (Murphy et al., 2016). A number of lakes have been investigated for microplastic pollution (Eriksen et al., 2013; Imhof et al., 2013; Free et al., 2014). The Great Lakes in North America for example, were

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found to have an average concentration of 43,157 particles per km^{-2} with the most populated lake found to have the highest microplastic count (Eriksen et al., 2013). Research undertaken on microplastic ingestion by freshwater organisms in natural populations (Faure et al., 2012; Sanchez et al., 2014; Biginagwa et al., 2016) found 12% of wild gudgeons sampled from French rivers (Sanchez et al., 2014) and 20% of Nile perch and Nile tilapia purchased in a harbor market in Lake Victoria contained microplastic (Biginagwa et al., 2016).

Several studies have looked at the potential uptake and effects of microplastics on freshwater organisms in the laboratory, these include invertebrate and vertebrate species (Rosenkranz et al., 2009; Imhof et al., 2013). Imhof et al. (2013) exposed a range of freshwater invertebrate species to microplastic and found 5 freshwater species capable of ingesting microplastic. *Daphnia* exposed to 20 nm and 1000 nm fluorescent polystyrene microspheres were found to uptake the spheres at concentrations of $2 \mu\text{m L}^{-1}$ (Rosenkranz et al., 2009). When placed in clean water after 4 h of exposure, 90% of the 1000 nm microspheres were cleared from the *Daphnia* and only 40% of the 20 nm in the same period. Despite its prevalence in the environment and the growing concern over its potential harmful effects there is currently no standardised bioassay for determining the toxicity of microplastic.

In the present study, we describe the development and use of a bioassay to investigate the impact of microplastic on the freshwater cnidarian *Hydra attenuata*. *H. attenuata* inhabits slow moving freshwater bodies where they regulate the planktonic structure through selective feeding of these habitats (Burnett, 1973; Schwartz et al., 1983) and reproduce asexually by budding every three days provided there is an adequate food supply (Burnett, 1973). *H. attenuata* is easily cultured and maintained in the laboratory and has been used extensively in toxicological assays as they are sensitive environmental indicators (Quinn et al., 2008a). The effects of wastewater, pharmaceuticals and heavy metals on *H. attenuata* have all been investigated previously (Karntanut and Pascoe, 2002; Quinn et al., 2004, 2008a). The hypothesis being tested in this study is that exposure to microplastic will reduce feeding, morphology and reproduction in the freshwater cnidarian *H. attenuata*. The objectives of this study were to expose *H. attenuata* to various microplastic concentrations, record ingestion of microplastic and prey species and observe changes in morphology and reproduction (hydranth numbers). A modified version of a previously developed protocol (Quinn et al., 2008a) was used to determine the impact of microplastic exposure on the ecologically relevant endpoints of (i) feeding rates (ii) morphology based on the Wilby, 1988 scoring system (Supporting Information (SI) Fig. 1) and (iii) hydranth number.

2. Methods

2.1. Test organism

Cultures of *H. attenuata* were sourced from a population in the Environment Canada St-Lawrence Centre (SLC), Montreal, Quebec, which have previously been used in various toxicity studies (Blaise and Kusui, 1997; Trottier et al., 1997; Quinn et al., 2007). *H. attenuata* were cultured in glass bowls containing 700 mL of Hydra medium ($147 \text{ mg L}^{-1} \text{ CaCl}_2 \cdot 2\text{H}_2\text{O}$, $110 \text{ mg L}^{-1} \text{ 2-}[(2\text{-Hydroxy-1,1-bis(hydroxymethyl)ethyl) amino] \text{ ethanesulfonic acid}$, pH 7) at $18 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ with an 8 h light (Polylux XL F58W/840, Made in Hungary, 58 W fluorescent tube outputting 5200 Lumens) and 16 h dark photoperiod, following the procedure described by Trottier et al. (1997) and were fed freshly hatched *Artemia salina* daily (used within approximately 3 h of hatching). All *H. attenuata* selected for the exposures had a morphological score of 10 per

Wilby (1988) scoring system (SI Fig. 1). Briefly the scoring systems determines toxicity by measuring drastic changes in morphology by observing the contraction of tentacles and the body and is scored from 10 (healthy, elongated tentacles and body) to 0 (disintegration). Scores of 10–6 (sub-lethal signs of toxicity such as shortened and clubbed tentacles) are reversible while scores of 5 and below are irreversible and considered endpoints of lethality.

2.2. Microplastic

A microplastic size class of $<400 \mu\text{m}$ was chosen as the freshly hatched *A. salina* nauplii that are fed to the *H. attenuata* are $<400 \mu\text{m}$ in size. Preliminary exposures showed that *H. attenuata* were capable of ingesting polyethylene flakes sourced from a commercially available face wash product which provided a cheap, plentiful and environmentally relevant (Eriksen et al., 2013) supply of microplastic within the appropriate size range (SI Table 1 & SI Method development). The face wash was passed through a $400 \mu\text{m}$ sieve to remove larger pieces of microplastic. The microplastics extracted were irregularly shaped, blue and clear in colour and their polymer type was confirmed using Fourier Transform Infrared spectrometry (FTIR). The extracted microplastic were washed 3 times with 70% ethanol, distilled H_2O and Hydra media then dried before the amounts used were weighed. The concentrations used in all the exposures were Control, 0.01, 0.02, 0.04, 0.08 g mL^{-1} .

2.3. Exposures and endpoints

Two different methods of agitating the microcentrifuge tubes to keep the microplastic in suspension were tested, a shaker (Stuart Shaking Incubator SI500) at 75 rpm used for the 0.5 mL tubes and a mechanical rotator (SI Fig. 2, built in the University of the West of Scotland by a laboratory technician using a Parvalux Electronic Motor manufactured in Wallisdown, Bournemouth, England) at 26 rpm used for the 2.0 mL tubes. Making comparisons between the two test was mainly confined to the practicality and usefulness of the separate exposure methodologies implemented. Two separate exposures were carried out in 0.5 and 2.0 mL plastic microcentrifuge tubes (Fisher Scientific). The relevant concentration of microplastic was weighed and placed into each tube, that was then filled with Hydra media and inverted 10 times to ensure the microplastic was homogeneously mixed. Healthy (morphology score 10) individual *H. attenuata* with 2 hydranths were selected from the population and carefully added to each tube (3 individuals per tube) using a pipette with each concentration being undertaken in triplicate. The exposures require that healthy *H. attenuata* (morphology score of 10) with 2 Hydranths are used, however these *H. attenuata* only make up a proportion of the total population. *H. attenuata* can have no Hydranths or more than two hydranths. In order to reduce variables, it is important to use similar *H. attenuata*. This meant there was a limit in the number of *H. attenuata* that met the exposure conditions that could be consistently removed from the population at any one time placing a limit on the number of replicates for each exposure.

The 0.5 mL exposure was repeated and the results combined for a total of 6 replicates for the 0.5 mL and 3 for the 2.0 mL exposure (SI Tables 2 and 3). *Artemia salina* cysts were left in aerated water ($700 \text{ mL ddH}_2\text{O}$, 9.8 g NaCl) at $18 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ for 48 h after which the cysts hatch. The freshly hatched *A. salina* nauplii was washed three times in Hydra media and 10 healthy (swimming) individuals were added to each microcentrifuge tube, care was taken to avoid adding *A. salina* directly onto the *H. attenuata* tentacles. The exposures began when the microcentrifuge tubes were added to the apparatus used to mechanically mix the tubes.

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