The effects of polyethylene microplastics on the growth, behaviour and cognition of juvenile convict cichlids

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A Thesis in the
Department of Biology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Science (Biology) at
Concordia University
Montreal, Quebec, Canada

January 2023

CONCORDIA UNIVERSITY

School of Graduate Studies

This is to certify that the thesis prepared

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Entitled: The effects of polyethylene microplastics on the growth, behaviour and cognition of juvenile convict cichlids

and submitted in partial fulfillment of the requirements for the degree of

Master of Science (Biology)

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ABSTRACT

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Miguel Eduardo L. Felismino

Microplastics, plastic particles between 0.0001 and 5 mm in diameter, are ubiquitous in the environment and are known to be consumed by organisms, leading to a variety of adverse effects. Our current study focused on identifying the effects of microplastic consumption on the growth, foraging and competitive interactions of juvenile convict cichlids (*Archocentrus nigrofasciatus*) and its effects on their behavioural decision making. We manipulated the levels of microplastic consumption among cichlids by feeding them brine shrimp (*Artemia* spp.) exposed to different concentrations of virgin polyethylene microspheres. Cichlids were exposed to microplastics for 10 days, during which we analyzed their foraging behaviour and competitive aggression during days 1, 6 and 10 of the experiment. Additionally, we measured their growth by mass. Following the 10-day exposure, we measured exploratory behaviour in a simple maze trial by quantifying latency to exploration, maze completion and shoaling. We performed the maze trial across two days and assessed differences in these metrics to make inferences on their learning ability. Initially, we found no impacts of microplastic exposure on foraging rate, growth, and competitive aggression. In contrast, we found significant effects on exploratory behaviour and maze performance. Fish exposed to microplastics exhibited higher latency to exploration, lower rates of maze completion and a larger change in their behaviour on the second day. Our current results show that virgin polyethylene microplastics at non-lethal levels have consequences on cichlid behaviour and cognition but not growth.

ACKNOWLEDGMENTS

I would like to extend my sincerest thanks to our lab's fearless leader and supervisor, Dr. Grant Brown, for his unwavering support of my academic, personal and professional endeavours. Thank you for providing me with the tools, both figuratively and literally, to build and complete this work. Thank you for your wisdom, guidance and, most importantly, blind optimism – I would not be here without it. I would also like to thank the members of my committee, Dr. Eric Pedersen and Dr. James W. Grant for their support and feedback. This work was also made possible by my funding sources: Concordia University's Faculty of Arts and Sciences, the Department of Biology, Concordia University's Sustainability Action Fund and the NSERC Discovery Grant (to Dr. Grant Brown). I also extend my thanks to Dr. Emma Despland, Dr. John Capobianco, Dr. John Oh and the Biochemistry Department for allowing me to use their instruments and laboratory space.

I find myself extremely fortunate to have been surrounded by an outstanding support system, the absence of which would have surely meant the demise of this project and my sanity (or whatever is left of it). Thank you to my colleagues, lab mates and friends, Denis Cao Van Truong, Veronica Groves, Alix Brusseau, Jenna Domenicano, Ségolène Chevalier-Rufigny, Félixe D. Synnott, Andrea Destarac and Abigail Stephens, for their assistance during experimental set-up and data collection. I could not have asked for a better team and I will never forget the days we shared in the dungeon. Thank you to the Animal Care Facilities team, Ivon Vassileva, Shannon Clarke and Olivier Godin, for helping me set-up my research in the most humane way and for helping me navigate the mysteries of our new facility.

I dedicate this work to my wonderful family, to the Felisminos, Torreses, Lapids and Bruans of the world, thank you for helping me pursue my passion, I love you all. Thank you to

my parents for all they have struggled through to bring our family over to better shores. I can only imagine the immense courage and perseverance it must have taken to immigrate to Canada, it continues to inspire me to make you both proud. To my sisters, Nikki, Raffi and Izzy, thank you for keeping me grounded and making sure I never forget to live my life. To my niece and nephew, Lia and Joaquin, thank you for always giving me an excuse to escape my grad school commitments and for being the most adorable little humans a *Tito* could ever ask for. I also want to thank my friends, my chosen family, Ryan, Neil, Clara, John and Sherwin, you are all salts of the earth and I appreciate you all more than you know. Last, I want to thank the glorious Toronto Maple Leafs for training my ability to endure failure, it has been truly instrumental to my success.

So long and thanks for all the fish. (Adams 1985)

Contribution of Authors

Study concept and experimental design: Felismino, Brown
Acquisition of data: Felismino, Chevalier-Rufigny
Analysis and interpretation of data: Felismino
Drafting of manuscript: Felismino
Critical revision: Felismino, Brown

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INTRODUCTION

Our planet has a persistent and growing plastic problem. Recent estimates suggest that global plastic inputs are far greater than any removal and mitigation strategies in place (Borrelle et al. 2020). Much of the problem is attributed to smaller plastic fragments including microplastics (MPs), defined as plastic particles between 0.00001 - 5 mm in diameter, which are now considered to be ubiquitous in the environment (Rochman 2018). As a result, the presence of microplastic pollution in natural environments has raised worldwide concerns about their potential harmful impacts on the biota (Bucci et al. 2020). The pathways and sources of this pollutant are complex and multi-faceted. For example, it includes inputs from terrestrial, aquatic and atmospheric sources (Rochman 2018). Aquatic environments globally have been identified as sinks for microplastic pollution, as reports in freshwater systems include the presence of microplastics in the sediment, surface water and middle water column of rivers, streams, and lakes (Eerkes-Medrano et al. 2015, Rochman 2018, Felismino et al. 2021). Aquatic organisms are being exposed to numerous anthropogenic pollutants at an increasing rate (Eerkes-Medrano et al. 2015). These pollutants include microplastics of different sizes, polymers and levels of biofouling and degradation. To no surprise, there is a growing body of evidence of the consumption and trophic transfer of microplastics among aquatic organisms (Batel et al. 2016, Athey et al. 2020, Stienbarger et al. 2021).

Once consumed, the dangers posed by microplastics are diverse and exhibit wide interspecies variation (Bucci et al. 2020). Some studies have shown that exposure to, and the consumption of, microplastics have no significant effects on the organism (Jovanović et al. 2018, Ogonowski et al. 2018, Jacob et al. 2019, Bucci et al. 2020). For example, gilt-head seabream (*Sparus aurata*) that were fed microplastics for 45 days exhibited no changes in growth and

histopathology (Jovanović et al. 2018). In contrast, others have shown that exposure to and/or consumption of microplastics can lead to adverse physiological and morphological changes including altered fat metabolism in Crucian carp (*Carassius carassius*) (Cedervall et al. 2012), reduced growth rate and increased mortality in *Daphnia magna* (Eltemsah and Bøhn 2019), and inhibition of key enzymes in the neurological pathways of common goby (*Pomastochistus microps*) (Oliveira et al. 2013). Additionally, ingestion of microplastics can cause physical damages to the digestive tract of fish including physical blockage and lacerations which could lead to poorer nutrition (Jovanović 2017). The consumption of hydrophobic plastic particles, such as the polyethylene spheres used in our study, are theorized to hinder bolus formation in fishes and reduce their ability to egest these particles and lead to impaction of the digestive tract (Miller et al. 2020). While these effects alone are enough to raise concern, our current understanding is that these effects may serve as ecotoxicological pathways for behavioral and cognitive impairments in fishes.

Despite the number of studies addressing the effects of microplastics, the complexities of its effects on fish behaviour are still poorly established. There is evidence that plastic ingestion can lead to behavioural changes including reduced feeding rate, reduced swimming activity, reduced exploration and increased shoaling behaviour in Crucian carp (Mattsson et al. 2015, 2017) as well as increased boldness (i.e., risk-taking behaviour) in Ambon damselfish (*Pomacentrus amboinensis*) (McCormick et al. 2020). The drivers of these effects are still not known but it has been hypothesized to involve hormonal changes, secondary effects of digestive tract issues or as a result of direct damage to brain tissue (Jovanović 2017, Mattsson et al. 2017, Ogonowski et al. 2018). Of these, we are particularly interested on the consequences of microplastics causing physical blockages of the digestive tract.

An impaction of the digestive tract can lead to a false feeling of satiation and a subsequent reduction in feeding (Cedervall et al. 2012, Jovanović 2017). It was shown that marine jacopever (*Sebastes schlegelii*) exposed to polystyrene microbeads exhibit reduced feeding activity (Yin et al. 2018). The resulting net decrease in the amount of nutrients consumed, combined with lower nutrient absorption, could lead to a starvation effect and an observable decrease in growth (Yin et al. 2018). Such effects could also result in reduced overall body condition. Lower nutrition has been shown to impact energy levels and can be reflected as a reduction of the fishes' swimming activity and ability to perform fitness-increasing behaviours such as exploring for, and competing for access to, food, territory and/or mates (Nunn et al. 2012). Therefore, it would be expected that the number of competitive interactions would decrease as the level of microplastic exposure increases.

Alternatively, the plastic-exposed, physiologically hungrier fish could perceive their decreased nutrient uptake as a sign of lower food quality. Outside the context of microplastics, brook charr (*Salvelinus fontinalis*) have been shown to increase competitive interactions when placed in environments with poorer food quality and/or availability thus increasing access to food (Dunbrack et al. 1996). In cases of equal availability of lower quality food, this could also manifest as an increase in foraging attempts. This compensatory increase in foraging could offset any nutritional deficits caused by microplastic consumption and thus negate the expected decrease in growth rate.

This duality of a potential response to a starvation effect is a well-established paradox in behavioural ecology (Boggs 1992, Careau and Garland 2012). Food-deprived individuals are expected to be energy-limited in their ability to find and compete for food. However, several studies show that some prey species exhibit increased activity under food-deprived environments

in an attempt to find more food (Peña-Villalobos et al. 2020). The Energy Budget Rule (EBR) postulates that individuals faced with a daily energy deficit are more likely to exhibit risk-prone behaviour (Stephens 1981). Empirical evidence of the EBR include studies which show that honeybees (*Apis mellifera*) (Mayack & Naug 2011) and juncos (*Junco phaeonotus*) (Caraco 1980) under starvation threat were more likely to choose a food source with higher reward variability (i.e. high risk, high reward). Choosing an unpredictable and more varied food source poses a higher risk of starvation and potentially death but presents the starving individuals the opportunity to offset their energy deficit. The same duality presents itself when predicting consequences of microplastic consumption on exploratory behaviour. While we expect microplastic-exposed individuals to be energy-limited in their willingness to explore, McCormick et al. (2020) demonstrated that Ambon damselfish exhibited the paradoxical response of increased exploration and risk-prone behaviours as a consequence of microplastic exposure. In these behavioural decisions, individuals must balance the trade-off between the energy cost of finding food and the associated rewards. A shift in food quality and/or their ability to process nutrients, potentially as a consequence of microplastic consumption, could lead to a shift in the cost-benefit analysis and therefore alter their behaviour.

As important as it is to understand the behaviour of freshwater fishes, it is equally important to understand how an individual modifies its behaviour as it accumulates knowledge of its surroundings. Learning can be broadly defined as an organism's ability to change their behaviour based on experience (Brown et al. 2011). The ability to process and respond to information about their surroundings can have large fitness consequences by learning the location of predators, food, mates or suitable habitats and responding accordingly. Spatial learning in an ecological context is described as the ability to associate biotic and abiotic cues

(e.g. environmental landmarks, predator encounters, scents) with opportunities to increase their fitness (e.g. locating food, territory or mates, avoiding predators). This cognitive ability is widely exhibited across several vertebrate taxa (Healy and Hurly 2004, Noble et al. 2012, Rosati et al. 2014, Beri et al. 2014). Acting on available information and learned associations reduces both lethal (e.g. being predated upon) and non-lethal (e.g. energetic expenditure) risks of exploration (Beri et al. 2014). In fishes, Trinidadian guppies (*Poecilia reticulata*) have been shown to improve their ability to reach a food source following training with a food reward (Lucon-Xiccato and Bisazza 2017). The literature on the complexities and drivers of spatial learning is vast and well-established. However, we still have an incomplete understanding of how it can be affected by anthropogenic pollutants such as microplastics. There is limited information on the effects of microplastics on learning and overall cognitive ability. Polyethylene and polystyrene microplastics have been shown to cause varying degrees of cognitive impairments including decreased memory and learning ability (Wang et al. 2022, Lee et al. 2022, Balzani et al. 2022). It is, however, important to note that these studies were done on mammalian and invertebrate models. To our knowledge there has been no published reports on a fish model. It is also equally important to point out that these studies were performed using smaller plastic particles up to two orders of magnitude smaller than those used in our study. Given that the effects of microplastics on organisms are largely variable, and dependent on the plastics' shape, size and polymer type, it is clear that there is a knowledge gap that needs to be filled (Rochman et al. 2019, Covernton et al. 2019).

The environmental stress and pollution brought about by the Anthropocene makes it paramount to understand the effects of human-induced stressors on important biological processes. The main goal of this thesis was to determine the impacts of microplastics on the

growth, behaviour, and cognition of juvenile convict cichlids (*Archocentrus nigrofasciatus*). We refer to our set of initial predictions as the satiation hypothesis (SH). We predicted that the trophic transfer and retention of microplastics will result in 1) reduced growth, 2) reduced foraging rate, 3) fewer competitive interactions and, 4) lower exploration. Our alternative hypothesis, the compensation hypothesis (CH), predicts 1) no effects on growth, 2) increased foraging rate, 3) increased competitive interactions and, 4) higher exploration. In both cases, we predict lower maze improvement as a signal of cognitive impairment caused by microplastic consumption. The predictions of both the satiation and compensation hypotheses are under the premise that microplastic consumption would elicit a nutritional deficit that would manifest in the cichlids' growth and foraging behaviour. We propose this to be the mechanism that would elicit a response in the fish's exploratory behaviour. In both hypotheses, we expect the effect sizes to be larger with higher microplastic concentrations.

METHODS

Study species

We used juvenile convict cichlids (*Archocentrus nigrofasciatus*) as the study species for this project. The cichlids used in this study were reared from a laboratory stock population. These fish were descendants of wild-caught individuals from Costa Rica. Cichlids in their wriggler stage, 1-2 days post-hatch, were housed in 20 L glass aquaria along with their siblings from the same brood ranging from 10 – 50 individuals per tank. Aquaria were maintained at constant water (dechlorinated water, aerated with a single air stone, temperature ~24 ºC, pH ~7.2) and photoperiod (12:12 Light:Dark cycle) conditions. Prior to the experiment, fish were fed with commercial flake food (Nutrafin Basix Staple Food) ad-libitum twice daily. The protocol used in the handling and care of fish were in accordance with the Concordia University Animal Research Ethics protocol #30000255.

We also reared brine shrimp (Artemia spp.) from commercially sourced frozen cysts (AAA Brine Shrimp Egg Hatch Grade A 90%+, AAA Aquatics Inc., Florida, USA) using an acrylic incubation apparatus (HATCH-RITE III, Florida Aqua Farms Inc., Florida, USA) (Figure S1). *Artemia* nauplii were harvested after 24 hours of incubation, transferred into glass jars and exposed to microplastics (see treatment description below). Shrimp were kept in warm aerated saltwater (26 g NaCl L^{-1}, ~26 ºC, pH ~ 7.2) with a 24-hour light source. The shrimp were then rinsed with freshwater and subsequently fed to juvenile convict cichlids during the experiment.

Microplastic quality assurance

We used virgin (i.e. unused), fluorescent yellow, polyethylene microspheres (Cospheric LLC, California, USA), also referred to as microbeads, with a size range of 10-20 μm. Polyethylene microplastics, as spheres and other shapes, are pervasive in aquatic environments

due to their wide use in plastic production, the fishing industry and as single-use plastics (Da Costa et al. 2018, Felismino et al. 2021). Additionally, fluorescent microspheres of this size range have been shown to be readily ingested by *Artemia* nauplii and easily visualized using an ultraviolet lamp and a microscope (Batel et al. 2016). Prior to using the microspheres, we confirmed their chemical composition using Fourier-transform infrared spectroscopy (FTiR) (Nicolet iS5 FTIR Spectrometer, Thermo Scientific). Spectroscopic analysis was done using manual matching of peaks and functional groups as well as via automatic comparison with an FTiR database using the KnowItAll Informatics System (version 10.0.22000). The microspheres were then sonicated in an ethanol bath to remove, or at least reduce the amount of, any plastic additives from the manufacturing process (Zimmermann et al. 2020). The spectroscopic information can be found in Figure S2 of the Supplementary Materials. Previous studies have shown that the leaching of chemical additives and plasticizers can have just as much an effect as the MPs themselves (Zimmermann et al. 2020). Here, we decided to clean the MPs to assign any observed effects to the polymeric composition of the microspheres specifically.

Microplastic treatments and exposure

Microplastics were introduced to the system by exposing brine shrimp to varying concentrations of polyethylene microspheres. In this study, we exposed the shrimp (one day post-hatch) to either a low concentration (10 MPs mL^{-1}), high concentration (100 MPs mL^{-1}) or a control (0 MPs mL^{-1}) in a 2 L glass jar for 24 hours. Microplastic concentrations for each treatment were achieved by adding aliquots of a prepared slurry with a concentration of 1000 MPs mL^{-1} to a glass jar. The high, low and control treatment received 100 mL, 10 mL and 0 mL of the slurry, respectively. For the low and control treatments, we added 90 mL and 10 mL aliquots from a “control slurry” (dechlorinated water with 0 MPs mL^{-1}), respectively, to keep the

total volume equal across the treatments. Each exposure treatment was then filled to a total final volume of 1 L of saltwater (26 g NaCl L^{-1}). Following the 24-hour exposure, the jar containing the shrimp was poured onto a 150 µm net and rinsed carefully with dechlorinated water. This process washed away saltwater as well as any unconsumed microplastics remaining in the water. Shrimp were then transferred to a 1 L glass jar filled with 750 mL of dechlorinated tap water and were used to feed cichlids twice daily. The shrimp solution was kept in a 1 ºC refrigerator when not in use. Aliquots of each batch of shrimp solution was viewed under a dissecting microscope (Leica EZ4 Stereo Microscope 8x - 35x, Leica Microsystems, Wetzlar, Germany), we used the average of three aliquots to determine the approximate number of shrimp and microplastics in the solution. Each jar contained an average of ~85,648 (sd ± 37,874; n = 81) shrimp.

The concentrations used here were approximated to be environmentally relevant based on field surveys of aquatic systems worldwide and through the recommendation of Bucci et al.'s (2020) review. It is difficult to determine the accuracy of our approximation as most field surveys use mesh sizes >100 µm and therefore provide little information on the environmental concentrations of smaller microplastics (Covernton et al. 2019, Bucci et al. 2020). Covernton et al. (2019) estimates that concentrations from lower size fractions could be one to four orders of magnitude higher than what is reported by current manta trawl studies. Given that the highest concentration observed in nature was 1.77 MPs mL^{-1}, we believe that the concentrations we used for this study are an acceptable approximation to represent microplastic concentrations in mildly to highly polluted aquatic systems (Dubaish and Liebezeit 2013, Bucci et al. 2020).

Fish digestions and microplastic quantification

To extract microplastics from the cichlids, we first performed a chemical digestion of the preserved fish using a 10% (w/v) potassium hydroxide (KOH) solution. The digestion procedure

was conducted for 24 hours in a 60 ºC oven. The temperature used in this procedure follow the recommendations of Munno et al. (2017) to maintain the physical and chemical integrity of the microplastics. The solution containing the dissolved fish (and microplastics) was then ran through vacuum filtration and any residual debris were collected in a qualitative filter paper with an 8 µm pore size (Supertek Grade 2 filter paper). The filter papers were then analyzed under a dissecting microscope and UV fluorescence to quantify the number of fluorescent microspheres. This procedure was conducted on a 33% subset of the total fish used in the maze trials.

Experiment 1: Impacts of microplastics on behaviour, growth and cognitive ability

This experiment was aimed at quantifying the effects of microplastics on a model aquatic organism. Here, we look at identifying the consequences on juvenile growth, foraging and exploratory behaviour and cognitive ability.

Experimental Design

The experiment was composed of six replicate blocks, each one with 42 cichlids split evenly across three treatments (i.e., 14 fish each for control, high and low). The fish were randomly assigned to treatments using a 14-faced dice. Here, we conducted two distinct but interconnected behavioural trials: first, a 10-day foraging trial and second, a 2-day maze trial using the same fish (Figure 1). Physical measurements of the fish were collected at the beginning and end of the experiment to assess juvenile growth. Behavioural observations were done through blinded video observations.

Juvenile growth and condition

Prior to the behavioural trials, the cichlids were lightly sedated using a diluted solution of emulsified clove oil (~15 mg L^{-1}). Each fish was then measured wet weight using a laboratory balance. We followed the same procedure to measure the fish at the conclusion of the

experiment. However, instead of just being sedated, the fish were euthanized using a more concentrated clove oil solution (>200 mg L^{-1}, according to Concordia University Animal Research Ethics protocol #30000255 and outlined in Concordia University A.C.F Standard Operating Procedure #D-11.0). Euthanized cichlids were then preserved in a 95% ethanol solution and stored in a 1 ºC refrigerator. Although tracking individual growth would have been a more informative metric for growth, it was not possible in this experimental set-up; instead, we tracked growth as a mean of the tank. The mean weight for each treatment were 0.150 g (± 0.092), 0.152 g (± 0.092) and 0.155 g (± 0.096) for the control, low and high treatments respectively. We also measured the fish's standard length (i.e. length from tip of snout to point of caudal insertion) and body condition (K factor) which generated similar information as the weight data we reported here (Appendix B).

Behavioural assay 1: Foraging and aggressive behaviours

Cichlids were kept in groups of 14 in 22 L tanks filled with approximately 16 L of dechlorinated water. Tanks were kept at ~24 ºC with a 12:12 light:dark schedule and were equipped with a filter and an air stone. The fish were fed 60 mL of shrimp from the respective MP-exposure treatments twice daily for 10 days. Foraging observations were conducted between 9:00 – 10:00 AM of days 1, 6 and 10 of the trial (Figure 1). Each foraging trial consisted of six consecutive 2-minute feedings with 10 mL of shrimp (suspended in water) injected in the tank at the beginning of each interval. Feedings were videotaped using GoPros (Hero3+ and Hero8 at 1080P: 60 FPS) for later analysis. We collected a total of 54 videos, each corresponding to a feeding trial for a tank from a specific day (day 1, 6 or 10), treatment (control, low or high) and block (one of six replicates). For each video, we followed and observed the foraging rate of at least 6 individuals to get the mean foraging rate and coefficient of variance for the tank.

We quantified foraging behaviour by counting the number of foraging attempts of an individual fish during the last minute of each 2-minute feeding period. We defined a foraging attempt as an open-mouthed lunge followed by a full stop. On a separate analysis of the same videos, we also quantified the number of aggressive interactions (attacks or chases between two or more individuals) within the tank for the entire duration of the foraging trial.

Behavioural assay 2: Exploration and behavioural decision making

Following the conclusion of the foraging trials, fish were assigned haphazardly into four groups of three fish and transferred into aerated holding tanks. Each group within a treatment was then assigned a group number which was used as a random effect in our statistical analysis. We then placed the cichlids in one of six identical maze set-ups (Figure S3). Each maze was set-up with a gravel substrate and three sections separated by white corrugated plastic walls with a 4 cm gap to allow for passage. We placed an air stone at the end of the maze to allow for aeration and water flow and to carry the food scent throughout the tank. The fish were placed in the first section and enclosed in a removable transparent cylindrical chamber to acclimate for 5 minutes. At the end of the acclimation period, we injected 60 mL of food scent (dissolved flake food) beside the air stone and lifted the acclimation chamber to release the fish into the maze. All trials were videotaped for further analysis using an overhead camera set-up (GoPro Hero3+ and Hero8 at 1080P: 60 FPS). Each trial was capped at 15 minutes excluding the acclimation period, regardless of the fish's progress in the maze. The fish were then removed from the maze and placed back in their holding tanks. We repeated the maze trial the following day using the same protocol. This two-day experimental set-up allowed us to make inferences on how the fish reacted to a novel versus non-novel environment. Additionally, their performance would be indicative of their exploratory behaviour and provide information on the fish's cognitive ability

or, more specifically, their ability to learn the maze. This then allows us to determine what role, if any, plastic consumption has in changing their behaviours in such scenarios.

In each trial, we assessed the fish's latency to exploration (LTE), defined as the amount of time it took for fish to first cross the first barrier fully (i.e., entire body completely crossed). We also assessed their ability to complete the maze by measuring the amount of time it took to fully cross the second barrier. A value of 900 seconds was assigned to any fish that did not cross the barriers by the end of the trial. We also recorded the barrier-crossings as a binary measure, assigning a value of 1 for crossing and 0 if not. Additionally, we quantified the shoal size when a fish first crossed a barrier.

Statistical analysis

All statistical analyses were performed using the 'nlme', 'lme4', 'ggplot2' packages on RStudio (Version 2022.02.2 Build 485). We used $p = 0.05$ as the cut-off for significance in our analyses. To analyze differences in juvenile growth, we used general linear mixed models (GLMMs) with a Gaussian distribution to quantify differences in weight between treatments and across days. We assigned block as a random factor in our model to account for individuals belonging to the same tank and to avoid pseudo-replication. GLMMs used in our statistical analysis were tested for normality of residuals and homoscedasticity visually using Q-Q plots. Weight data were log-transformed to fit the assumptions of the model.

We analyzed the foraging rate of the tank using the mean foraging attempts of fish across all six 2-minute feeding periods and calculating the mean for the whole tank. We use a generalized linear mixed model (GzLMM) with a Poisson distribution with mean attempts as the response variable. We assigned treatment and feeding day as fixed effects and we used block as a random factor. Additionally, we looked at the trends across the six feeding periods to compare

the rates of foraging decline associated with satiation. We performed separate GLMMs with a Gaussian distribution for each of the three observation days (Days 1, 6 and 10) with attempts as a response variable. We assigned treatment and feeding period as fixed effects. Again, we used block as a random factor. To determine the effects of microplastics on competitive aggression within the shoal, we used a two-way ANOVA with total number of interactions as the response variable and treatment and day as explanatory variables. We then used a Tukey Test with a 95% confidence level for our post-hoc analysis.

We used mixed models to analyze differences in maze performance with treatment and day as fixed effects and group and block as random effects. We used a GzLMM with a Gamma distribution to analyze differences in the amount of time it takes for them to first cross a barrier. We reported this as latency to exploration (i.e. crossing the first barrier) and time to completion (i.e. crossing the second barrier). We also analyzed differences in willingness to explore and maze completion, reported as a binary measure of crossing the first and second barrier, using a GzLMM with a Binomial distribution. In addition, we performed individual GzLMMs for each treatment and each day as a post-hoc test to determine significant differences while still accounting for group and block as random factors. Last, we used a GzLMM with a Poisson distribution to analyze differences in shoal size when they first cross a barrier.

Experiment 2: Plastic retention in juvenile convict cichlids

This experiment was aimed at determining the degree as to which the cichlids retained plastics in their system. The treatments and tank set-up were similar to *Experiment 1*. We used a blocked experimental design consisting of 4 replicates. Each replicate consisted of a control (0 MP mL^{-1}), low (10 MP mL^{-1}) and high (100 MP mL^{-1}) treatment with 10 fish each. Juvenile cichlids were randomly assigned to tanks using dice. The mean standard lengths for the fish in

each treatment were 1.273 cm (± 0.216), 1.273 cm (± 0.203) and 1.283 cm (± 0.219) for the control, low and high treatments respectively. Fish were fed twice daily with a brine shrimp that were exposed to the respective treatments. The fish were exposed to their feeding regimes for 6 days, half of the fish were euthanized and preserved 24 hours after the last feeding and the rest at 48 hours after last feeding. Fish digestion and microplastics quantification was performed following the same procedures as described in *Experiment 1*.

RESULTS

Microplastic quantification

The results of the wet counts of aliquots of our feeding solution (i.e. brine shrimp in water) showed that we successfully transmitted the differences in microplastic concentrations across our treatments even after going through a trophic level (Figure 2). The low treatment had higher MP concentration than the control and the high treatment was an order of magnitude higher than the low treatment. Whilst we maintained the ratio of MP concentrations between treatments, the amount of microplastics that were fed to the juvenile cichlids were an order of magnitude less than what the brine shrimp were originally exposed to. The control feeding solution had an average microplastic concentration of 0.012 (± 0.064) MPs mL^{-1}, the low feeding solution had 1.3333 (± 1.1473) MPs mL^{-1}, and the high feeding solution had 17.0247 (± 9.8149) MPs mL^{-1}. Despite our best attempts to limit contamination, we attribute the non-zero microplastic concentration in the control treatment to this as there are no other immediately obvious sources for these particles. Nevertheless, we believe that the impacts of a few stray particles would have been negligible.

After processing 24 fish per treatment, we were only able to retrieve a total of two microplastic spheres with one belonging to the low treatment and one from the high treatment. While our pilot studies confirmed that juvenile cichlids consume these plastic spheres, our experimental design limited us from specifically confirming the presence of microplastics in the cichlids. Additionally, this showed that the accumulation of plastic spheres in the bodies of the juveniles at this timescale is minimal and most are released at least within 48 hours.

Impacts on juvenile growth and condition

We performed initial measurements of weight on 252 fish split evenly across three treatments and six blocks. Of these fish, 247 survived until the end of the experiment and were the subjects of the final measurements. Fish survival was high and similar across treatments with two deaths from the low and control treatments and one death from the high treatment.

Juveniles grew over the experimental period. Final weight measurements were significantly different from initial measurements ($p < 0.0001$, $F = 43.53$, $df = 1, 488$) (Figure 3). However, we found no significant main effect of treatment ($p = 0.84$, $F = 0.17$, $df = 2, 488$) or interaction effect between day and treatment ($p = 0.98$, $F = 0.015$, $df = 2, 488$). Our results show that exposure to virgin polyethylene microspheres did not result in different average weight and did not impact juvenile growth rate during the 12-day duration (10-day exposure, 2-day maze) of our experiment (Figure 3).

Effects on foraging rate and competitive interactions

Microplastics had no effect on the foraging rate of juvenile cichlids (Figure 4). We had foraging information for a total of 345 individuals. In comparing baseline (day 1) versus final (day 10) foraging rates, we found no significant treatment x day interaction effect ($p = 0.97$, $\chi^2 = 0.061$, $df = 2, 217$) and we saw no significant change across days ($p = 0.71$, $\chi^2 = 0.14$, $df = 1, 217$). There was a significant effect of treatment ($p = 0.0007$, $\chi^2 = 14.42$, $df = 2, 217$) though mostly driven by the difference of control vs. high ($p = 0.00069$, $z = 3.39$, $df = 1, 334$); control vs. low was non-significant ($p = 0.65$, $z = 0.46$, $df = 1, 334$). It is however important to reiterate that fish in the high treatment already had an average foraging rate that was higher on day 1. Therefore, the significant effect of treatment between control and high is most likely a result of

the high treatment's higher baseline foraging rate rather than an impact of microplastic consumption.

Interestingly, when we included day 6 in our analysis, we found a significant difference in the tanks' average foraging attempts across treatments ($p = 0.0004$, $\chi^2 = 15.52$, $df = 2, 334$), across feeding days ($p = 0.01$, $\chi^2 = 9.14$, $df = 2, 334$) as well as a treatment x day interaction ($p = 0.001$, $\chi^2 = 18.33$, $df = 4, 334$). While the low and control treatments exhibited similar foraging rates throughout the experiment, the high treatment had a higher initial (day 1) foraging rate, followed by a large decrease in day 6 and a final (day 10) foraging rate similar to their initial (Figure 4).

We found no significant treatment x day interaction effect in the number of competitive interactions ($p = 0.92$, $F = 0.24$, $df = 4, 45$). These results suggest that microplastic consumption had no impact on competitive interactions within the tank. Additionally, we observed a 2- to 4-fold increase in aggressive behaviour from day 1 to days 6 and 10 across all three treatments (Figure 5). The drastic difference in competitive interactions from day 1 to day 6 combined with the similarity between days 6 and 10 shows the presence of an initial latency before competitive dynamics were established. Once established, the competitive dynamics remained unchanged throughout the experiment regardless of microplastic exposure. The ANOVA confirmed the results observed from Figure 5 with a significant main effect of feeding day ($p = 0.0096$, $F = 5.16$, $df = 2, 45$).

We observed a downward trend in foraging rate from the first to the last feeding which was similar across treatments (Figure 6). Whilst feeding period was significant across all three feeding days, treatment had no significant effect on these trends (Table 1). This suggests that microplastic exposure had no effect on the cichlids' rate of satiation.

Effects on exploration and behavioural decision making

We found a significant interaction between day and treatment in the cichlids' LTE ($p = 0.038$, Table 2). Microplastic-exposed fish had a larger change in their LTE between day 1 and day 2 of the maze trial compared to the control (Figure 7). Post-hoc comparisons show a significant difference between high and control treatments, ($p = 0.015$, $t = 2.43$, $df = 1, 423$), but not low and control treatments ($p = 0.08$, $t = 1.73$, $df = 1, 423$). Both high and low treatment had a significant decrease in LTE from day 1 to day 2 (high: $p = 0.0002$, low: $p = 0.003$) while control were similar between day 1 and day 2 ($p = 0.62$) (Table 3). In contrast, across all four metrics analyzed in the maze trials, the overall main effect of treatment was not significant (Table 2).

Quantifying the crossing of the first barrier as a binary measure provided additional information on the fish's willingness to explore. This analysis provided similar results to that of their LTE with a significant treatment x day interaction effect for the high treatment compared to the control ($p = 0.02$, $z = 2.25$, $df = 1, 424$) and a non-significant treatment x day interaction for the low treatment compared to the control ($p = 0.15$, $z = 1.4$, $df = 1, 424$) (Figure 8A). The overall treatment x day interaction was non-significant ($p = 0.07$, Table 2). Only the high treatment had a significant difference from day 1 to day 2 (high: $p = 0.037$, $z = 2.08$, $df = 1, 140$; low: $p = 0.37$, $z = 0.9$, $df = 1, 140$; control: $p = 0.18$, $z = -1.3$, $df = 1, 140$).

Our results also suggest that exposure to microplastics had an effect on the fish's completion of the maze (Figure 8B). The effect of microplastics on maze completion across the trial days (treatment x day interaction effect) was non-significant ($p = 0.08$, Table 2). Despite this weak interaction term, we found that the high vs. control had a significant treatment x day interaction effect ($p = 0.02$, $z = 2.26$, $df = 1, 424$) while the difference between low and control

was non-significant ($p = 0.21$, $z = 1.26$, $df = 1, 424$). Plastic-exposed fish show an initial deficit in their maze performance with a lower completion ratio on day 1 of the trial (Figure 8B). On day 1, only the difference between the control and the high was significant (control vs. high: $p = 0.02$, $z = -2.34$, $df = 1, 211$; control vs. low: $p= 0.14$, $z = -1.46$, $df = 1, 211$). Both the high and low microplastic treatments had an increase in completion ratio across days with their day 2 performance matching that of the control. The ratio of control fish that completed the maze did not change from day 1 to day 2 ($p = 1.0$, $z = 0$, $df = 1,140$). Of the microplastic treatments, only the high treatment had a significant difference across days ($p = 0.0009$, $z = 3.33$, $df = 1, 140$). The low treatment shared a similar trend to the high treatment and was marginally significant ($p = 0.058$, $z = 1.89$, $df = 1, 140$).

Surprisingly, these results on maze performance were not consistent when looking at total elapsed time to reach the end of the maze (Figure 7). Similar to their LTE, both high and low treatment had a significant decrease in time to maze completion from day 1 to day 2 (high: $p = 0.01$, $t =2.50$, $df = 1, 423$; low: $p = 0.007$, $t = 2.72$, $df = 1, 423$) while fish under the control treatment remained similar across both days ($p = 0.46$, $t = 0.75$, $df = 1, 423$). However, results of the overall analysis yielded no significant treatment x day interaction effects ($p = 0.31$, Table 2). Within-day comparisons also showed no significant differences in time to complete the maze between treatments on either day (Table 3).

We also found that microplastic exposure had no impacts on shoaling behaviour during the maze trials (Figure 9). We found an increase in shoaling from day 1 to day 2 in the initial crossing of the first barrier; the difference was not significant ($p = 0.068$, $\chi^2 = 3.32$, $df = 1, 426$). Additionally, we found a significant increase in shoaling between days in the initial crossing of the second barrier ($p = 0.038$, $\chi^2 = 4.32$, $df = 1, 426$). However, the change was not significantly

different across treatments for either barrier (Barrier 1 treatment x day effect: $p = 0.81$, $\chi^2 = 0.43$, df = 2, 424; Barrier 2 treatment x day effect: $p = 0.70$, $\chi^2 = 0.71$, df = 2, 424).

Experiment 2: Microplastic retention

Out of the 120 fish (SL = 1.41 cm ± 0.25) that we started with, we processed and analyzed 117 fish. All three missing fish belonged to the 48h group. One fish was missing per treatment. We found no microplastic spheres in the control treatments. Additionally, and as expected for both low and high treatments, we found more individuals with microplastics in their bodies in the 24h group compared to the 48h group (Table 4). We found that 6 out of 20 fish had plastic spheres in their bodies in the low:24h group compared to 2 out of 19 in the low:48h group. Similarly, 6 out of 20 fish from the high:24h group had plastic spheres in their bodies compared to 4 out of 19 in the high:48h group. Among those that had retained plastics, there was a higher number of plastics found in individuals from the 24h group especially in the high treatment. The highest number of plastic spheres found in a single fish was 46.

DISCUSSION

The results of our study show that the consumption of virgin polyethylene microplastics spheres had no effects on the growth, foraging rates and competitive interactions of juvenile convict cichlids after 10 days of exposure. However, we found strong evidence that microplastics consumption did impact exploration and behavioural decision making. We found that microplastic-exposed fish exhibited more risk-averse behavior on the first day by having longer latency to exploration and lower maze completion ratio. We also found that microplastic-exposed fish exhibited a larger change in exploration from day 1 to day 2. Additionally, we found that these effects were more pronounced in the high microplastic concentration.

The absence of effects on juvenile growth was contrary to our initial predictions but was not entirely surprising. Similar studies have reported that polyethylene microbeads, of different size classes, alone do not have an impact on juvenile growth and body condition (Batel et al. 2016, Ferreira et al. 2016, Critchell and Hoogenboom 2018, Jakubowska et al. 2020). The satiation hypothesis was based largely on the premise of physical blockage and intestinal damage. However, the results of our microplastic quantification from *Experiment 1* and our plastic retention experiment (*Experiment 2*) show that the majority of the plastics were egested by the fish. The small number of plastics that were retained by the fish seem to not have enough of an obstructive effect to have observable consequences on juvenile growth and body condition; at least not within this timescale. While it has been previously shown that microplastics can have acute impacts (7-day exposure) on the growth of planktivorous fish (*Acanthochromis polyacanthus*) (Critchell and Hoogenboom 2018), the accumulation of plastics in the gut might become more of an issue for prolonged chronic exposures, in the order of weeks and months instead of days. Long-term exposure (12-weeks) of Japanese medaka (*Oryzias latipes*) to

polyethylene microbeads showed a significant reduction in growth rate in plastic-exposed fish (Chisada et al. 2019). There would therefore be merit in identifying the long-term accumulation and retention of microbeads in juvenile convict cichlids to quantify the effects of chronic exposure on juvenile growth and body condition.

Similarly, the apparent lack of plastic retention and gut obstruction might explain why there was no effect of microplastic exposure on foraging or satiation rate. We failed to support our initial prediction that overall foraging rates would decrease due to a satiation effect caused by the impaction of the digestive tract. If microplastics did cause an obstruction, we should have observed a steeper decrease in foraging attempts throughout the feeding periods for the plastic-exposed fish. This was, however, not the case here. Alternatively, we posited that an increase in foraging rate and a slower satiation rate (shallower slope) was also possible in response to perceived lower food quality. This compensatory response would offset any nutritional/food deficits that microplastic consumption might be causing and thus not lead to a decrease in growth. Given our negative results on fish growth and body condition, this scenario seemed more likely. While we do see that the high microplastic treatment had an overall higher average foraging rate on day 10, the high treatment started with a higher baseline (day 1) average foraging rate (Figure 4). We therefore cannot associate the higher day 10 foraging rate of the high treatment as an effect of microplastic consumption alone. This highlights the importance of establishing baseline measurements before generating conclusions on foraging rates.

It remains unclear why we observed a large decline in foraging rate for the high microplastic treatment on day 6. It is possible that high levels of microplastic consumption can lead to a more variable foraging strategy with large peaks and dips in foraging attempts. It could be that the lower foraging rate observed during the morning feeding on day 6 was accompanied

by a much higher foraging rate in the afternoon. Unfortunately, we only have observations for the morning feedings. To our knowledge, there are no studies that have looked at the within-day temporal variability of foraging rates as a consequence of microplastic exposure. This therefore remains as a potential avenue for future research. Regardless, after accounting for baseline foraging rates, foraging attempts did not differ among the three treatment groups on day 10. Future experiments should examine multiple observation times within the day to test for possible effects on daily activity budgets.

Overall, we failed to support neither the satiation nor the compensation hypothesis as the exposure to polyethylene microplastics did not show a clear positive or negative impact on overall foraging and satiation rates after 10 days. Similar results have been found with the exposure of post-larvae (i.e. larvae under metamorphosis) convict surgeonfish (*Acanthurus triostegus*) to polystyrene microspheres for up to 8 days (Jacob et al. 2019) and the exposure of gilt-head seabream to low density polyethylene (LDPE) particles for 21 days (Rios-Fuster et al. 2021). Both studies found no effect of microplastics on foraging rates. Additionally, previous studies have shown that the effects of polyethylene spheres on predatory performance could be mediated by other environmental factors such as temperature. Fonte et al.'s (2016) study on juvenile common goby showed a non-significant reduction in predatory performance (quantified as total prey eaten versus total prey provided) as a result of microplastic exposure in their low temperature (20 ºC) treatment. In contrast, the reduction in predatory performance was more intense and was statistically significant under a higher temperature (25 ºC) treatment.

We also found no significant effects of microplastic exposure on competitive interactions within a group when comparing total instances of competitive aggression during foraging periods. This result is consistent with behavioural observations on LDPE-exposed gilt-head

seabream showing no effects of microplastic exposure on the frequency of competitive interactions (i.e. bites and chases) within a group (Rios-Fuster et al. 2021). In both the satiation and compensation hypothesis, we predicted that microplastic consumption would impose a diet-induced nutritional stressor that would elicit a change in competitive dynamics. The apparent absence of this stressor, as seen in the growth and foraging behaviour results from this study, is a likely explanation for the absence of a significant effect (in either direction) of microplastic exposure on competitive interactions.

Despite finding negative results in our proposed mechanism for the drivers of change in exploration, we did find significant results in our maze experiments. While we found significant results in our maze trials, our results failed to support either the satiation or the compensation hypothesis. For both hypotheses, we predicted an overall effect of treatment; instead, we found a significant treatment x day interaction effect and a consequent reversal of the direction of the difference between the control and the microplastic treatments (Figure 7 and 8). This suggests that the effects of microplastic consumption on exploration and behavioural decision making might be more complex than we had predicted.

During day 1 of our maze trials, we found that microplastic-exposed fish seemed to express a more risk-averse phenotype in their exploratory behaviour. Fish from the high microplastic treatment took longer before starting to explore and fewer completed the maze compared to the control. This could indicate a shift in the fish's perceived costs and benefits of exploration. The decrease in exploratory behaviour might suggest that microplastic consumption has increased the cost or decreased the benefit of exploring a novel space. The resulting risk-averse phenotype is contrary to the findings of McCormick et al. (2020) where Ambon damselfish exposed to polystyrene microbeads were found to exhibit more risk-prone

behaviours; they were more active and travelled further away from shelter. While the results of our study seem to contradict their results, it is important to highlight that McCormick et al. (2020) used microplastics that were different in size and polymeric composition. They used polystyrene microbeads which were more than an order of magnitude larger than the ones used in this study (200-300 µm vs. 10-20 µm). Given the established dependence of the effects of microplastics on polymer type and particle size, this may be the main driver of the opposing results (Covernton et al. 2019, Zimmermann et al. 2020). Further, polystyrene plastics are generally assumed to be more hazardous compared to polyethylene strictly based on their chemical properties and have been empirically shown to cause more genotoxicity in larval sea trout (*Salmo trutta*) (Jakubowska et al. 2020). Lithner et al.'s (2011) study ranks polystyrene and its associated monomer, styrene, higher in the hazard rankings than polyethylene and its monomer, ethylene. In contrast, a study more comparable to ours in terms of polymer type and particle size, looked at the effects of trophically-transferred polyethylene beads (with a slightly higher size range 38-45 µm) and found no effects on the boldness and exploration of Krefft's frillgobies (*Platorchestia smith*) (Tosetto et al. 2017). This discrepancy highlights the species-specificity of the impacts of microplastics and the overall difficulty of generalizing the results of such studies.

The results of our maze trials are consistent with increased spatial neophobia as a result of microplastic exposure. Exploring a novel environment poses potential risk given the absence of complete information on local conditions (Dall et al. 2005, McNamara and Dall 2010). The 'dangerous niche' hypothesis posits that, to manage this risk, individuals may express behaviours linked to spatial neophobia, defined as the hesitancy or fear to enter a new environment (Greenberg and Mettke-Hofmann 2001, Greenberg 2003). The Error Management Theory adds

that when faced with a decision in a novel environment with unknown outcomes, individuals should lean towards the choice with the least costly outcome (Johnson et al. 2013). Neophobic behaviours, such as reduced activity, reduced exploration and increased shoaling, are costly in terms of reducing their chances to forage, secure territory and/or finding a mate. However, the choice to explore (i.e. to be neophilic) comes with the cost of energetic resources and the risk of death from the abiotic and biotic dangers of the new environment. Individuals must balance the trade-off between caution and missing fitness-increasing opportunities. Microplastics in the environment could lead to a shift in food quality and/or the fish's ability to process it. This could lead to a shift in their cost-benefit analysis and therefore be the cause of the shift in exploratory behaviour that we observed here.

In the absence of support for our prediction that microplastic consumption will lead to a food-related stressor and therefore alter the fish's cost-benefit analysis, we are unable to make a direct determination of the mechanism(s) behind the results of the maze trials. However, it remains that we saw a decrease in exploratory behaviour as a consequence of microplastics. It is possible that microplastic exposure leads to a deficiency in nutrients that may not be reflected in their growth but enough to change their cost-benefit analysis and therefore their decision making. It is also possible that the exposure to, and consumption of, polyethylene beads caused a physiological or hormonal change in the juveniles which could have led to the behavioural effects seen here. For example, plasmic hormone levels are closely linked with animal behaviours (Baker et al. 1999). In the context of neophobic behaviour, higher cortisol levels have been associated with neophobia including reduced activity and feeding in a novel environment. A few studies have looked at the relationship between microplastic consumption and plasma cortisol levels with varied results (Jakubowska et al. 2020, Shi et al. 2020). It could be that the

stress of microplastic consumption could trigger behaviours linked to spatial neophobia even in the absence of background risk. This could have larger implications since it is well-established that spatial neophobia is heavily influenced by background risk with higher background predation risk increasing potential cost to exploring novel spaces (Elvidge et al. 2016, Crane et al. 2020, 2022). Providing empirical evidence for this mechanism would be a useful addition to the study of this well-documented phenomena in the context of an emerging pollutant.

In our analysis of the effects of microplastics on the cognitive ability of juvenile cichlids, we found a larger improvement between the two days in the plastic-exposed fish. Both high and low microplastic treatments had a significant decrease in their LTE, a significant decrease in their time to completion and a significant increase in the number of fish that finished the maze. In comparison, the control fish had no significant changes in those metrics. While the plastic-exposed fish seemed to be more risk-averse on day 1, our results show that microplastics leads to increased risk-prone tactics (i.e. faster exploration on day 2 vs. day 1). This increase in exploration on day 2 could be a compensatory response for not getting the food reward at the end of the maze on the day 1. Since fewer of the plastic-exposed fish finished on day 1, more of them would have been hungrier on day 2 and thus more willing to take risks, explore and find food.

Previous studies have shown that microplastic exposure can lead to reduced or impaired learning in mice and honeybees (*A. mellifera*) (Wang et al. 2022, Lee et al. 2022, Balzani et al. 2022). For example, Lee et al. (2022) found that exposure to polystyrene microplastics resulted in a decrease in the learned fear responses of mice. In contrast, we do not see the same decrease in learning ability in our study. Instead, our results show that while cichlids exposed to plastics can learn, the pattern of spatial learning differs. While the control group exhibited consistent patterns on test days 1 and 2 for maze performance metrics, microplastic-exposed cichlids

appeared to shift from a risk-averse to risk-prone response patterns. This could indicate a learned response or it could indicate a shift in perception of risk and resulting behavioural trade-offs. While further experiments are needed to tease out the mechanisms involved, functionally our results are consistent with cognitive differences. To our knowledge, these results are the first to report on the effects of microplastics on the cognitive ability of fish.

In the results of the maze trials, the high and low microplastic treatments shared similar trends across all the metrics. For both slopes and mean values on each day, the difference between control and high are always larger compared to control vs. low. This was consistent with previous studies that associate larger effect sizes, or sometimes simply the presence of one, with higher microplastic doses (Bucci et al. 2020). In this study we were able to show that the effects of microplastics on exploration and learning can be observed at both environmentally relevant concentrations as well as an elevated concentration.

It is important to highlight that by using virgin polyethylene microspheres which have undergone a cleaning process under sonication, we negated the potential effects of plastic additives and adsorbed contaminants. Exposure to those chemicals through microplastic consumption is one of the major pathways for microplastics to cause ecotoxicological effects (Anbumani and Kakkar 2018). In some cases, the effects of these chemicals can be larger than the effects of the plastic particles themselves (de Ruijter et al. 2020). Additionally, the uniformity in shape, size, color and polymer type of the particles used here is not representative of the suite of microplastic pollution in natural environments (Rochman 2018, Rochman et al. 2019, Felismino et al. 2021). By using pristine/virgin plastic spheres, we also fail to represent the true nature of microplastics in the environment as environmental microplastics are subjected to various degradation processes including photo-, thermal-, and bio- degradation (Gewert et al.

2015). These physical and chemical characteristics impact the rate of consumption of plastics, their ability to sorb chemicals and, ultimately, how they impact organisms (Lee et al. 2014, Rochman et al. 2019, Liu et al. 2020, Zimmermann et al. 2020). It is likely that the results of this study would have been different if non-virgin, environmental, or weathered plastics were used instead of, or in addition to, our particles (Liu et al. 2020). While it is important to isolate and determine the impacts of certain shape and polymer type combinations, future work on the topic should aim to incorporate a more diverse contaminant suite to improve environmental relevance.

REFERENCES

Adams, D. 1985. So long, and thanks for all the fish. London, Pan Books.

Anbumani, S., and P. Kakkar. 2018. Ecotoxicological effects of microplastics on biota: a review. Environmental Science and Pollution Research 25:14373–14396.

Athey, S. N., S. D. Albotra, C. A. Gordon, B. Monteleone, P. Seaton, A. L. Andrady, A. R. Taylor, and S. M. Brander. 2020. Trophic transfer of microplastics in an estuarine food chain and the effects of a sorbed legacy pollutant. Limnology and Oceanography Letters 5:154–162.

Baker, J. D., S. L. McNabb, and J. W. Truman. 1999. The hormonal coordination of behavior and physiology at adult ecdysis in *Drosophila melanogaster*. Journal of Experimental Biology 202:3037–3048.

Balzani, P., G. Galeotti, S. Scheggi, A. Masoni, G. Santini, and D. Baracchi. 2022. Acute and chronic ingestion of polyethylene (PE) microplastics has mild effects on honey bee health and cognition. Environmental Pollution 305:119318.

Batel, A., F. Linti, M. Scherer, L. Erdinger, and T. Braunbeck. 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. Environmental Toxicology and Chemistry 35:1656–1666.

Beri, S., B. W. Patton, and V. A. Braithwaite. 2014. How ecology shapes prey fish cognition. Behavioural Processes 109:190–194.

Boggs, C. L. 1992. Resource allocation: exploring connections between foraging and life history. Functional Ecology 6:508–518.

Borrelle, S. B., J. Ringma, K. L. Law, C. C. Monnahan, L. Lebreton, A. McGivern, E. Murphy, J. Jambeck, G. H. Leonard, M. A. Hilleary, M. Eriksen, H. P. Possingham, H. De Frond, L. R. Gerber, B. Polidoro, A. Tahir, M. Bernard, N. Mallos, M. Barnes, and C. M. Rochman. 2020. Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. Science 369:1515–1518.

Brown, G. E., M. C. O. Ferrari, and D. P. Chivers. 2011. Learning about danger: chemical alarm cues and threat-sensitive assessment of predation risk by fishes. Pages 59–80 Fish Cognition and Behavior. John Wiley & Sons, Ltd.

Bucci, K., M. Tulio, and C. M. Rochman. 2020. What is known and unknown about the effects of plastic pollution: a meta-analysis and systematic review. Ecological Applications 30:1–16.

Caraco, T. 1980. On foraging time allocation in a stochastic environment. Ecology 61:119–128.

Careau, V., and T. Garland. 2012. Performance, personality, and energetics: correlation, causation, and mechanism. Physiological and Biochemical Zoology 85:543–571.

Cedervall, T., L. A. Hansson, M. Lard, B. Frohm, and S. Linse. 2012. Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. PLoS ONE 7:1–6.

Chisada, S., M. Yoshida, and K. Karita. 2019. Ingestion of polyethylene microbeads affects the growth and reproduction of medaka, *Oryzias latipes*. Environmental Pollution 254:113094.

Covernton, G. A., C. M. Pearce, H. J. Gurney-Smith, S. G. Chastain, P. S. Ross, J. F. Dower, and S. E. Dudas. 2019. Size and shape matter: a preliminary analysis of microplastic sampling technique in seawater studies with implications for ecological risk assessment. Science of The Total Environment 667:124–132.

Crane, A. L., G. E. Brown, D. P. Chivers, and M. C. O. Ferrari. 2020. An ecological framework of neophobia: from cells to organisms to populations. Biological Reviews 95:218–231.

Crane, A. L., E. E. Demers, L. E. A. Feyten, I. W. Ramnarine, and G. E. Brown. 2022. Exploratory decisions of Trinidadian guppies when uncertain about predation risk. Animal Cognition 25:581–587.

Critchell, K., and M. O. Hoogenboom. 2018. Effects of microplastic exposure on the body condition and behaviour of planktivorous reef fish (*Acanthochromis polyacanthus*). PLoS ONE 13:1–19.

Da Costa, J. P., A. R. Nunes, P. S. M. Santos, A. V. Girão, A. C. Duarte, and T. Rocha-Santos. 2018. Degradation of polyethylene microplastics in seawater: insights into the environmental degradation of polymers. Journal of Environmental Science and Health, Part A 53:866–875.

Dall, S. R. X., L.-A. Giraldeau, O. Olsson, J. M. McNamara, and D. W. Stephens. 2005. Information and its use by animals in evolutionary ecology. Trends in Ecology & Evolution 20:187–193.

Dunbrack, R. L., L. Clarke, and C. Bassler. 1996. Population level differences in aggressiveness and their relationship to food density in a stream salmonid (*Salvelinus fontinalis*). Journal of Fish Biology 48:615–622.

Eerkes-Medrano, D., R. C. Thompson, and D. C. Aldridge. 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Research 75:63–82.

Eltemsah, Y. S., and T. Bøhn. 2019. Acute and chronic effects of polystyrene microplastics on juvenile and adult *Daphnia magna*. Environmental Pollution 254:112919–112919.

Elvidge, C. K., P. J. C. Chuard, and G. E. Brown. 2016. Local predation risk shapes spatial and foraging neophobia patterns in Trinidadian guppies. Current Zoology 62:457–462.

Fatehi Dubaish and Gerd Liebezeit. 2013. Suspended microplastics and black carbon particles in the Jade System, Southern North Sea. Water, air, and soil pollution 224:1352–1352.

Felismino, M. E. L., P. A. Helm, and C. M. Rochman. 2021. Microplastic and other anthropogenic microparticles in water and sediments of Lake Simcoe. Journal of Great Lakes Research 47:180–189.

Ferreira, P., E. Fonte, M. E. Soares, F. Carvalho, and L. Guilhermino. 2016. Effects of multi-stressors on juveniles of the marine fish *Pomatoschistus microps*: gold nanoparticles, microplastics and temperature. Aquatic Toxicology 170:89–103.

Fonte, E., P. Ferreira, and L. Guilhermino. 2016. Temperature rise and microplastics interact with the toxicity of the antibiotic cefalexin to juveniles of the common goby (*Pomatoschistus microps*): post-exposure predatory behaviour, acetylcholinesterase activity and lipid peroxidation. Aquatic Toxicology 180:173–185.

Gewert, B., M. M. Plassmann, and M. MacLeod. 2015. Pathways for degradation of plastic polymers floating in the marine environment. Environmental Science: Processes & Impacts 17:1513–1521.

Greenberg, R. 2003. The role of neophobia and neophilia in the development of innovative behaviour of birds. S. M. Reader and K. N. Laland, editors. Animal Innovation. Oxford University Press.

Greenberg, R., and C. Mettke-hofmann. 2001. Ecological aspects of neophobia and neophilia in birds. Pages 119–178 *in* V. Nolan and C. F. Thompson, editors. Current Ornithology. Springer US, Boston, MA.

Healy, S. D., and T. A. Hurly. 2004. Spatial learning and memory in birds. Brain, Behavior and Evolution 63:211–20.

Jacob, H., A. Gilson, C. Lanctôt, M. Besson, M. Metian, and D. Lecchini. 2019. No effect of polystyrene microplastics on foraging activity and survival in a post-larvae coral-reef fish, *Acanthurus triostegus*. Bulletin of Environmental Contamination and Toxicology 102:457–461.

Jakubowska, M., M. Białowąs, M. Stankevičiūtė, A. Chomiczewska, J. Pažusienė, K. Jonko-Sobuś, A. Hallmann, and B. Urban-Malinga. 2020. Effects of chronic exposure to microplastics of different polymer types on early life stages of sea trout *Salmo trutta*. Science of the Total Environment 740:1–11.

Johnson, D. D. P., D. T. Blumstein, J. H. Fowler, and M. G. Haselton. 2013. The evolution of error: error management, cognitive constraints, and adaptive decision-making biases. Trends in Ecology & Evolution 28:474–481.

Jovanović, B. 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integrated Environmental Assessment and Management 13:510–515.

Jovanović, B., K. Gökdağ, O. Güven, Y. Emre, E. M. Whitley, and A. E. Kideys. 2018. Virgin microplastics are not causing imminent harm to fish after dietary exposure. Marine Pollution Bulletin 130:123–131.

Lee, C.-W., L.-F. Hsu, I.-L. Wu, Y.-L. Wang, W.-C. Chen, Y.-J. Liu, L.-T. Yang, C.-L. Tan, Y.-H. Luo, C.-C. Wang, H.-W. Chiu, T. C.-K. Yang, Y.-Y. Lin, H.-A. Chang, Y.-C. Chiang, C.-H. Chen, M.-H. Lee, K.-T. Peng, and C. C.-Y. Huang. 2022. Exposure to polystyrene

microplastics impairs hippocampus-dependent learning and memory in mice. Journal of Hazardous Materials 430:128431.

Lee, H., W. J. Shim, and J.-H. Kwon. 2014. Sorption capacity of plastic debris for hydrophobic organic chemicals. Science of The Total Environment 470–471:1545–1552.

Lithner, D., A. Larsson, and G. Dave. 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Science of the Total Environment 409:3309–3324.

Liu, P., X. Zhan, X. Wu, J. Li, H. Wang, and S. Gao. 2020. Effect of weathering on environmental behavior of microplastics: properties, sorption and potential risks. Chemosphere 242:125193.

Lucon-Xiccato, T., and A. Bisazza. 2017. Complex maze learning by fish. Animal Behaviour 125:69–75.

Mattsson, K., M. T. Ekvall, L. A. Hansson, S. Linse, A. Malmendal, and T. Cedervall. 2015. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Environmental Science and Technology 49:553–561.

Mattsson, K., E. V. Johnson, A. Malmendal, S. Linse, L. A. Hansson, and T. Cedervall. 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Scientific Reports 7:1–7.

McCormick, M. I., D. P. Chivers, M. C. O. Ferrari, M. I. Blandford, G. B. Nanninga, C. Richardson, E. P. Fakan, G. Vamvounis, A. M. Gulizia, and B. J. M. Allan. 2020. Microplastic exposure interacts with habitat degradation to affect behaviour and survival of juvenile fish in the field: effects of plastic and coral degradation. Proceedings of the Royal Society B: Biological Sciences 287.

McNamara, J. M., and S. R. X. Dall. 2010. Information is a fitness enhancing resource. Oikos 119:231–236.

Miller, M. E., M. Hamann, and F. J. Kroon. 2020. Bioaccumulation and biomagnification of microplastics in marine organisms: a review and meta-analysis of current data. PLOS ONE 15:e0240792.

Noble, D. W. A., P. Carazo, and M. J. Whiting. 2012. Learning outdoors: male lizards show flexible spatial learning under semi-natural conditions. Biology Letters 8:946–948.

Nunn, A. D., L. H. Tewson, and I. G. Cowx. 2012. The foraging ecology of larval and juvenile fishes. Reviews in Fish Biology and Fisheries 22:377–408.

Ogonowski, M., Z. Gerdes, and E. Gorokhova. 2018. What we know and what we think we know about microplastic effects – A critical perspective. Current Opinion in Environmental Science and Health 1:41–46.

Oliveira, M., A. Ribeiro, K. Hylland, and L. Guilhermino. 2013. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecological Indicators 34:641–647.

Peña-Villalobos, I., I. Casanova-Maldonado, P. Lois, V. Palma, and P. Sabat. 2020. Costs of exploratory behavior: the energy trade-off hypothesis and the allocation model tested under caloric restriction. Scientific Reports 10:4156.

Rios-Fuster, B., P. Arechavala-Lopez, K. García-Marcos, C. Alomar, M. Compa, E. Álvarez, M. M. Julià, A. Solomando Martí, A. Sureda, and S. Deudero. 2021. Experimental evidence of physiological and behavioral effects of microplastic ingestion in *Sparus aurata*. Aquatic Toxicology 231:105737.

Rochman, C. M. 2018. Microplastics research—from sink to source. Science 360:28–29.

Rochman, C. M., C. Brookson, J. Bikker, N. Djuric, A. Earn, K. Bucci, S. Athey, A. Huntington, H. McIlwraith, K. Munno, H. De Frond, A. Kolomijeca, L. Erdle, J. Grbic, M. Bayoumi, S. B. Borrelle, T. Wu, S. Santoro, L. M. Werbowski, X. Zhu, R. K. Giles, B. M. Hamilton, C. Thaysen, A. Kaura, N. Klasios, L. Ead, J. Kim, C. Sherlock, A. Ho, and C. Hung. 2019. Rethinking microplastics as a diverse contaminant suite. Environmental Toxicology and Chemistry 38:703–711.

Rosati, A. G., K. Rodriguez, and B. Hare. 2014. The ecology of spatial memory in four lemur species. Animal Cognition 17:947–961.

de Ruijter, V. N., P. E. Redondo-Hasselerharm, T. Gouin, and A. A. Koelmans. 2020. Quality criteria for microplastic effect studies in the context of risk assessment: a critical review. Environmental Science & Technology 54:11692–11705.

Shi, W., Y. Han, S. Sun, Y. Tang, W. Zhou, X. Du, and G. Liu. 2020. Immunotoxicities of microplastics and sertraline, alone and in combination, to a bivalve species: size-dependent interaction and potential toxication mechanism. Journal of Hazardous Materials 396:122603.

Stephens, D. W. 1981. The logic of risk-sensitive foraging preferences. Animal Behaviour 29:628–629.

Stienbarger, C. D., J. Joseph, S. N. Athey, B. Monteleone, A. L. Andrady, W. O. Watanabe, P. Seaton, A. R. Taylor, and S. M. Brander. 2021. Direct ingestion, trophic transfer, and physiological effects of microplastics in the early life stages of *Centropristis striata*, a commercially and recreationally valuable fishery species. Environmental Pollution 285:117653–117653.

Tosetto, L., J. E. Williamson, and C. Brown. 2017. Trophic transfer of microplastics does not affect fish personality. Animal Behaviour 123:159–167.

Wang, S., Q. Han, Z. Wei, Y. Wang, J. Xie, and M. Chen. 2022. Polystyrene microplastics affect learning and memory in mice by inducing oxidative stress and decreasing the level of acetylcholine. Food and Chemical Toxicology 162:112904.

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

Zimmermann, L., S. Göttlich, J. Oehlmann, M. Wagner, and C. Völker. 2020. What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*. Environmental Pollution 267:115392.

FIGURES AND TABLES

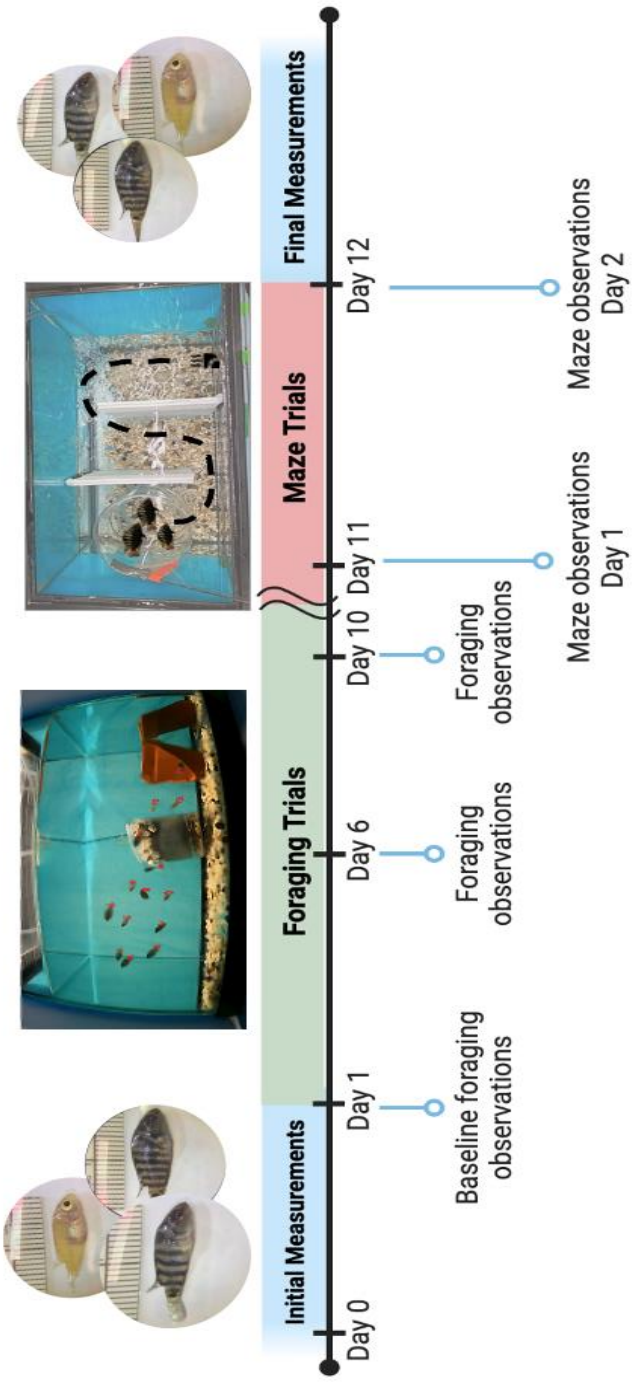


Figure 1: Timeline of Experiment 1. Initial measurements were conducted before the start of foraging trials and fish were given 1 day to acclimate. Foraging experiment lasted 10 days with observations on days 1, 6 and 10. Maze trials began a day after the foraging trials. Final measurements were conducted at the conclusion of the maze trials

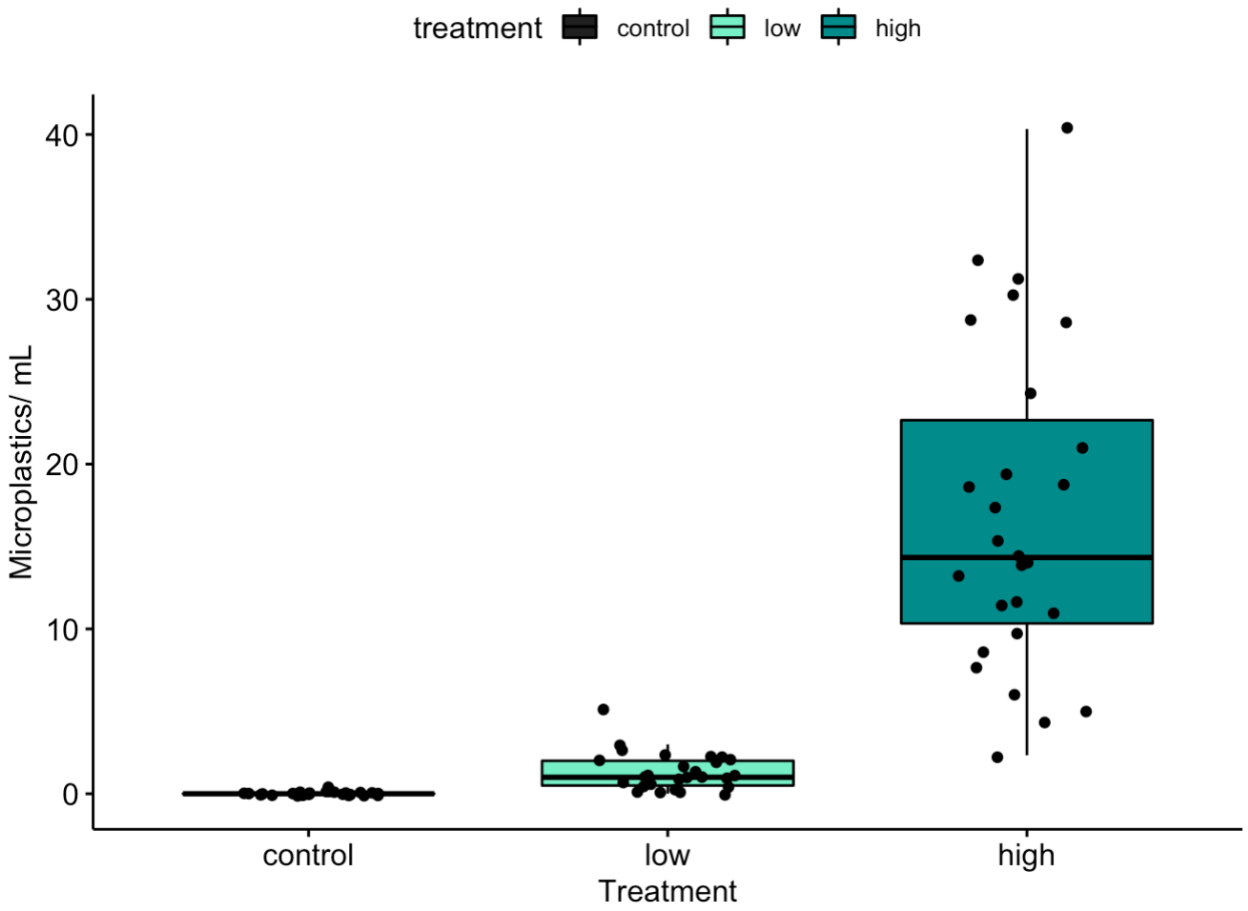


Figure 2: Boxplot showing the extrapolated concentrations of microplastics in the brine shrimp feeding solutions. See text for description of calculation. Control is shown in black, low treatment in light teal and high in dark teal.

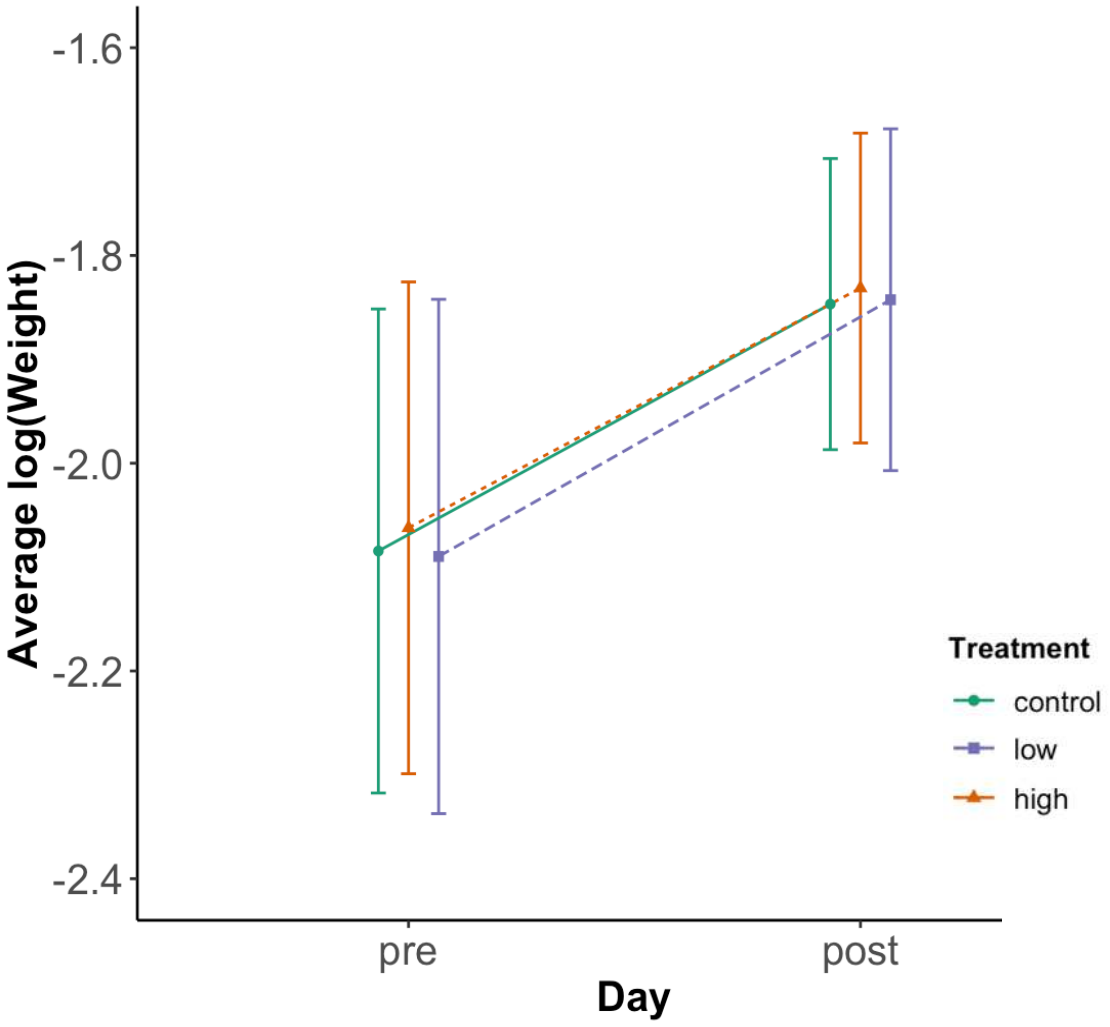


Figure 3: Mean ± SE of the average log (Weight) of all cichlids in a tank. Trends show an increase between the initial (pre) and final (post) measurement across all treatments. Control (0 MP mL^{-1}) is shown in solid green line, low treatment (10 MP mL^{-1}) in long purple dashes and high treatment (100 MP mL^{-1}) in short orange dashes.

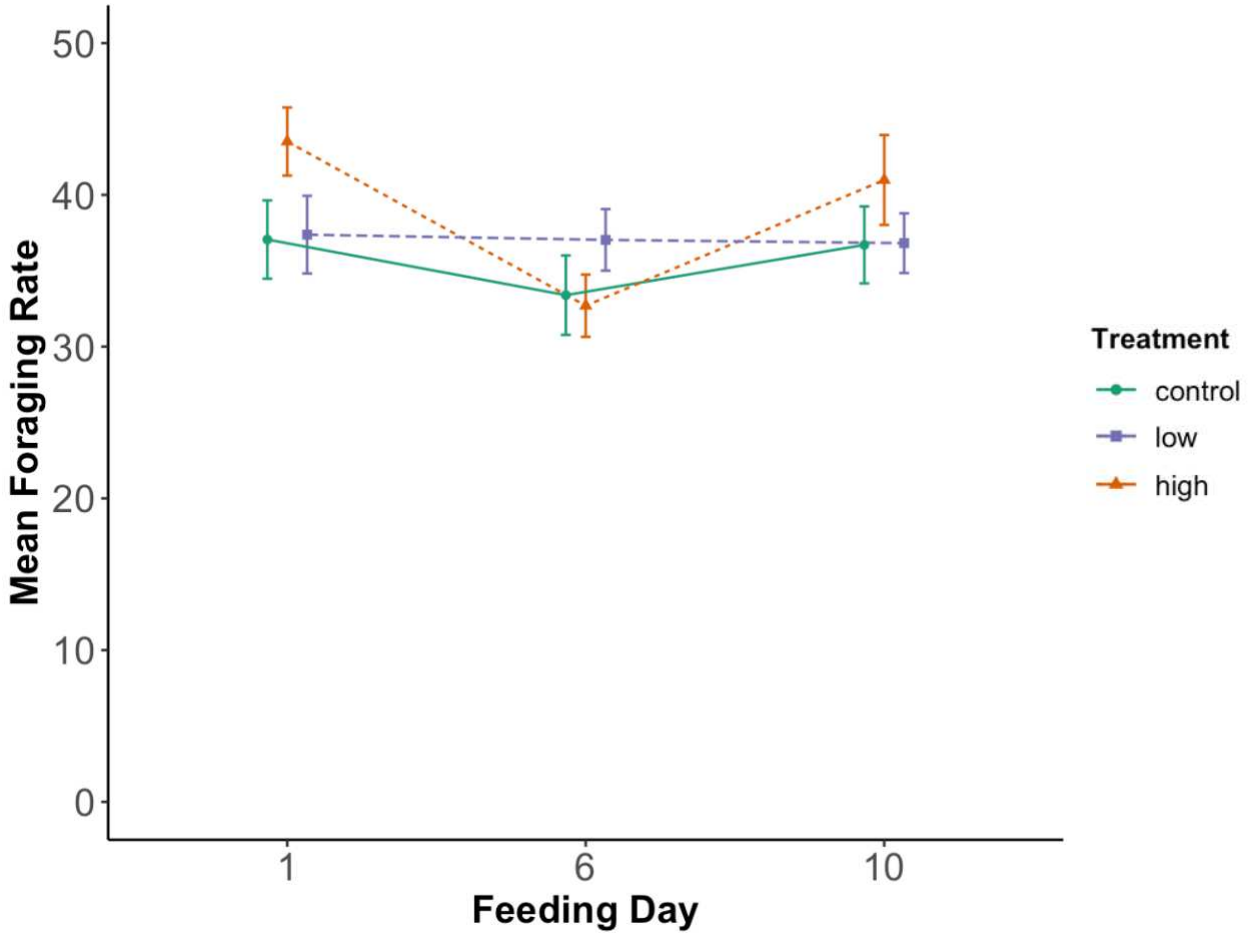


Figure 4: Mean ± SE of the mean foraging rate of cichlids within a tank. Mean foraging rate reflects the total foraging attempts across the six feeding periods divided by 6. Control (0 MP mL^{-1}) is shown in solid green line, low treatment (10 MP mL^{-1}) in long purple dashes and high treatment (100 MP mL^{-1}) in short orange dashes.

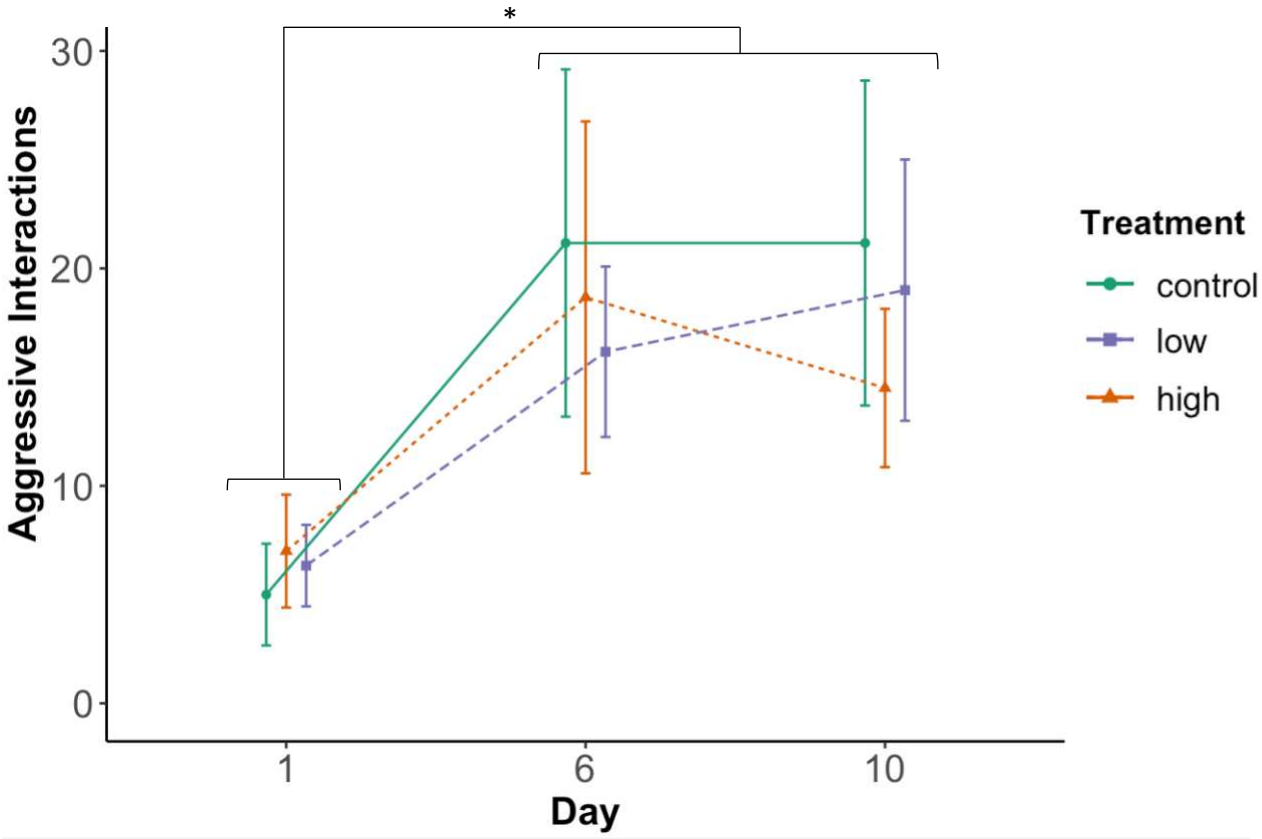


Figure 5: Mean ± SE of the total number of aggressive interactions within a tank during the foraging trials. Values are shown for days 1, 6 and 10 of the foraging trial. An asterisk (*) indicates a significant ($p < 0.05$) difference between day 1 and days 6 and 10 across all treatments. Day 6 and day 10 are statistically similar. Control (0 MP mL^{-1}) is shown in solid green line, low treatment (10 MP mL^{-1}) in long purple dashes and high treatment (100 MP mL^{-1}) in short orange dashes.

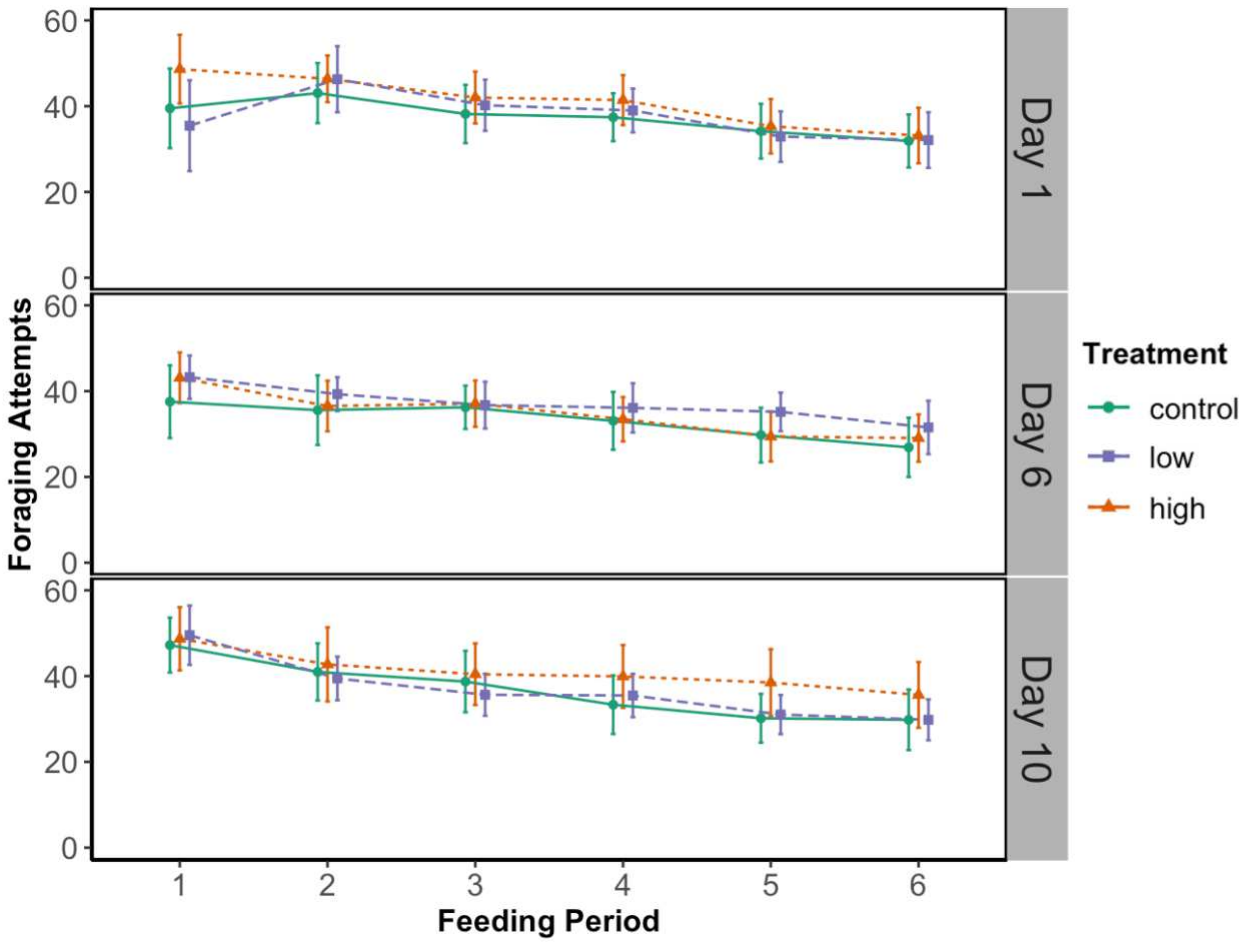


Figure 6: Mean ± SE of the foraging attempts during each feeding period across days 1, 6 and 10 of the foraging trial. Trends indicate a satiation effect across all treatments that are not significantly different from each other. Control (0 MP mL^{-1}) is shown in solid green line, low treatment (10 MP mL^{-1}) in long purple dashes and high treatment (100 MP mL^{-1}) in short orange dashes.

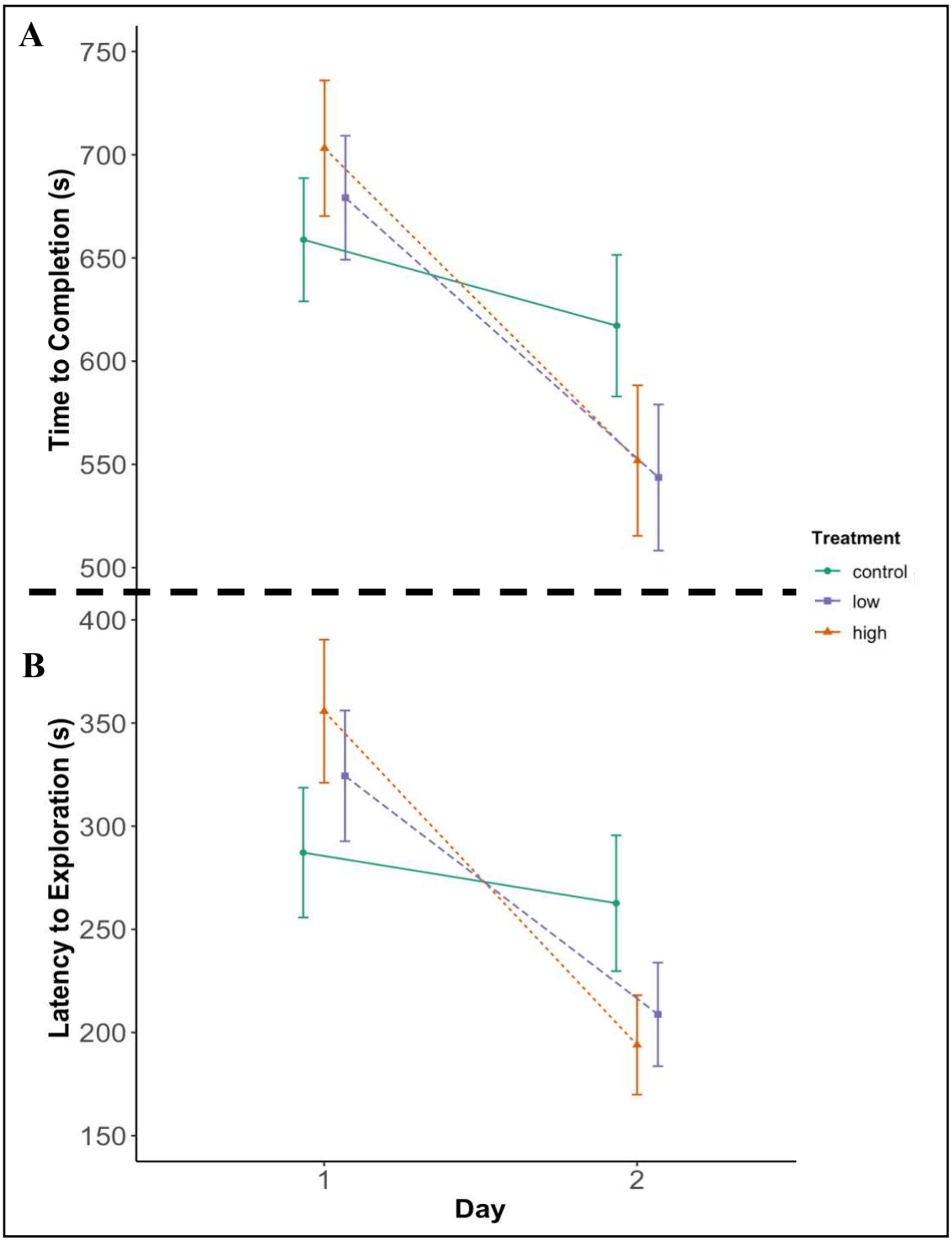


Figure 7: Mean ± SE of the cichlids' A) latency to exploration and B) time to complete the maze across the two days of the maze trial. Time is indicated in seconds. Note the discontinuity on the y-axis between 400 and 500 seconds indicated by a black dashed line. Control (0 MP mL^{-1}) is shown in solid green line, low treatment (10 MP mL^{-1}) in long purple dashes and high treatment (100 MP mL^{-1}) in short orange dashes.

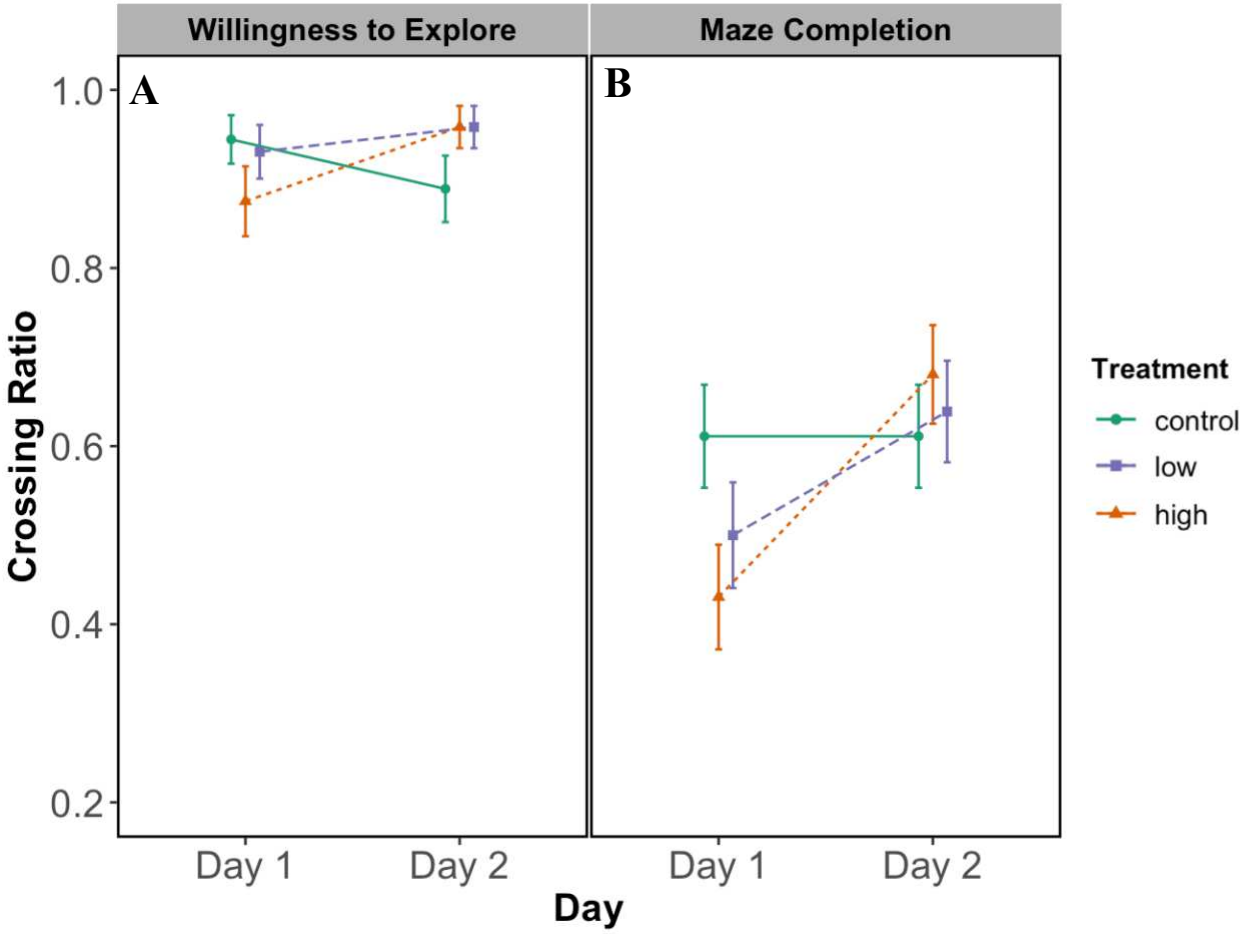


Figure 8: Mean ± SE of the cichlids' crossing ratio of the A) first and B) second barriers of the maze across the two days of the maze trial. Crossing was scored as a binary measure with a value of 1 assigned if they crossed and 0 if not. Results are interpreted as A) willingness to explore and B) maze completion. Control (0 MP mL^{-1}) is shown in solid green line, low treatment (10 MP mL^{-1}) in long purple dashes and high treatment (100 MP mL^{-1}) in short orange dashes.

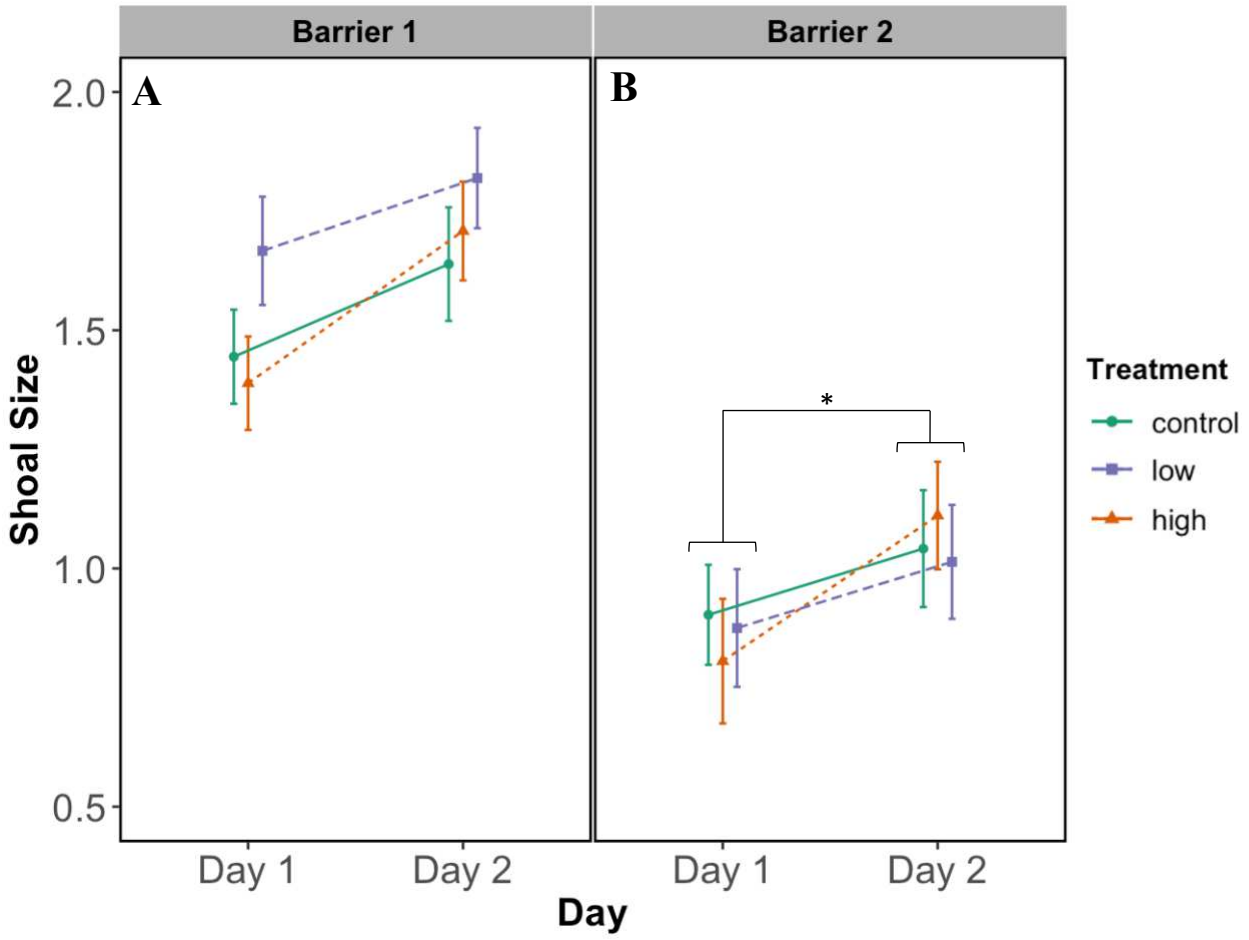


Figure 9: Mean ± SE of the shoal size during their first crossing of the A) first and B) second barriers of the maze across the two days of the maze trial. The asterisk indicates the significant ($p < 0.05$) increase in shoal size from day 1 to day 2 when crossing the second barrier. The difference is not affected by treatment. The increase seen in A across all treatments is not significant ($p = 0.068$). Control (0 MP mL^{-1}) is shown in solid green line, low treatment (10 MP mL^{-1}) in long purple dashes and high treatment (100 MP mL^{-1}) in short orange dashes.

Table 1: Results from the separate GLMMs for each day on the effect of treatment, feeding period and the treatment x period interaction on the cichlids' foraging attempts. Significant values are shown in bold.

	Effect	dF	F-value	p
Day 1	Treatment	2	2.2172	0.1152
	Feeding period	5	5.6112	**0.0002**
	Treatment x Period	10	0.5376	0.8588
Day 6	Treatment	2	1.9771	0.1448
	Feeding period	5	5.0231	**0.0004**
	Treatment x Period	10	0.1910	0.9965
Day 10	Treatment	2	2.3058	0.1059
	Feeding period	5	7.1455	**<0.0001**
	Treatment x Period	10	0.2311	0.9924

Table 2: Results from the GzLMMs for each metric analyzed in the maze trials. The effect of day, treatment and treatment x day interaction on the cichlids' latency to exploration, time to completion, willingness to explore and maze completion. Significant values are shown in bold.

Metric	Effect	dF	χ^2	p
Latency to Exploration (LTE)	Treatment	2, 423	1.8643	0.3937
	Day	1, 423	0.3392	0.5603
	Treatment x Day	2, 423	6.5508	**0.0378**
Time to Completion	Treatment	2, 423	0.4886	0.7833
	Day	1, 423	0.4907	0.4836
	Treatment x Day	2, 423	2.3148	0.3143
Willingness to Explore	Treatment	2, 424	2.9021	0.2343
	Day	1, 424	1.6722	0.1960
	Treatment x Day	2, 424	5.2789	0.0714
Maze Completion	Treatment	2, 424	2.3058	0.5877
	Day	1, 424	7.1455	**0.0048**
	Treatment x Day	2, 424	0.2311	0.0773

Table 3: Results from the separate GzLMMs of the effect of day and treatment on the cichlids' latency to exploration and time to completion. Significant values are shown in bold. Results show differences within days (treatments compared to control) and within treatments (day 2 compared to day 1).

Metric	Grouping	**Effect**	**t**	**p**
Latency to exploration	Within Day 1	High	-1.350	0.177
		Low	-0.763	0.446
	Within Day 2	High	1.921	0.0547
		Low	1.385	0.1660
	High Treatment	Day 2	3.735	**0.00019**
	Low Treatment	Day 2	2.985	**0.00283**
	Control Treatment	Day 2	0.494	0.621
Time to completion	Within Day 1	High	-0.77	0.441
		Low	-0.36	0.719
	Within Day 2	High	1.106	0.269
		Low	1.253	0.210
	High Treatment	Day 2	2.503	**0.0123**
	Low Treatment	Day 2	2.715	**0.00664**
	Control Treatment	Day 2	0.715	0.475

Table 4: Results from the conclusion of Experiment 2. We show the number of surviving fish (out of 20), the number of fish that had microplastics in their body and the range of the number of microplastics found within a fish. Results are shown from both the 24h and 48h treatment.

Last feeding	Treatment	Surviving fish	Fish with MPs	Range
24h	Control	20	0	0
	Low	20	6	0 - 2
	High	20	6	0 - 46
48h	Control	19	0	0
	Low	19	2	0 - 2
	High	19	4	0 - 8

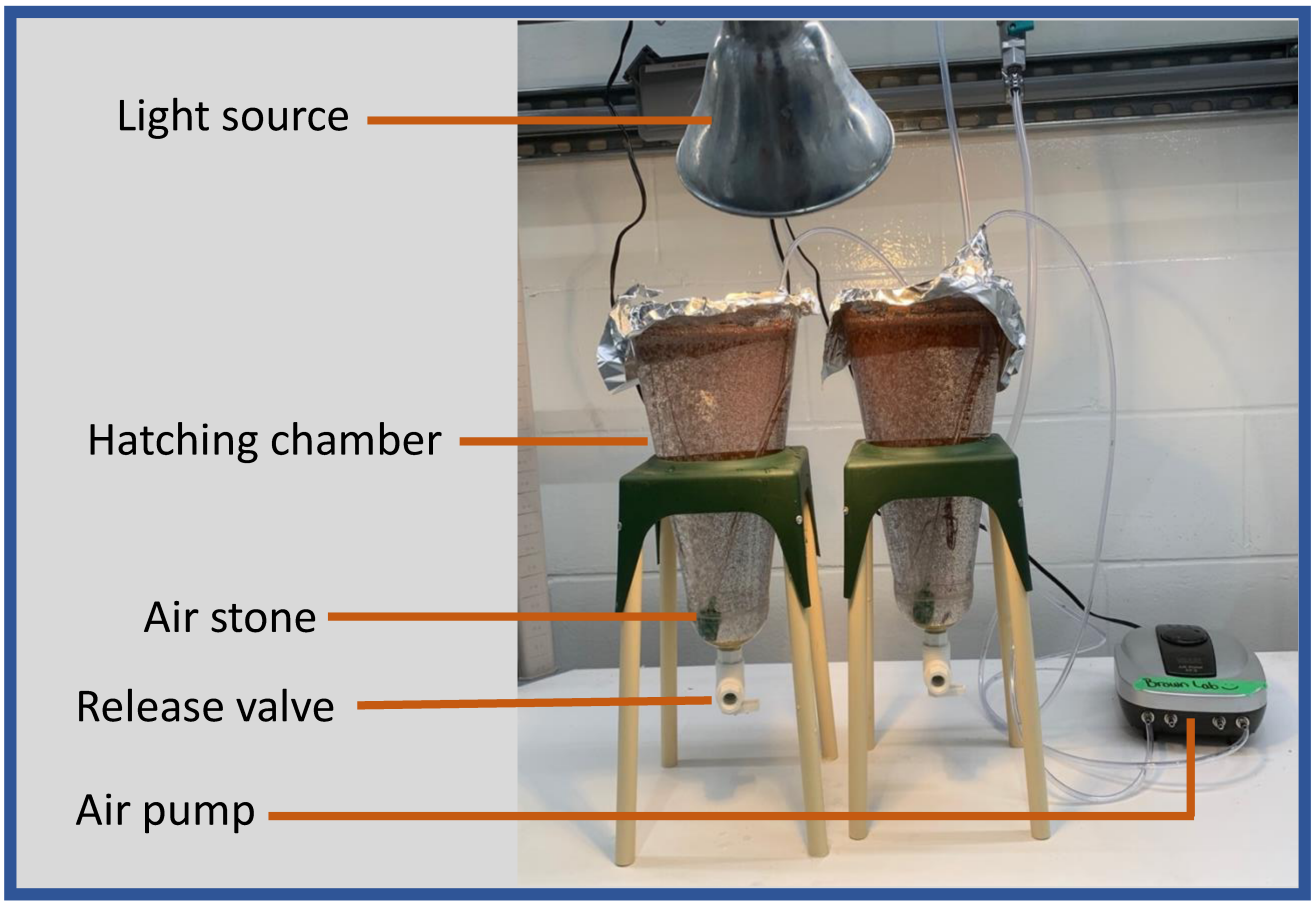
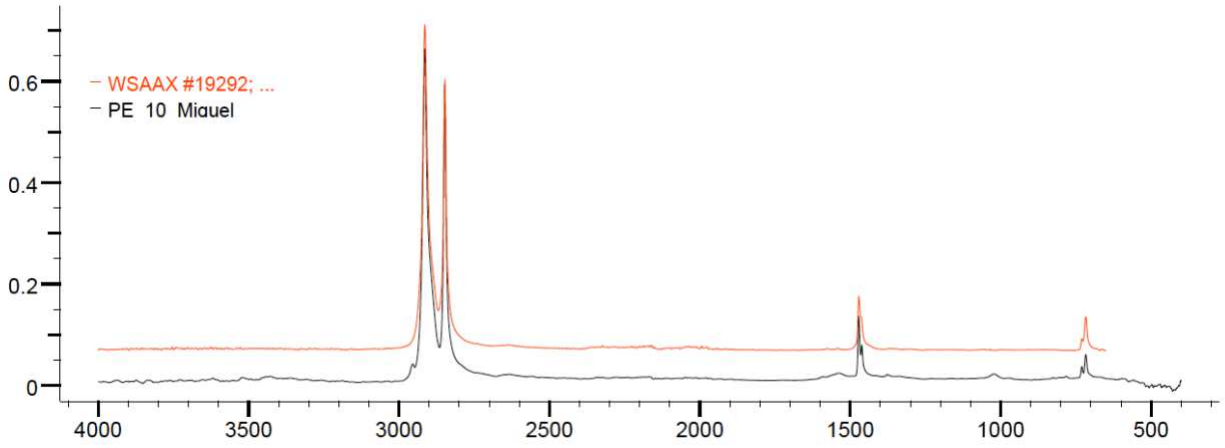


Figure S1. General set-up for brine shrimp rearing. Each hatching chamber/cone is placed under a light source (lamp with incandescent bulb) which also serve as a heat source. The cone is set-up with an air stone attached to an air pump. Harvesting is done by opening the release valve at the bottom of the chamber.



Name	Value
Resulting HQI	93.77
Database Abbreviation	WSAAX
Database Title	ATR-IR - Sigma-Aldrich Library of ATR-IR Spectra - Wiley
Record ID	19292
Name	Polyethylene
CAS Registry Number	9002-88-4
Catalog Number	429015
Formula	(C2H4)n
Purity	Ultra-high molecular weight, average Mw 3,000,000-6,000,000
Source of Sample	Aldrich
Synonyms	Polyethylene as; Carlona PXB
Wiley ID	SIAL_ATR-IR_040032

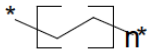


Figure S2. Results of spectroscopic analysis performed on the polyethylene microspheres used in this study. The spectrum was collected using Fourier-transform infrared spectroscopy and analyzed using the KnowItAll Informatics System. Results show high hit quality index when compared with stock polyethylene.

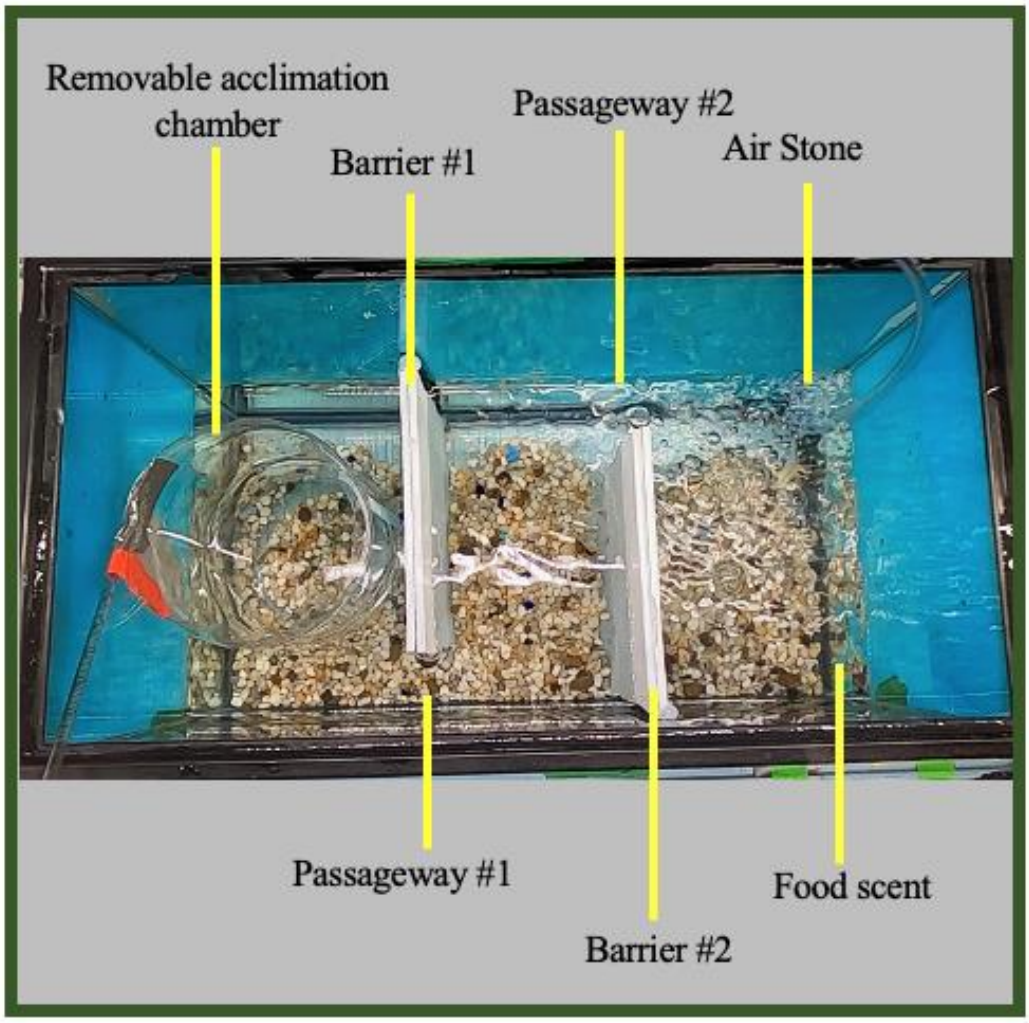


Figure S3. General set-up for the maze trials. Fish are placed in a removable acclimation chamber made from PET. The maze is divided into three chambers by two barriers made from corrugated plastic (polypropylene) adhered to the walls with silicone. Each barrier has a 4 cm gap to allow for passage. The maze is set-up with an air stone attached to an air pump.

Appendix B: Coefficients of Variance

Previous studies have established the relationship between the coefficient of variance within a group's foraging behaviour and competitive interactions within a group (Blanckenhorn et al. 1998). Resource monopolization by dominant individuals through acts of aggression leads to both more variation in foraging success and fish size (i.e., higher CV) and more competitive interactions (Noël et al. 2005). We found no significant effects of microplastic exposure on competitive interactions within a shoal both when comparing coefficients of variance within shoals and total instances of competitive aggression during foraging periods. This result is consistent with the established assumption that the coefficients of variance in body size, length and/or foraging attempts can be used as a proxy for competitive interactions within a shoal (Blanckenhorn et al. 1998, Noël et al. 2005).

References:

Blanckenhorn, W. U., J. W. A. Grant, and D. J. Fairbairn. 1998. Monopolization in a resource queue: water striders competing for food and mates. Behavioral Ecology and Sociobiology 42:63–70.

Noël, M. V., J. W. A. Grant, and J. G. Carrigan. 2005. Effects of competitor-to-resource ratio on aggression and size variation within groups of convict cichlids. Animal Behaviour 69:1157–1163.

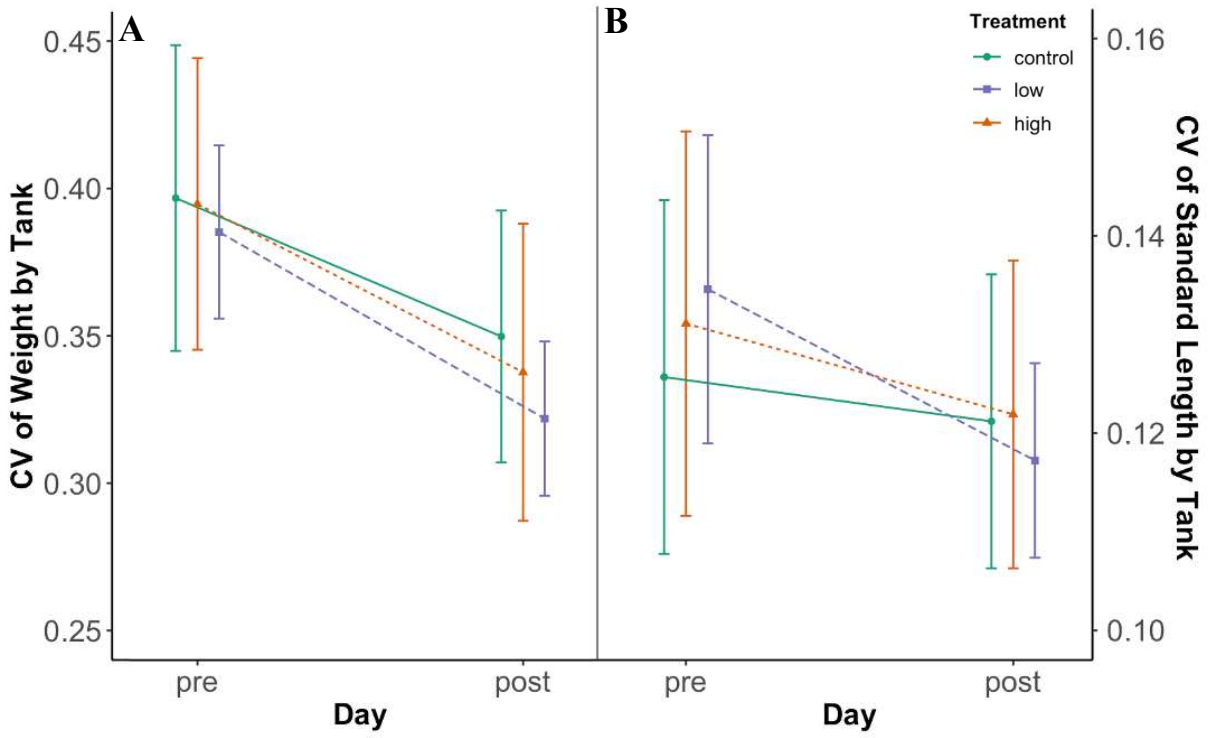


Figure S4: Mean ± SE of the coefficient of variance (CV) of A) Weight and B) standard length of cichlids within a tank. CV is calculated as *CV = (Standard Deviation / Mean)*. Control (0 MP mL^{-1}) is shown in solid green line, low treatment (10 MP mL^{-1}) in long purple dashes and high treatment (100 MP mL^{-1}) in short orange dashes.

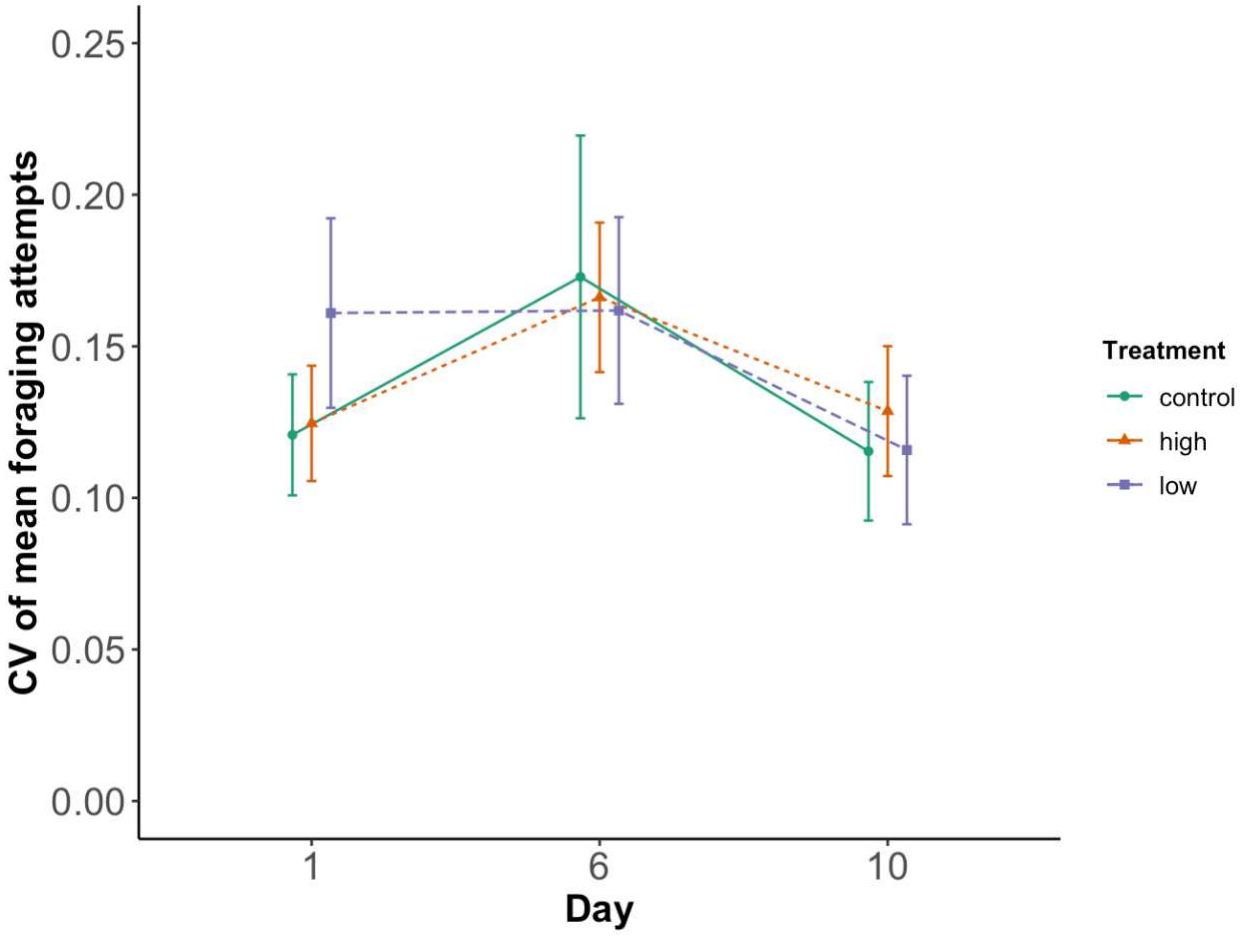


Figure S5: Mean ± SE of the coefficient of variance (CV) of mean foraging rate of cichlids within a tank. CV is calculated as *CV = (Standard Deviation / Mean)*. Control (0 MP mL^{-1}) is shown in solid green line, low treatment (10 MP mL^{-1}) in long purple dashes and high treatment (100 MP mL^{-1}) in short orange dashes.

Appendix C: Standard Length and Body Condition

While sedated, fish were photographed under a dissecting microscope (Leica EZ4 Stereo Microscope 8x - 35x, Leica Microsystems, Wetzlar, Germany) to measure standard length (i.e. length from tip of snout to point of caudal insertion). Analysis of the fish images were done using ImageJ (version 1.53k). Measures of weight and standard length also allowed us to provide information on body condition using the formula: $K = 10 * (Weight * (Length-3))$ (Froese 2006). The mean standard lengths for each treatment were 1.613 cm (± 0.338), 1.612 g (± 0.356) and 1.626 g (± 0.349) for the control, low and high treatments respectively.

References:

Froese, R. 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. Journal of Applied Ichthyology 22:241–253.

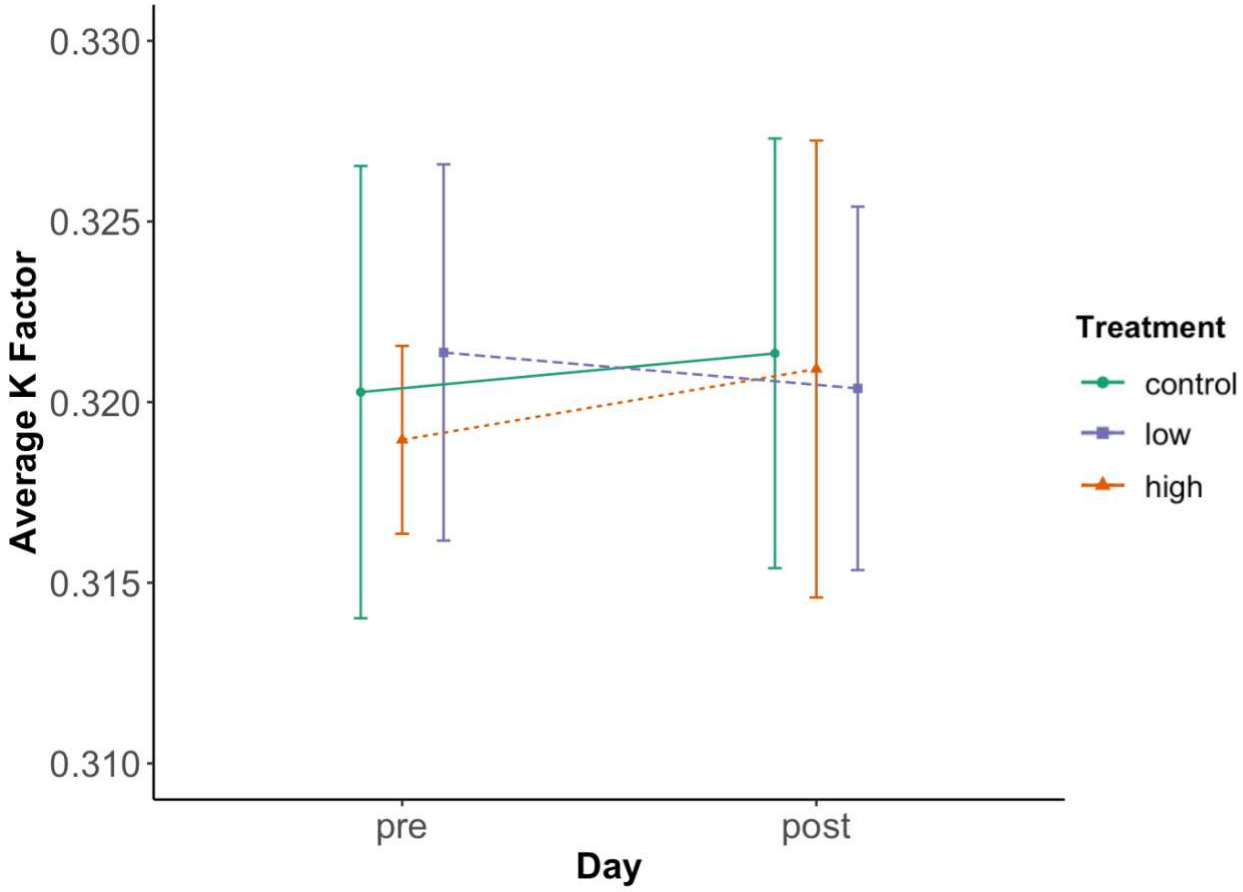


Figure S6: Mean ± SE of the average condition (K) factor of all cichlids in a tank. We compared values from the initial (pre) vs final (post) measurements. Condition factor calculated as $K = 10 * (Weight * Length^{-3})$. Control (0 MP mL^{-1}) is shown in solid green line, low treatment (10 MP mL^{-1}) in long purple dashes and high treatment (100 MP mL^{-1}) in short orange dashes.

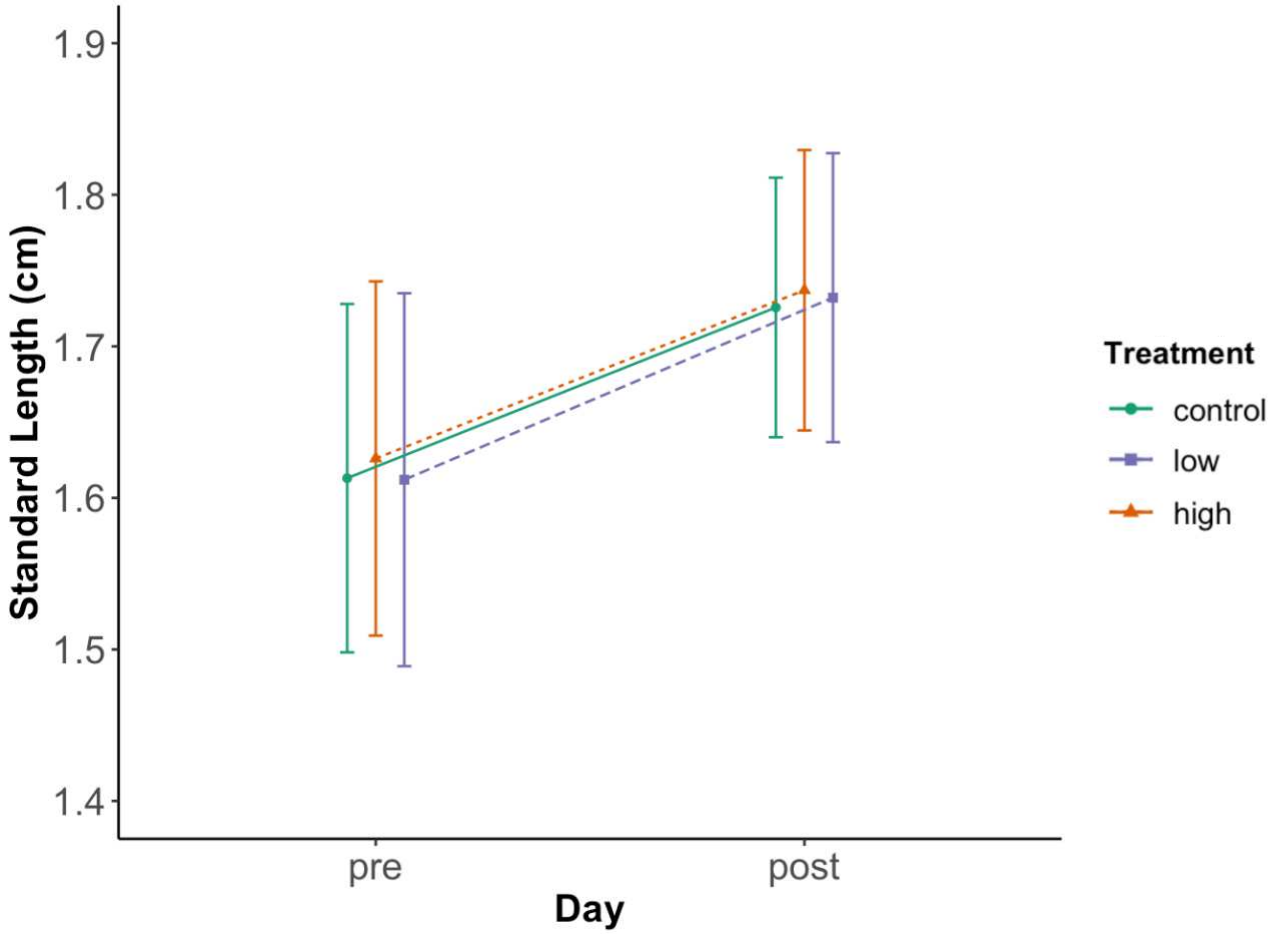


Figure S7: Mean ± SE of the mean standard length of all cichlids in a tank. We compared values from the initial (pre) vs final (post) measurements. Control (0 MP mL^{-1}) is shown in solid green line, low treatment (10 MP mL^{-1}) in long purple dashes and high treatment (100 MP mL^{-1}) in short orange dashes.