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Short communication

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Rendani Mbedzi, Tatenda Dalu, Ryan J Wasserman, Florence Murungweni, Ross N Cuthbert

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Functional response quantifies microplastic uptake by a widespread African fish species

Rendani Mbedzi ^{a, +}, Tatenda Dalu ^{a, +}, Ryan J Wasserman ^b, Florence Murungweni ^a, Ross N Cuthbert ^{c, *}

^a *Aquatic Systems Research Group, Department of Ecology and Resource Management, University of Venda, Thohoyandou 0950, South Africa*

^b *Department of Biological Science and Biotechnology, Botswana International University of Science and Technology, Palapye, Botswana*

^c *School of Biological Sciences, 19 Chlorine Gardens, Queen's University Belfast, Belfast BT9 5DL, Northern Ireland, United Kingdom of Great Britain and Northern Ireland*

***Corresponding author-email address:** *rcuthbert03@qub.ac.uk*

⁺ Equal contributors

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Rendani Mbedzi ^{a, +}, Tatenda Dalu ^{a, +}, Ryan J Wasserman ^b, Florence Murungweni ^a, Ross N Cuthbert ^{c, *}

^a *Aquatic Systems Research Group, Department of Ecology and Resource Management, University of Venda, Thohoyandou 0950, South Africa*

^b *Department of Biological Science and Biotechnology, Botswana International University of Science and Technology, Palapye, Botswana*

^c *School of Biological Sciences, 19 Chlorine Gardens, Queen's University Belfast, Belfast BT9 5DL, Northern Ireland, United Kingdom of Great Britain and Northern Ireland*

***Corresponding author-email address:** *rcuthbert03@qub.ac.uk*

⁺ Equal contributors

Abstract

Ecological impacts of microplastic remain poorly understood, despite their ubiquity across all habitat types globally. Microplastic concentrations vary significantly across spatiotemporal gradients, however we lack quantitative methodologies to predict species-level responses to differential environmental concentrations. In the present study, we expose a key species, the banded tilapia *Tilapia sparrmanii* Smith, 1940, to different concentrations of microplastic particles. We apply and develop the functional response approach for quantifications of microplastic uptake by the fish across different environmental densities. Tilapia consumed microplastic even when relatively rare in their environment, and consumption rates related negatively to concentrations supplied, conducive with a saturating Type II (i.e., inversely-density-dependent) functional response. Attack rate (i.e., search efficiency), handling time and maximum feeding rate estimates towards microplastic were estimated, providing key information on feeding behaviour in relation to exposure concentrations. We propose the utility of functional response approaches for predictive quantifications of microplastic uptake rates. In turn, this can better-link laboratory exposure studies to environmental concentrations which are known to cause ecological impact, and provide a means of comparing uptakes among species and across environmental contexts.

Keywords: attack rate; handling time; pollution; freshwaters; consumer-resource; polyethylene

Introduction

Microplastic (< 5 mm in size) pollution continues to proliferate across all habitat types and regions globally (Mason et al., 2018; Wagner and Lambert, 2018). Principally driven by the production of artificial plastic materials for human use (Cole et al., 2011), primary (i.e., *via* direct microplastic release) and secondary (i.e., *via* macroplastic degradation) origin microplastic enters the environment in a variety of shapes, sizes and densities (Rocha-Santos and Duarte, 2014), with ecosystem ramifications mostly unknown. Enormous quantities of microplastic are released into the environment through household wastewaters (Mason et al., 2016; Wagner and Lambert, 2018), with many particles subsequently transported *via* riverine systems into seas or lakes, where high concentrations accrue (Eriksen et al. 2013; Fischer et al., 2016; Su et al., 2016). Whilst microplastics have exerted negative ecological impacts on marine ecosystem biota, such as increased mortality (Reichert et al., 2018) and decreased reproduction (Cole et al., 2015), knowledge concerning the impacts of microplastic on freshwater biota remains rudimentary, despite the particular vulnerability of freshwaters to environmental change (Al-Jaibachi et al., 2018; Blettler et al., 2018; Provencher et al., 2018; Cuthbert et al., 2019a; Windsor et al., 2019).

Laboratory-based exposure trials are routinely used to examine the effects of microplastic on flora and fauna, although such studies have recently come under scrutiny for using excessive, environmentally-unrealistic concentrations (Cunningham and Sigwart, 2019). Nevertheless, concentrations of microplastic in aquatic environments are known to be highly variable spatiotemporally (Hurley et al., 2018; Tibbetts et al., 2018; Nel et al., 2018), and therefore uptake of microplastic by biota may vary rapidly over their range in aquatic ecosystems. In turn, impacts of microplastic uptake on organisms may vary substantially owing to potential density-dependent effects (Cuthbert et al., 2019a). Yet, there is currently a lack of

understanding of how uptake rates by biota respond to differential environmental microplastic concentrations, hampering predictive quantifications of how uptake rates, and thus their ecological impact, relate to environmental heterogeneity in microplastic pollution concentrations.

Functional responses quantify resource use as a function of resource density (Holling, 1959). Whilst functional responses have been widely used to quantify the nature of consumer-resource interactions in many ecological fields (e.g., Abrams 1982; Cuthbert et al., 2019b), applications are lacking for quantifications of direct microplastic uptake by organisms (but see Woods et al., 2018). Three forms of functional responses are commonly described: the density-independent linear Type I response; inversely density-dependent hyperbolic Type II response, where consumption rates are high at low densities, and; positively density-dependent Type III response, which is sigmoidal due to low consumption rates at low densities (Hassell, 1978). In theory, Type II functional responses are destabilising for resources (e.g., prey), owing to a lack of low density refuge. By extension, in a microplastic uptake context, a Type II functional response may be indicative of high consumption rates even under low environmental concentrations.

In the present study, we therefore employ a functional response approach to quantify the density-dependence of microplastic uptake by the banded tilapia *Tilapia sparrmanii* Smith, 1940, which is a widespread and key species in warm freshwater habitats of southern Africa. This species has been introduced to many freshwater basins, and is distributed extensively outside of its native range (Ellender and Weyl, 2014). Banded tilapia readily consumes animal prey, however its diet is also known to include plant material (Zengeya and Marshall 2007; Marshall 2011). Given its broad diet and extensive distribution, banded tilapia

represent a suitable model species for examination of microplastic uptake. We hypothesise that microplastic uptake by juvenile stages of the fish will relate positively to environmental microplastic concentrations. Further, we expect that tilapia will uptake microplastic even when present at relatively low densities in aquatic environments.

Collection of captive-bred fish and all experiments were carried out in compliance with the ethical clearance approved by the University of Venda Research Committee (no. SES/18/ERM/10/1009), South Africa.

Materials and methods

Experimental design and analysis

Banded tilapia, *Tilapia sparrmanii* Smith, 1840 were supplied from Netshituni Fishery Project Phiphidi, Vhembe District, South Africa. All fish were transported to the Department of Ecology and Resource Management, University of Venda, Thohoyandou and were housed in 30 L buckets with continuously aerated water (23.2 ± 0.3 °C; 12:12 light:dark photoperiod). Fish were allowed to acclimate to the system for at least 48 hours prior to use in microplastic trials. Experiments were conducted in individual 10 L buckets filled with 4 L of filtered (63 µm mesh sieve) and matured tap water. Banded tilapia were size matched with respect to standard length (SL $\pm$ SD: 32.4 ± 2.1 mm) and were starved for 30 hours prior to the experiment to standardise hunger levels. Fish were randomly selected two hours prior to use and placed individually in 10 L white plastic buckets to settle. After the acclimatisation period, fish were presented with microplastic (i.e., ultra-high molecular weight, surface-modified multi-coloured polyethylene powder, 125 µm particle size (Sigma-Aldrich, UK)) at four densities (0.05 g (~155 particles/39 particles L⁻¹), 0.1 g (~309 particles/77 particles L⁻¹), 0.5 g (~1545 particles/386 particles L⁻¹), 1 g (~3090 particles/773 particles L⁻¹)), each mixed

with 2 g of tilapia fish food (Rainbow Kingdom, Louis Trichardt) and with five replicates per density. Therefore, 25 individual fish were used in total for the experiment. To initiate the feeding period, fish flakes were added evenly to the surface of the water with the allocated microplastic treatments. Whilst recent studies have been criticised for using unrealistic microplastic concentrations (see Cunningham and Sigwart, 2019), quantities used in the present study span both 'low' (i.e. < 100 particles L^{-1}) and 'high' (i.e. > 100 particles L^{-1}) dosages, with 100 particles L^{-1} regarded as the highest measured environmental concentration (Burns and Boxall, 2018). Microplastic treatment presentations were fully crossed and randomised so that all combinations were trialled simultaneously. Feeding trials were run for 4 hours and were initiated at midday, after which microplastic consumption was examined. Controls were run simultaneously and consisted of five replicates in the absence of microplastic, with only fish food.

At the end of the experiment, all fish were euthanised by immersion in 40 mg L^{-1} of clove oil and fixed in 99 % ethanol for stomach contents analysis to determine the numbers of microplastic eaten. The entire gut was removed from each fish for quantification of microplastic uptake, using new petri dishes that were rinsed with distilled water for each individual. Laboratory blanks were used to ensure that there were no microplastic particles in the distilled water. As the microplastics are relatively resistant to digestion, individual consumption within fish was inferred by quantifying numbers of microplastic in stomach contents under a Carl Zeiss Stemi dissecting microscope (Carl Zeiss MicroImaging GmbH, Göttingen). Owing to the relatively large size and thus high detectability of experimental microplastic particles, it was not necessary to use digestion on fish and we did not expect microplastic to be lodged in the gut lining.

Data analysis

Differences in fish lengths (mm; integers) among microplastic treatments (5 levels) were examined using generalised linear models (GLMs) assuming Poisson distributions of error with log links. A nested likelihood ratio test was used to infer the treatment effect size and significance level. Similarly, raw microplastic consumption (counts) was analysed using Poisson GLMs according to microplastics treatment (4 levels, excluding microplastic-free control). Residuals were examined in each model to ensure there was no significant evidence for overdispersion, allowing a Poisson rather than e.g. quasi-Poisson error family to be used.

Functional response analyses followed two stages. First, logistic regression was used to analyse proportional microplastic consumption (counts) as a function of initial microplastic abundance (i.e., density; continuous predictor) in order to categorise the functional response (i.e., into Types I, III, III). From this regression, a significantly negative first order term indicates a Type II functional response (Juliano, 2001). Second, functional responses were modelled using Rogers' random predator equation owing to the non-replacement of microplastic particles during the experiment (Rogers, 1972):

$$N_e = N_0(1 - \exp(a(N_e h - T))) \quad (1)$$

where N_e is the number of microplastic particles consumed, N_0 is the initial density of particles, a is the attack rate, h is the handling time and T is the experiment duration (fixed at 1, i.e., 4 hours). Attack rates corresponds to the functional response curve slope (i.e., area or volume cleared per unit time by fish), whilst the handling time, inversely, corresponds to the functional response asymptote (i.e., fish maximum feeding rate). The Lambert W function was used to allow for model fitting, owing to the recursive nature of the random predator equation (Bolker, 2008). A non-parametric bootstrapping procedure ($n = 2000$) was followed

to generate 95 % confidence intervals (CIs) around the functional response curve (Pritchard et al., 2017). In all analyses, significance was inferred at a threshold of $p < 0.05$.

Results

The length of fish used in the experiment did not differ significantly depending on microplastic exposure group (GLM: $\chi^2 = 0.29$, $df = 4$, $p = 0.99$). Therefore, size-related differences likely did not significantly alter propensities for microplastic consumption by fish. No microplastic particles were consumed in control fish, and it was thus unnecessary to adjust consumption to account for any background levels of plastics. Microplastic was found in 100 % of exposed tilapia, and counts tended to increase slightly with greater exposure concentrations (Figure 1). Nevertheless, there were no statistically clear differences in microplastic consumption among exposure concentrations (GLM: $\chi^2 = 4.57$, $df = 3$, $p = 0.21$), with fish consistently consuming particles even when relatively sparse in the environment.

The proportion of microplastic consumed (i.e., numbers eaten relative to numbers remaining) was significantly negatively related to the initial experimental particle density (GLM: linear coefficient = 0.0009, $z = 19.55$, $p < 0.001$). Therefore, fish exhibited significant evidence for a hyperbolic Type II functional response, characterised by high proportional consumption rates at low densities (Figure 2). This enabled functional response attack rate (a) and handling time (h) parameters to be estimated (random predator equation: $a = 0.63$, $z = 1.88$, $p = 0.06$; $h = 0.04$, $z = 14.52$, $p < 0.001$). Accordingly, fish exhibited maximum consumption rates ($1/h$; i.e., functional response asymptote) of approximately 25 particles over the 4 hour experimental period (Figure 2).

Discussion

The present study demonstrates the potential for *Tilapia sparrmanii* to uptake microplastic across a gradient of environmental concentrations. Tilapia were found to uptake microplastic even when relatively rare in their aquatic environment, with increasing concentrations having a positive but statistically unclear effect on uptake levels. Under higher concentrations than tested in the present study, microplastic particles have been shown to accrue across all tissues of freshwater tilapia following consumption (Ding et al., 2018). In turn, adverse impacts on juvenile fish stages have been reported following exposure to concentrations aligned with the lowest levels in this study, such as anemia (e.g., Hamed et al., 2019). Further, uptake of microplastic has been shown to interact with, and potentially alter, toxicity levels of other pollutants upon tilapia (Zhang et al., 2019). Therefore, the quantified propensity for *T. sparrmanii* to uptake microplastic in the present study may drive biotic impacts even under relatively low environmental concentrations, with the present study spanning both low and high dosages from empirical measurements (Cunningham and Sigwart, 2019).

Microplastic uptake by *T. sparrmanii* exhibited significant non-linear density-dependence in the present study, with uptake rates falling asymptotically with increasing environmental concentrations. This aligns with a Type II functional response, where proportional uptake rates are highest under low environmental densities (i.e., concentrations) (Holling, 1959). This suggests that *T. sparrmanii* actively uptakes microplastic even where relatively rare in the environment. Functional responses offer great utility in quantifications of resource uptake under different densities (e.g., Cuthbert et al., 2019b), and the approach thus lends itself to microplastic studies where density-dependences of uptake may be deciphered. In turn, feeding behaviours may be related against microplastic concentrations documented to caused impact. Here, the functional response approach returned attack rate (i.e., search efficiency)

and handling time parameters towards microplastic by *T. sparrmanii*. In this context, the attack rate corresponds to the volume cleared per unit time by fish (i.e., curve initial slope), whilst the handling time is the time taken to capture, consume and digest. Inversely, the handling time estimate enabled maximum feeding rates (i.e., curve asymptote) of microplastic to be calculated for *T. sparrmanii*, providing important quantitative information relating to the propensity for this species to uptake microplastic over time. Whilst not evidenced in the present study, further studies should examine excretion rates of microplastic, and how these may influence uptake, retainment and distribution in organisms under different experimental durations

Just as functional responses can be used to quantify interaction strengths in consumer-resource (e.g., predator-prey) systems (Kalinowski and DeLong, 2016), we propose the use of functional responses in quantifications of microplastic uptake rates. Functional responses can be subsequently compared among species or across environmental contexts to better understand key drivers which may alter uptake rates (Cuthbert et al., 2019b). Further, differences in uptake rates across microplastic polymer types and sizes could be examined using a functional response approach. Ultimately, understandings of species-level feeding responses to different environmental concentrations will assist in our understanding of microplastic impacts, and help to better-link laboratory exposure studies to real-world concentrations.

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Figure 1. Mean (± 1 SE) numbers of microplastic consumed (i.e., counted in gut) by individual fish among different initial exposure weights ($\text{g } 4 \text{ L}^{-1}$) following 4 hour experimental period. Letters above indicate a lack of significant differences.

Figure 2. Type II functional response of fish towards microplastics at different exposures (no. particles 4 L⁻¹) following 4 hour experimental period. Grey areas are bootstrapped 95 % CIs and points are underlying raw consumption data.

- Banded tilapia *Tilapia sparrmanii* consumed microplastic in aquatic environment.
- Consumption did not significantly relate to exposure concentration.
- Tilapia exhibited a saturating Type II functional response towards microplastic.
- Functional responses are powerful in microplastic uptake assessments.