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# No effects of plasticized microplastics on the body condition and reproduction of a marine fish

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This study experimentally explored the influence of periodic consumption of polystyrene (PS) microplastic fragments on the body condition and fitness of a tropical marine fish. Adult damselfish, *Acanthochromis polyacanthus*, were pulse fed microplastic fragments bound with one of two different common plasticizers [di-2-ethylhexyl phthalate (DEHP), di-2-ethylhexyl terephthalate (DEHT)] together with virgin-plastic and no-plastic controls. Ingestion of plastic over a 150d period had no detectable effect on growth, indices of body condition, or gonadosomatic indices. Histology of the liver showed no detrimental effects of ingesting any of the plastic treatments on hepatocyte density or vacuolation. Plastic consumption had no effect on the number of clutches produced over the breeding period, the number of eggs, or the survival of embryos. It is believed that the relatively inert nature of PS, the low amount of plasticizers leached from the fragments and fast gut through-put times meant fish were exposed to low levels of toxic compounds.

**Keywords:** body condition, coral reef fish, microplastic, phthalate plasticizer, plasticizers, polystyrene, reproduction.

## Introduction

Pollution of the oceans by plastic waste has become one of the major environmental issues of the 21st century (Vo and Pham, 2021; Wootton *et al.*, 2021). Estimates of the problem suggest that 19–23 million tonnes of plastic entered aquatic ecosystems in 2016 (Borrelle *et al.*, 2020), where further degradation into small particles occurred through chemical and physical erosion. As plastic particles become smaller with decomposition, their relative surface area increases, and they become bioavailable to a broader range of marine organisms, including fishes [for a review, see Markic *et al.* (2020)]. Upon ingestion, organisms can be exposed to chemicals that leach from the degrading plastic, as well as adsorbed chemicals (e.g. persistent organic pollutants) and biofilms that coat the surface of the particles (Zarfl and Matthies, 2010; Jacquin *et al.*, 2019; Vo and Pham, 2021). So prevalent are plastics throughout the world's oceans (Oberbeckmann and Labrenz, 2020) that plastic bi-products are found at every trophic level (Teuten *et al.*, 2009; Ziccardi *et al.*, 2016), and have been regularly found to accumulate in commercial fishes consumed by humans, which has galvanized efforts to reduce marine plastic pollution (Neves *et al.*, 2015; Lusher *et al.*, 2017; Farady, 2019).

The ingestion of plastics can be harmful to marine organisms through occlusion of the gastrointestinal tract, but can also be detrimental to organisms that can pass plastic particles through their guts (Law, 2017). Plastics, such as polyvinylchloride, polyethylene, and polystyrene (PS) are largely inert, but are usually manufactured to contain chemicals that enhance mechanical flexibility and toughness, known as plasticizers. These compounds are usually of low to medium-high molecular weight and are relatively non-volatile under normal environmental temperature ranges (Jamarani *et al.*, 2018). Plasticizers can comprise up to 80% of the plastic product in flexible products (Rahman and Brazel, 2004). Because most of these plasticizers are not bound into the polymer matrix, they are readily released through diffusion, abrasion, and leaching. These plasticizers can potentially represent the most dangerous chemical component of the plastic matrix due to their bioreactive nature (Jamarani *et al.*, 2018). In the last decade, the main plasticizers have been phthalate compounds (e.g. diethylhexyl phthalate, DEHP), but experiments have shown these to have acute toxic effects on development and reproduction due to their role as endocrine and DNA disruptors (Rowdhwal and Chen, 2018; Sedha *et al.*, 2021). Moreover, because of their lipophilic properties phthalates can accumulate in lipids within the liver and may cause serious damage (Park *et al.*, 2020). Non-phthalate plasticizers [e.g. di-2-ethylhexyl terephthalate (DEHT)] are becoming increasingly popular (Katsikantami *et al.*, 2016), and while they are proposed to have fewer toxic side-effects, few studies have been conducted that assess their effects.

It is likely that for most marine organisms, ingestion of plastics will be relatively rare and may lead to sub-lethal

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**Figure 1.** The spiny chromis, *Acanthochromis polyacanthus*, is a fish common to Indo-Pacific tropical reefs and is one of the few marine fishes to brood its young. Here, we see a parent with a brood. Photographic credit: M. McCormick.

effects. If ingestion of plastic particles results in gut fullness (McCauley and Bjorndal, 1999), or toxic effects such as endocrine disruption (Rowdhwal and Chen, 2018) or oxidative damage (Kim *et al.*, 2021), then changes in activity and behaviour of adults could be predicted. These behavioural changes may lead them to make suboptimal decisions in relation to foraging, courtship, or predation risk, leading to lower fitness and/or survival [e.g. McCormick *et al.* (2020)]. Recent reviews of gut contents studies have shown that ingestion of microplastics by fishes may be low but common, and, despite high variability, ingestion loads average 2.7 pieces per fish in marine environments and are even higher in freshwater systems (8 pieces per fish) (Wootton *et al.*, 2021).

The current study is one of the first to explore the influence of periodic microplastic fragment consumption, and the plasticizers incorporated into them, on the body condition and fitness of a tropical marine fish. Here, we pulse fed a brooding serial spawning coral reef fish, the spiny chromis *Acanthochromis polyacanthus*, PS microplastic fragments bound with one of two different common plasticizers (DEHP or DEHT) together with controls. Breeding pairs were monitored for their reproductive output, and aspects of their behaviour, body condition, and health were measured at the end of the 5-month experiment. Our predictions were that consumption of a diet with virgin PS would have little influence on the fish due to its inert nature, but consumption of plastic fragments containing plasticizers may alter fish characteristics through their potentially toxic effects. Based on previous research, it was expected that the consumption of plastics that incorporated a DEHP plasticizer may have a greater impact than the less studied DEHT.

## Materials and methods

### Study site and animal husbandry

*Acanthochromis polyacanthus* is a common Indo-Pacific damselfish that deposits a benthic egg mass with no dispersive larval phase. In the wild, females spawn egg clutches within caves, and both adults take turns in protecting the embryos, and later the juveniles, from predators (Figure 1).

The present experiment was conducted in the marine and aquaculture research facilities unit at James Cook University from April 2019 to September 2019, with the first feeding of treatment diets to the fish on 22-April 2019. Reproductive output was monitored daily until 1-September 2019. Adults were collected using barrier nets from shallow reefs around Lizard Island in the northern Great Barrier Reef, Australia (14°41′S, 145°27′E) during November 2016. Fish used in this study averaged 98 mm total length and 53 g wet body weight. Fish were transported to the laboratory and placed in breeding pairs into 200 L opaque cylindrical tanks (60 × 45 cm) on a flow-through water system (at a flow rate of ∼10 L per min per tank), filtered with 100-micron bags, a UV sterilizer, protein skimmer, and biofilter. Tanks were not aerated to minimize noise disturbance. Tanks were maintained at 28.5–29.5°C and a 14:10-h light:dark regime. Tanks contained a half-terracotta pot as a shelter and nesting surface. The occurrence of new egg clutches was recorded for the first 124 d of treatment (see Supplementary Figure S1 for a timeline summary).

All experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 7th Edition, 2004 and in compliance with the Queensland Animal Care and Protection Act, 2001. Animal collection and the experimental procedure were

conducted under an animal ethics approval from James Cook University (A2361, A2408).

## Microplastic composition and dosage

A total of 15% of the two plasticizers by weight was chosen as the plasticizer concentration for logistical reasons (see Supplementary file for details). Greater amounts made grinding of the plastics difficult using the methodology adopted due to their enhanced flexibility. It was difficult to determine the best dosage rate of the plastics to include within food due to the lack of detailed information on the quantities of plastic types within tropical environments and how these differ across microhabitats and through time. Available evidence suggests that microplastic concentrations are highly variable among geographic locations and sites (Harris, 2020; Stanton *et al.*, 2020; Alfaro-Núñez *et al.*, 2021; Wootton *et al.*, 2021), with their position in the water column dependent upon the density of the plastic and any biofouling that may occur (see McCormick *et al.*, 2020 supplementary for a review), together with the environmental conditions that may lead to resuspension from the sediment [e.g. Hitchcock (2020)]. For this reason we decided to mimic a scenario, where some microplastics are present in the environment, but fishes only have access to these periodically, possibly due to resuspension, rarity in the environment, or as a result of being a non-preferred food item.

## Microplastic and diet production

Polystyrene was chosen as the plastic for the study because it is the third most commonly produced polymer and contributes a significant portion of the plastic entering the oceans (Andrady, 2011). With a specific density of 1040–1090 kg $m^{-3}$, it is denser than seawater and so poses a potential threat to benthic and demersal marine organisms. It was also chosen for comparative purposes as the few previous experiments undertaken on marine fishes have used PS (Lu *et al.*, 2016; Jacob *et al.*, 2019; Assas *et al.*, 2020; McCormick *et al.*, 2020). While styrene can migrate from PS into oils, it has not been found to migrate into water (Paraskevopoulou *et al.*, 2012). In the current study, two types of common plasticizers were incorporated with the plastic: di-2-ethylhexyl phthalate (DEHP) and DEHT. DEHP has historically been one of the most commonly used phthalate plasticizers, but has been demonstrated to be an endocrine disruptor through its action as an androgen antagonist (Rowdhwal and Chen, 2018; Czogała *et al.*, 2021). It has been shown to affect sperm motility (Huang *et al.*, 2012), chromatin DNA integrity, and is known to disrupt development (Gray Jr *et al.*, 2000; Lombó and Herráez, 2021). DEHT, on the other hand, is viewed as a non-toxic non-phthalate alternative (Tyler *et al.*, 2018; Den Braver-Sewradj *et al.*, 2020), though few studies have been undertaken to gauge the extent of its potential effects on organisms.

Two millimetre microplastic fragments were created from PS beads that had been dissolved and reconstituted with one of two plasticizers to produce three plastic treatments: (a) virgin PS, (b) PS + DEHT, and (c) PS + DEHP. Gel permeation chromatography determined that the PS contained 14.96 and 14.72% by weight of the respective plasticizers (see Supplementary file for details of plastic production and characterization; Supplementary Figure S2). A Nutribullet$^{TM}$ blender was used to fracture the plastic, which was then sieved down to a 0.89 to 2.9 mm maximum width (mean = 1.89 mm, SD = 0.44; Supplementary Figure S3).

Fish were fed a protein based hatchery diet (INVE NRD G12,1200 μm), previously shown to sustain growth, reproduction, and lead to viable offspring in *A. polyacanthus* (Rodgers *et al.*, 2018). Feed was crushed with a blender, and plastic fragments from each treatment were mixed evenly into one of four equal portions, which were then rebound using 10% gluten (see Supplementary file for diet preparation details). This created four diets, one consisting of the hatchery food with no plastic, while the others contained various plastics to an average concentration of 11.3% by weight (± 6.2 SD, concentrations among plastic treatments, $F_{2,55} = 2.25$, $p = 0.11$). To avoid any diet rejection, fish had been previously weened onto the pelletized control diet for ∼3 months prior to the start of the experiment.

## Diet manipulation

Five to six breeding pairs were randomly assigned to one of the four feeding treatments, and fed twice per day (morning and late afternoon) ($n_{pairs}$ = Control 6, Virgin 6, DEHP 5, and DEHT 5). Fish that received the plastic treatments were fed the appropriate plastic-containing diets in the morning feed on Monday, Wednesday, and Friday, meaning that they received a plastic pulse three out of every 14 feeds, emulating a variable availability of plastic in the environment. For the treatments fed diets with PS, concentrations of plastic fragments averaged 5.38 particles (0.014 g) per fish per feed. Observations during feeding indicated that fish would immediately swim up to the food and bite at it when first added. The food would sink to the bottom, but was consumed within 10 min. There was no indication that fish selectively avoided the plastic fragments or rejected them after consumption. The tanks were cleaned with a syphon twice a week, or more frequently if required.

## Adult characteristics and body condition

A number of measures of adult body condition were measured at the end of the experiment (17th and 19th September 2019; i.e. ∼150 d). These included sex, morphological attributes (length, body weight, liver weight, gonad weight, and spleen weight), and derived body condition indices (carcass weight, relative body mass, gonadosomatic index, hepatosomatic index, spleenosomatic index) (see Table 1 for descriptions). Ratios were not calculated as power functions because there was no evidence of non-linear relationships between the variables and body weight. Variables were not measured at the start of the experiment as stress would delay reproduction. Because breeding pairs were randomly allocated to treatments, any difference in mean values among treatments can be attributed to treatment differences.

Liver cell density and vacuolation were recorded as measures of energy storage, and previous studies of fish that have ingested plastic have found detrimental effects (Rochman *et al.*, 2013). Liver cells store glycogen and lipids within their cytoplasm, and studies have shown that their size and density respond rapidly to variations in energy demands and diet, with lower liver cell density represents higher glycogen stores (Pratchett *et al.*, 2004). Vacuolation may also reflect a degenerative change caused by fluid distension (Wolf and Wheeler, 2018). Livers were preserved in a buffered formalin-acetic acid mixture (McCormick and Molony, 1992), prepared for histology following Green and McCormick (1999), and serially sectioned. Densities of liver cells were counted within randomly

**Table 1.** Summary of the variables measured on each *A. polyacanthus* at the end of the 5-month experiment, or during breeding, where fish received one of four diets that intermittently contained plastic fragments ($n = 22$ pairs, 44 individuals).

| Variable | Description | Mean | SD |
|---|---|---|---|
| Total length (mm) | Length from snout to end of intact caudal fins | 98.33 | 7.59 |
| Body wet weight (g) | Blotted wet weight of intact body | 53.14 | 12.03 |
| Carcass weight (g) | Body weight—(gut weight + gonad weight + fat weight) | 50.02 | 11.11 |
| Gut weight (g) | Weight of complete alimentary canal | 227.04 | 42.80 |
| Liver weight (g) | Weight of liver | 0.85 | 0.42 |
| Spleen weight (g) | Weight of spleen | 0.033 | 0.017 |
| Gonad weight (g) | Weight of ovaries or testes | 0.19 | 0.26 |
| Number of clutches | Total number of egg clutches over reproductive monitoring (124d) | 3.23 | 2.49 |
| Relative weight (g/mm) | Carcass weight/Total length | 0.53 | 0.09 |
| Gonadosomatic index (%) | Gonad weight x 100/Carcass weight | 0.4 | 0.54 |
| Hepatosomatic index (%) | Liver weight x 100/Carcass weight | 1.67 | 0.70 |
| Spleenosomatic index (%) | Spleen weight x 100/Carcass weight | 0.067 | 0.036 |
| Hepatocyte vacuolation | Histological assessment of vacuolation of liver hepatocytes (%) | 75.55 | 18.92 |
| Hepatocyte density | Density of liver hepatocytes | 75.19 | 24.5 |
| Days under treatment (d)[1,2] | Days under treatment prior to first spawning | 37.63 | 20.8 |
| Time in shelter(s)[1] | Time in the shelter of the nesting site while eggs were present out of a 10 min measurement period | 443.6 | 121.8 |
| Time fanning(s)[1] | Time that either parent spent fanning eggs out of a 10 min measurement period | 194.5 | 86.4 |

[1]recorded per breeding pair.
[2]prior to the first egg clutch under treatment.

placed 300 × 300 µm quadrats. The proportion of vacuoles in hepatic tissues was estimated from the proportion of points that intersected vacuoles under a grid of 25 points randomly placed four times (Supplementary Figure S6).

### Brood characterization and adult maintenance behaviour

During the 5-month experimental period, the nest sites of the breeding pairs were checked for egg clutches at the start of each day prior to feeding. When a new clutch of eggs had been laid, it was photographed so the total number of eggs could be determined. Each clutch was re-photographed at day 9 (just prior to hatching), and the difference in the number of eggs was used as a measure of embryo mortality. Photography of the clutch was the same across treatments, and observations suggested that parents rapidly returned to tending their eggs after the disturbance.

Two video cameras (GoPro Hero 3+) were used to assess parental behaviour in the presence of a clutch. One camera was positioned 45 cm above the tank looking down to encompass the entire tank in the field of view, while a second camera was positioned inside the tank looking into the nest. The cameras recorded (at 30 fps) for a minimum of 35 min and the last 10-min section was used to assess parental behaviours. Fish showed minimal interest in the cameras. Videos were deployed 1 h prior to feeding. Video footage allowed two variables to be quantified during the 10-min sampling period: (a) total time spent within the nest site; and (b) total time spent fanning the egg clutch by either parent. Variables were measured using Solomon Coder software.

### Statistical analyses

Morphological and body condition variables were first analysed for differences between treatments and sex with a permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2017) on Mahalanobis distance, including the following variables: carcass weight, relative body weight, gonadosomatic index, hepatosomatic index, spleenosomatic index, density of hepatocytes, and percentage of liver vacuoles (Table 1 for definitions). Canonical discriminant analysis (CDA) was used to display and interpret the differences found. This was followed by univariate, two-factor general linear models to further explore the variables responsible for any difference. The normality and homogeneity of variance of all data were checked using residual analysis and transformed where appropriate for univariate analyses (spleenosomatic index was $\log_{10}$ transformed). Initially, models included a covariate of fish weight, but this covariate did not account for a significant amount of variance in the datasets and so was dropped. Effect sizes are given as partial eta-squared ($\eta^2_p$), which represents an estimate of the proportion of the total variance in a dependent variable that is associated with the membership of different groups (Richardson, 2011).

Parental behaviour while tending embryos (time spent in the nesting cave and spent fanning embryos) and number of eggs produced in the first clutch were compared (separately) among treatments (fixed) with a linear mixed effects model accounting for the identity of the breeding pairs (random) using restricted maximum likelihood. The number of days from the start of the experiment until the first clutch, and between the first and second egg clutches were compared among treatments using a one-factor ANOVA. The number of egg clutches was also compared among treatments using a one-factor ANOVA, though the variable was $\log_{10}(x + 1)$ transformed to improve homogeneity of variance.

Egg mortality was compared among treatments with a linear mixed effects model with clutch identity (clutch 1 or 2) as a random factor.

## Results

### Morphological and body condition variables

Overall, there was no difference in the morphology or body condition measures of fish among plastic treatments, but there was an effect of sex (PERMANOVA: treatment $F_{3,36} = 0.500$,

**Table 2.** Summary of univariate analyses testing for effects of plastic treatment by sex on the morphology or body condition variables of *A. polyacanthus* adults.

| Variable | Sex | Treatment MS (df 3) | Error MS (df 17 female, 19 male) | F | $p$ | Effect size ($\eta^2_p$) |
|---|---|---|---|---|---|---|
| Total length | Female | 19.7 | 50.7 | 0.39 | 0.76 | 0.06 |
| Carcass weight | Female | 21.89 | 125.2 | 0.17 | 0.91 | 0.03 |
| Relative body weight | Female | 0.00 046 | 0.0077 | 0.059 | 0.98 | 0.01 |
| Gonadosomatic index* | Female | 0.08 | 0.059 | 1.37 | 0.29 | 0.19 |
| Hepatosomatic index | Female | 0.032 | 0.27 | 0.12 | 0.95 | 0.02 |
| Spleenosomatic index* | Female | 0.027 | 0.038 | 0.72 | 0.55 | 0.11 |
| Hepatocyte density | Female | 180.7 | 423.1 | 0.43 | 0.74 | 0.07 |
| Vacuolation (%) | Female | 206.8 | 729.4 | 0.28 | 0.84 | 0.05 |
| Total length | Male | 37.6 | 72.3 | 0.52 | 0.67 | 0.06 |
| Carcass weight | Male | 91.06 | 144.2 | 0.63 | 0.60 | 0.09 |
| Relative body weight | Male | 0.0046 | 0.0088 | 0.53 | 0.67 | 0.08 |
| Gonadosomatic index* | Male | 0.0022 | 0.001 | 2.34 | 0.11 | 0.27 |
| Hepatosomatic index* | Male | 0.0051 | 0.038 | 0.13 | 0.94 | 0.02 |
| Spleenosomatic index* | Male | 0.0031 | 0.045 | 0.07 | 0.98 | 0.01 |
| Hepatocyte density | Male | 69.3 | 597.7 | 0.12 | 0.95 | 0.02 |
| Vacuolation (%) | Male | 178.6 | 361.9 | 0.49 | 0.69 | 0.07 |

*log10 transformed.
Effect size is given as a partial eta-squared.

$p = 0.968$; sex, $F_{1,36} = 2.630$, $p = 0.001$). There was also no interaction between factors ($F_{3,36} = -0.371$, $p = 0.590$). A CDA bi-plot suggests that males are heavier than females (Supplementary Figure S7). Univariate analyses of variables found neither males nor females were affected by plastic treatment (Table 2), with gonadosomatic index having the highest plastic treatment effect sizes of 0.19 (female) and 0.27 (male). There was no effect of treatment on any of the body condition variables, and effect sizes were low (Table 2).

### Clutch maintenance behaviour

There was no difference among treatments in the amount of time fish spent within the shelter containing the eggs ($F_{3,14} = 2.91$, $p = 0.07$), though there was a trend for parents from the DEHP treatment to spend a lower time in the shelter with the egg clutches (Figure 2). There was also no difference among treatments in the parental fanning of the embryos ($F_{3,14} = 1.65$, $p = 0.22$).

### Number of clutches

Seventeen breeding pairs produced 71 egg clutches over the 133-d sampling window during which reproduction was recorded, with an overall average of 3.2 clutches per pair. Number of clutches laid ranged between 0 and eight clutches per breeding pair, with fish from the Control treatment being slightly above average (4.3 clutches) and DEHP being slightly below average (1.8 clutches). There was no difference among treatments in the number of clutches produced over the experiment ($F_{3,13} = 0.315$, $p = 0.814$, $\eta^2_p = 0.07$; Supplementary Figure S8).

### Clutch size

There was no difference among treatments in the number of clutches produced ($F_{3,18} = 0.99$, $p = 0.42$, $\eta^2_p = 0.14$; Figure 3). A mean of 370 eggs per clutch (SD 88.5) were produced over the first two clutches. There was no difference in the number of eggs in the first clutch among the plastic treatments ($F_{3,12} = 0.21$, $p = 0.89$).

### Days between clutches

The average number of days from the start of treatment until the first egg clutch was the same as between the first and second clutches at 24 d. There was no difference among treatments in the number of days between the first and second egg clutches ($F_{3,13} = 0.157$, $p = 0.92$, $\eta^2_p = 0.03$).

### Embryo mortality

Mean mortality varied substantially among treatments and between clutches. There was no difference in embryo mortality among plastic treatments ($F_{3,12} = 0.767$, $p = 0.53$), with mortality ranging from 3.5 to 100% over the first 9 d of embryogenesis. The DEHP treatment tended to have the highest average mortality (Supplementary Figure S9), though this was not significant.

## Discussion

The ubiquity of plastics in all habitats around the globe and its visually obvious nature have led to the recent focus on plastics as one of the key sources of environmental contamination (Barrett *et al.*, 2020). Our study is one of the first to examine the effects of the periodic consumption of plastic fragments by fishes, and the effects of the plasticizers that were incorporated into those plastics. Fish were fed PS fragments (with or without DEHP or DEHT) sporadically (3 out of 14 feeds) to represent a scenario where plastic fragments are relatively rare with respect to availability in their environment, which appears to be the general case in the tropics (Abayomi *et al.*, 2017; Wang *et al.*, 2020). We found no evidence of detectable detrimental effects of plastic consumption on the morphology, liver stores, reproductive behaviour, or reproductive output of an adult damselfish, despite the fish being intermittently exposed to the plastics for 5 month. This is a positive finding, but further studies are required to determine whether there are more subtle physiological effects of plastic consumption, such as oxidative stress or toxin deposition (Kim *et al.*, 2021), that may accumulate to produce future detrimental effects or affect the quality of their offspring.

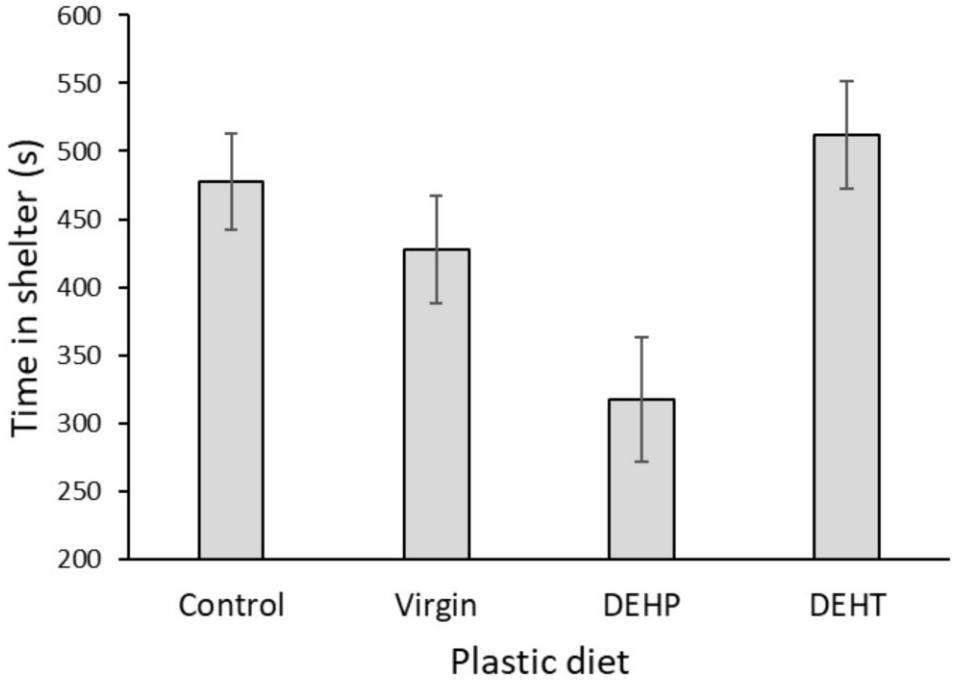


**Figure 2.** Time spent in a shelter with an egg clutch for *A. polyacanthus* sporadically fed a diet containing PS plastic fragments with or without one of two plasticizers (DEHP or DEHT). N = 10, 8, 6, and 8 video recordings of different clutches (left to right).

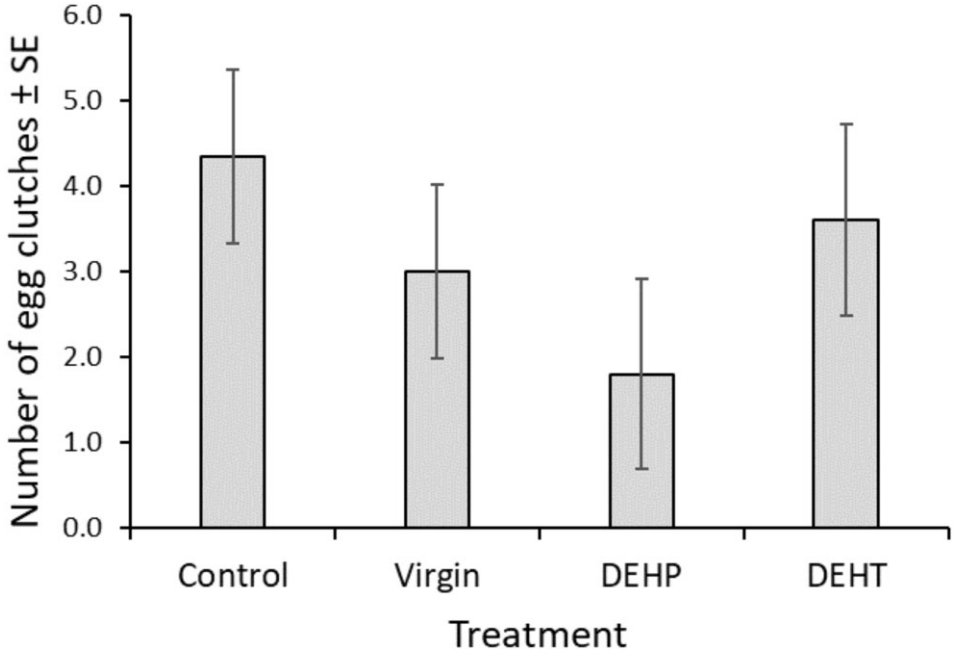


**Figure 3.** Number of egg clutches laid over 133 d by adult *A. polyacanthus* sporadically fed a diet containing PS plastic fragments with or without one of two plasticizers (DEHP or DEHT). N = 6, 6, 5, and 5 breeding pairs (left to right).

Our study contrasts with others that have examined the effect of common plasticizers on aquatic organisms, which have found strong detrimental effects of phthalate plasticizers (Rowdhwal and Chen, 2018), leading to calls for less toxic alternatives (Katsikantami *et al.*, 2016). The current study is one of the few studies to examine the morphological and behavioural influences of plasticizers when the compounds are incorporated within microplastics, rather than as the original chemical dissolved in a water source. Most other studies have used high concentrations of aqueous plasticizers *in vitro* or in live fish assays where the chemicals are directly absorbed through tissues of embryos or larval fish (such as the gills and epidermis) directly exposed to the water (Zanotelli *et al.*, 2010; Yuen *et al.*, 2020; Cui *et al.*, 2021; Jia *et al.*, 2021). These early larval stages are particularly vulnerable due to their large surface area/volume ratios and unarmored skin (usually scales are poorly developed in these early developmental stages). Here we found that neither the phthalate (DEHP) nor the

non-phthalate plasticizer (DEHT) treatment had a detectable detrimental effect on the adult stages of our target fish for the variables measured.

The amount of the plasticizer that is available for release during digestion will depend on the type of plastic, the way the plasticizer is incorporated (bonded or in vesicles), temperature, degree of weathering, or anything that alters the surface area of the plastic fragments or the exposure of new plasticizer, such as mechanical abrasion (Liu *et al.*, 2020) or biofilm accumulation (Zettler *et al.*, 2013). In the present experiment, pilot studies suggested that the amount of plasticizer leached from the PS fragments with ∼15% plasticizer was very low, which is not unexpected at the temperatures used in the supplementary leaching experiment (37°C), or in the main experiment (28°C) (Hahladakis *et al.*, 2018; Wei *et al.*, 2019). Coffin *et al.* (2019) found negligible amounts of DEHP leached from PS foam fragments under agitated control (seawater) or simulated fish digestion, despite being present in high quantities. The expectation is that plasticizers will diffuse to the surface of the fragments to form a hydrophobic film (Shashoua, 2003), which is relatively stable in water. Any processing during capture or ingestion, such as mastication or grinding by a pharyngeal jaw [a characteristic of many fish groups, including marine wrasse and freshwater cichlids; Burress *et al.* (2019), Evans *et al.* (2019)] is likely to accelerate the migration of plasticizers from the plastic matrix during digestion. The fish species used in the current study is an omnivorous damselfish that does not secondarily process food particles, with small particles being swallowed whole for digestion. It may be that the lack of secondary processing of plastics and relatively fast gut throughput rates (∼4.6 h) (Marnane and Bellwood, 1997) means that exposure to the potentially toxic effects of plasticizers would be minimal. The effect of consuming plastics that incorporate plasticizers (i.e. most plastics) may therefore be dependent in part on the feeding biology of the species and be species- and life-stage specific. It is worthwhile noting that phthalate, such as DEHP, can occur freely in the water column in polluted areas (Fatoki *et al.*, 2010; Gugliandolo *et al.*, 2020), and these may potentially represent more bioavailable forms of toxins than those bound in degrading plastic (Salvaggio *et al.*, 2019).

Our study found that plastic consumption, regardless of whether plasticizers were added, had no detectable effect on reproductive behaviour or output. Until recently, there have been very few studies that experimentally examined the impact of microplastic consumption on adult fishes. For instance, a meta-analysis by Foley and colleagues found no experimental studies that have looked at the effects of microplastics on fish reproduction (Foley *et al.*, 2018). Most previous studies that have found detrimental effects of microplastic ingestion on reproduction have been on invertebrates (see Anbumani and Kakkar, 2018). Studies undertaken more recently on fishes have tended to continually expose fish to very high levels of very small PS beads, with their findings being variable between studies, but loosely dependent on bead concentration and exposure duration. For instance, Assas *et al.* (2020) exposed Japanese medaka (*Oryzias latipes*) for 3 weeks to a very high level of small PS beads (2 μm; 100 million beads per L), together with a no-bead control, and found no effect on fish growth, egg output, or juvenile survival. Likewise, Marana *et al.* (2022) found no influence of high levels (i.e. many trillions/L) of 0.5 μm PS beads for 110 d on reproductive output in zebrafish (*Danio rerio*). Other studies using continuous, prolonged exposure to PS beads also found a negligible effect on reproductive output, with no differences in egg production or survival (Anubumani and Kakkar 2018, Foley *et al.*, 2018).

Two recent studies have found some effects of PS bead consumption on the reproductive function of fishes, but these have used very high loadings of small beads. Wang *et al.* (2021) continuously exposed marine medaka (*Oryzia melastigma*) embryos through to adults to 2 μm PS beads at very high levels ( 352,000–34 million particles/L) and found microplastic exposure decreased the weight and gonadosomatic index of adults, but accelerated the sexual maturity of females. Microplastic exposure damaged the gonads, decreased egg production and fertilization rates, with steroid hormone biosynthesis being affected. Qiang and Cheng (2021) fed 1 μm PS beads to adult zebrafish (*D. rerio*) for 21 d, finding no affects at 18.2 billion beads/L, but impacts on testes histology, though not on ovaries at concentrations of 1.82 trillion beads/L. Given the particle sizes used in these experiments are well below the particle sizes that would be the targets for foraging, it is unclear what ramifications these findings have for fish living in the wild.

Not only was there no impact of plastic ingestion on gross morphological measures of fish body condition, but our study found no detectable effect on the liver, which plays a key role in detoxification processes, energy metabolism, and storage. The amount of energy products stored within the liver, as indicated by hepatocyte density and vacuolation, was also not affected by plastic ingestion. Other studies have found similarly negligible effects on the liver, except at extremely high plastic loads. For instance, Lu *et al.* (2016) found high doses (11 trillion/L, 29 million/L) of small PS beads (70 nm, 5 μm) caused lesions and lipid accumulation in liver of adult zebrafish (*D. rerio*). Abarghouei *et al.* (2021) found that liver lesions developed in adult goldfish (*Carassius auratus*) after 168 h exposure to even their lowest PS treatment of ∼0.7 million 8 μm beads/L. Jacob *et al.* (2021) found seabream (*Sparus aurata*) exposed to high concentrations of small beads (i.e. ∼48,000 10–20 μm PE spheres per day) through their food (*Artemia*) showed higher rates of hepatocyte vacuolation and lower densities of hepatocytes than controls. Research suggests that at high concentrations, these small beads are able to enter the blood through the gills and intestine and then reach the liver (Ma *et al.*, 2021) where they can have detrimentally affect the liver when at concentrations that are well above present environmental concentrations. Lastly, Ašmonaitė *et al.* (2018) fed PS fragments in diets at high concentrations (500–2411 particles/fish/day) to rainbow trout (*Oncorhynchus mykiss*) and found no impact on the hepatic stress markers. Our study supports existing literature, which suggests that there are few detrimental effects on the liver when PS plastics occur at potentially more environmentally relevant levels, let alone the much lower levels that are likely to be ingested by foraging fishes.

The current experiment used newly made plastics so that the levels of plasticizer could be controlled. Other potentially detrimental aspects of the ingestion of naturally occurring microplastic have not been incorporated, including the concentration of hydrophobic contaminants that may sorb to plastic more easily than natural sediments and facilitate the transfer of these toxicants to organisms (Wardrop *et al.*, 2016). The high potential throughput of plastics through the gastrointestinal tract of a fish may mean that toxic effects from these secondary-sorbed chemicals may accumulate with time.

As noted by Jovanović (2017), the total load of micro- and nanoplastics that will pass through the gastrointestinal tract of a fish in its lifetime is likely high in some locations and will keep increasing in the future. Our study has found no effect of periodically ingesting plastic fragments for 5 month. However, recent studies have suggested that there are likely to be sublethal physiological impacts, such as oxidative stress (Coffin *et al.*, 2020), that will disrupt energy allocation and may alter the energy available for reproduction or make fish more susceptible to other environmental challenges, such as environmental warming.

To date, most studies that have examined the influence of plastic ingestion have examined the effects of constant exposure to very small plastic spheres (i.e. <5 μm) at environmentally unrepresentative concentrations (i.e. millions to trillions/L). These particles enter the body during gill ventilation or as a bycatch during feeding, and their small size precludes them being primary targets of foraging. Despite the high concentrations of particles used by most studies, there is a surprising correspondence between our findings of no effect of plastic ingestion on body condition or reproduction and previous studies for all but those treatments using the highest plastic densities. While our findings are positive for the survival of fishes in an ocean increasingly polluted by plastic, they should be used cautiously as they do not account for the cocktail of pollutants that sorb to the surface of plastics in polluted waters (Ziccardi *et al.*, 2016). They are, however, likely to be representative of the effects that occur from the ingestion of other types of plastics that show similar plasticizer leaching characteristics.

## Conclusions

To date, most studies have examined the effects on fishes of constant exposure to very small plastic spheres at environmental unrepresentative concentrations. While this was pointed out in 2016, it appears that it is an ongoing feature of the research exploring the biological effects of micro- and nanoplastic exposure (Lenz *et al.*, 2016; Bucci *et al.*, 2020). These particles enter the body during gill ventilation or as a bycatch during feeding, and their small size precludes them being primary targets of foraging. In our five-month laboratory study, we simulated a scenario where some microplastic fragments were present in the environment, but fishes only had access to these periodically, possibly due to resuspension, rarity in the environment, or as a result of being a non-preferred food item. This sporadic plastic consumption over 150 d was found to have no impact on any of the body condition indices measured, reproductive output or egg survival. Interestingly, despite the high concentrations of particles used by most studies, there is a surprising correspondence between our findings of no effect of plastic ingestion on body condition or reproduction and previous studies for all but those treatments using the highest plastic densities. While our findings are positive for the survival of fishes in an ocean increasingly polluted by plastic, they should be used cautiously as they do not account for the cocktail of pollutants that sorb to the surface of plastics in polluted waters (Ziccardi *et al.*, 2016). They are, however, likely to be representative of the effects that occur from the ingestion of other types of plastics that show similar plasticizer leaching characteristics. Clearly, further studies are warranted that examine the long-term effects on fishes of realistic levels of environmentally sourced plastics of an ecologically appropriate size.

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## Supplementary Data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

## Competing interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Mark McCormick reports financial support was provided by Australian Research Council Centre of Excellence for Coral Reef Studies. Bridie Allan reports a relationship with Ian Potter Foundation that includes: funding grants.

## Authors contribution statement

M.I.M., B.J.M.A., and E.P.F. conceived and designed the study. K.P. and G.V. created the plastics fragments and assessed their structure and chemical composition. E.P.F. and J.E. prepared the fish diets. E.P.F., C.M., E.S., S.B., J.E., P.G., and L.V. looked after adult fish, checked embryos, and photographed clutches. M.I.M., E.P.F., C.M., E.S., and L.V. processed the fish at the end of the experiment. M.I.M., E.P.F., J.E., E.S., E.S., and S.B. analysed the data. M.I.M. wrote the first draft of the manuscript and all authors contributed substantially to the final version.

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## Data availability statement

Data is available on FigShare: https://doi.org/10.6084/m9.figshare.19930220.v1.

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