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Supplementary file

PART 1. Ethical considerations

Transportation of juvenile fish from light traps to the Lizard Island research station aquarium system occurred in 60-L containers with lids to reduce stress by subduing the ambient light conditions and took 10-20 min. The station aquarium system pumps water straight from the lagoon and is a similar chemistry and temperature to ambient seawater. If the journey from the light traps to the laboratory was greater than ~10 min then seawater was carefully exchanged using a bucket to make sure that oxygen and water temperature were maintained at ambient seawater conditions. Target fishes were carefully removed from the catch by means of hand nets and the bycatch was returned to the reef after being temporarily maintained (< 4 h) in a flow-through seawater system. Focal fish were tagged to identify experimental fish in the field following the protocol detailed in Hoey and McCormick [1]. Briefly, this involved placing fish in a plastic bag of aerated seawater such that the fish could be held still using the bag but still ventilate their gills. This allowed a fluorescent elastomer tattoo to be delivered under the scales using a 27-gauge hypodermic needle, leaving a 1.5-2 mm long stripe of (red) colour. Tagging of this species and developmental stage of damselfish using this method has been shown to not affect growth or mortality [1]

PART 2. Rationale for microplastic concentrations

Obtaining reliable data on the availability of microplastics to animals is very difficult because it not only requires good estimates of the concentrations of microplastics in the different habitats where the target animals may be present, but also requires information on the likelihood of the animal feeding on the particles when available. Currently, estimates of concentrations typically come from surface water or sediment samples. Very few estimates of microplastic concentrations in the waters around tropical coral reefs are available and surprisingly few are available for inshore coastal areas near urban centres of the tropics [2, 3]. Wang et al. [4] used a manta tow plankton net to sample 18 remote open ocean stations across the tropical mid-west Pacific and found very low quantities of microplastics in surface waters (6,028 to 95,335 particles/km^2;

equivalent to ~ 0.015 x 10^{-3} to 0.24 x 10^{-3} particles/L). Abayomi et al. [5] found higher surface microplastic particle concentrations of 4.38 x 10^{4} to 1.46x 10^{6} p/km^2 (equivalent to 0.1095 x 10^{-3} to 2.92 x 10^{-3} p/L) in coastal waters of the Arabian Gulf, while sediment samples from beaches revealed concentrations of 1,800 to 11,400 p/m^3. Surface water samples from in and outside Kingston harbour, Jamaica revealed higher concentrations of up to 2.7 x 10^{6} p/km^2 (equivalent to 5.73 x 10^{-3} p/L). Manalu et al. [6] found extremely high concentrations of microplastics in the coastal sediments of Jakarta Bay, off Indonesia's national capital (range 18,405 to 38,790 p/kg dry sediment). Reisser et al. [7] undertook surface net tows at 4 sampling stations within the Great Barrier Reef, Australia and found plastic concentrations of 956 to 13,518 p/km^2 (~5.6x10^{-3} to 0.79 / L) (though only floating plastic particles were counted in this study).

More research has been undertaken in non-tropical coastal areas and the microplastic concentrations found tend to be much higher. We believe that this indicative of the lack of sampling around tropical urban areas and waterways rather than necessarily a difference with latitude. For instance, 38,450 and 126,200 /m^3 were the upper ranges of particle concentrations in the sediment of two urban estuaries in South Carolina, USA, with 11 and 88 particles/L in the water column [8]. Sampling of water from the Pearl River and River estuary in China obtained upper ranges of 7.8 and 53.3 p/L respectively [9]. Size analyses showed that 80% of the particles are less than 0.5mm in size in the latter study. Interestingly, a recent paper for an Australian Estuary using towed surface nets found moderate concentrations of water column microplastic particles (0.4 p/ L) in general, but these concentrations increased 43-times after a storm event (17.4 p/L) [10]. It is suggested that such dramatic increases stem from two components: new particles being washed into the system, and the resuspension of existing particles from the sediment. Resuspension of materials within sediments is a common feature on coral reefs [11, 12], and will be of particular importance to dense types of plastic such as polystyrene, which at a specific density of 1.04 g/cm^3 is negatively buoyant [13]. This resuspension of microplastics within the upper layers of the sediment will be particularly important to tropical organisms associated with coral reefs that feed at or close to the bottom. Furthermore, high rainfall and storm events are typical of the tropical regions and research shows that these events can lead to the transport of the discharge from estuaries >50 km offshore and >100 km north on the Great Barrier Reef (GBR) [14] and at least 20km offshore in typical summer conditions [15]. About

65% of the almost 3000 reefs of the GBR are within 50km of the coast, which means that most receive regular inputs of suspended material, some of which will be microplastics.

Currently, what we can say of the concentrations available to fishes in tropical waters is that they are likely to be spatially variable and will pose an increasing threat in the future as the amount of microplastics within the environment increases. Concentrations around tropical South-East Asia are likely to be high given the prevalence of visible plastic in their coastal zone and waterways [16, 17], and the very high concentrations in beach sediment recorded off Jakarta Indonesia by Manalu et al. [6]. Our current study was conducted at Lizard Island (14° 40' S, 145° 28' E), on the northern GBR, Australia. This study site is ideal as it is in a remote part of the GBR with no urbanisation inshore. This means that it is likely that neither the parents of the offspring studied nor the offspring have a history of microplastic consumption. This reduces the confounding influence of exposure history on our results. Given the importance that the parental transfer of pollutant effects can have to offspring characteristics (e.g., [18]) the remote nature of the study site in the present study can be seen as advantageous. To date, no information is available on the concentrations of microplastic particles in specific microhabitats foraged by the focal fish used for the study. Other studies have found that fish can preferentially select and consume microplastics within their environment [19], suggesting that even if microplastics are in relatively low concentrations in the environment, they may be preferentially consumed. The experimental scenario used in the present study may be analogous to the microplastic levels found near inshore fringing reefs and inshore reefs near urban areas within the tropical Indo-Pacific. However, given the current uncertainty of the availability of microplastics to specific organisms in nature, caution must be exercised in the interpretation of the results of our experiments and what they mean for fishes on reefs.

Microbeads of 200-300 μm were used in the current study for a number of reasons. These beads are of a similar size to a newly hatch brine shrimp (*Artemia* spp.), which was also to be used in the experiment. This size also represents the mean size of beads used in many commercial face cleansers (60 - 800 μm, mean 264 μm) [20].

PART 3. Chemical analysis of polystyrene beads

Methods

Polystyrene microbeads (Polysciences, 200-300 μm), Polystyrene (Sigma Aldrich, average molecular weight = 192K Daltons) and deuterated water (D_2O) (Sigma Aldrich, 99.9 atom%) were used as received. Thermal Gravimetric Analysis (TGA) was performed using a TA Instruments SDT 650 instrument at a heating rate of 10 ºC/min up to 500 ºC under constant flow of nitrogen (50 mL/min). The initial weight of the samples was between 1.88 and 3.98 mg. Infrared spectra of the neat samples were recorded using a Fourier Transform – Infrared Spectrometer (FT-IR) equipped with a SMART iTR attenuated total reflectance attachment. Proton Nuclear Magnetic Resonance (^{1}H-NMR) of the samples were recorded as solutes in D_2O at 298 K on a Bruker 400 MHz NMR spectrometer using standard Bruker pulse sequences. In a glass reaction vile, 1.5 mL of D_2O and ~15 microsphere particles were added and left for 24 hours under ambient conditions. A ^{1}H-NMR spectrum of the D_2O leachate after exposure to the microsphere particles was obtained, and compared to a pure D_2O spectrum to examine the leachate.

Results

Figure S1 compares the TGA analysis of the microspheres with pure polystyrene. Both thermograms revealed a single thermal decomposition at 384 ºC, indicating a good match. A small decrease just above 100 ºC in the spheres was attributed to loss of moisture. The FT-IR analysis of the microspheres (Figure S2) were compared with commercially available pure polystyrene. The essentially perfect match of the commercially available polystyrene and the microspheres indicate that the microspheres are pure polystyrene. Figure S3 compares the proton nuclear magnetic resonance (NMR) spectra of pure deuterated water (D_2O) and the D_2O leachate after exposure of the microspheres for 24 hours. The NMR spectra show no evidence of leaching as both before and after spectra match perfectly.

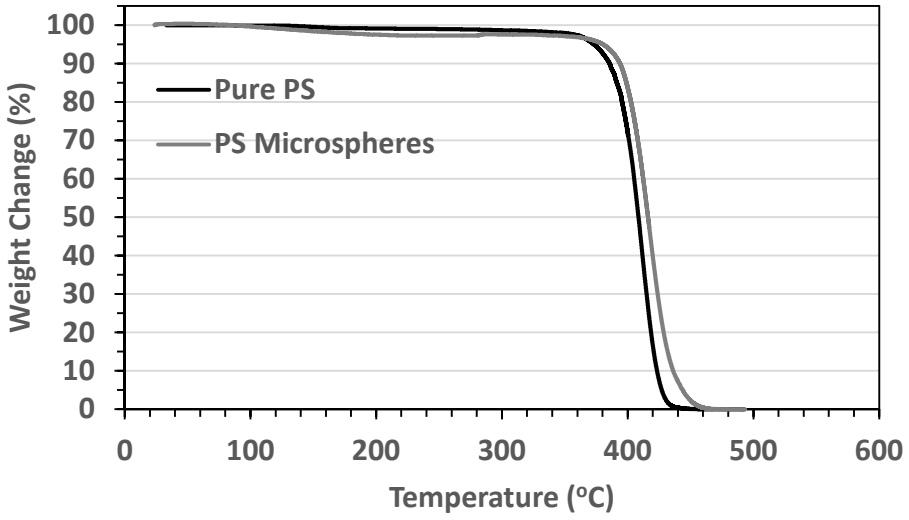

Figure S1. Thermal gravimetric analyses of pure polystyrene (solid black) and the polystyrene microspheres (solid grey).

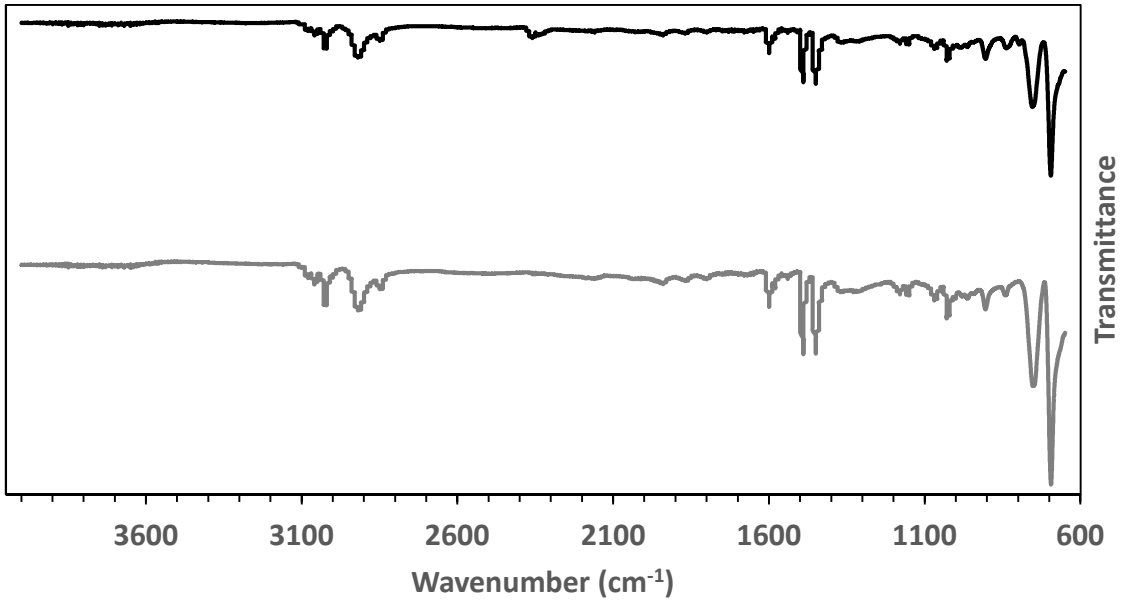

Figure S2. The infrared spectra of pure polystyrene (solid black) and the polystyrene microspheres (solid grey).

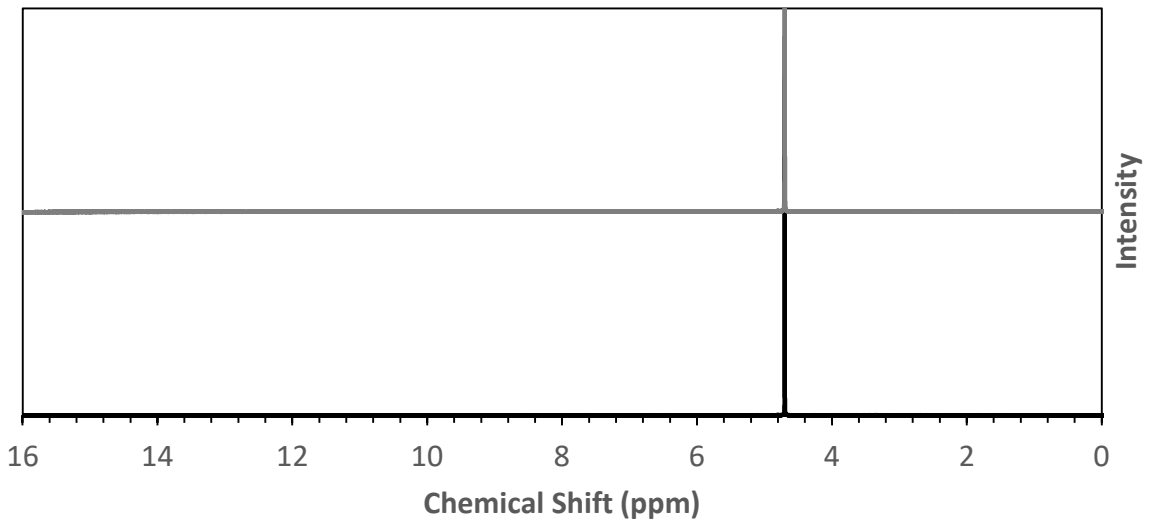

Figure S3. The proton nuclear magnetic resonance spectra of pure deuterated water (D_2O) and the D_2O leachate after exposure to polystyrene microspheres for 24 hours.

PART 4. Ingestion trials

To examine the variability in the consumption of microplastic spheres by *P. amboinensis*, light trap caught fish were starved for a minimum of 6 h, placed into 0.8 L glass beakers in groups of 4 fish and left to acclimate for 1 h. After the acclimation period 40 polystyrene spheres were added (i.e., 50 p/L). An airstone kept the seawater well oxygenated and plastic particles in suspension. Fish were then left undisturbed for 1 h, after which they were immediately euthanized via cold shock. The fish were maintained on ice until dissection, which took place within 12 h of death. The fish were placed under a dissecting microscope (Zeiss Discovery V.8) and their digestive tracks were dissected under 2.5x magnification. Spheres retrieved from the guts were counted by two observers and photographed with an AxioCam ERc5s (Zen Blue Edition 2011 imaging software).

All but 9 out of 60 fish were found to have consumed plastic spheres (85%), with ingestion rates ranging between 1 and 33 particles (Fig. S4) with a mean of 4.5 spheres per fish (median of 1). The distribution was markedly right skewed with 60% of fish eating 3 or fewer spheres (Fig. S4).

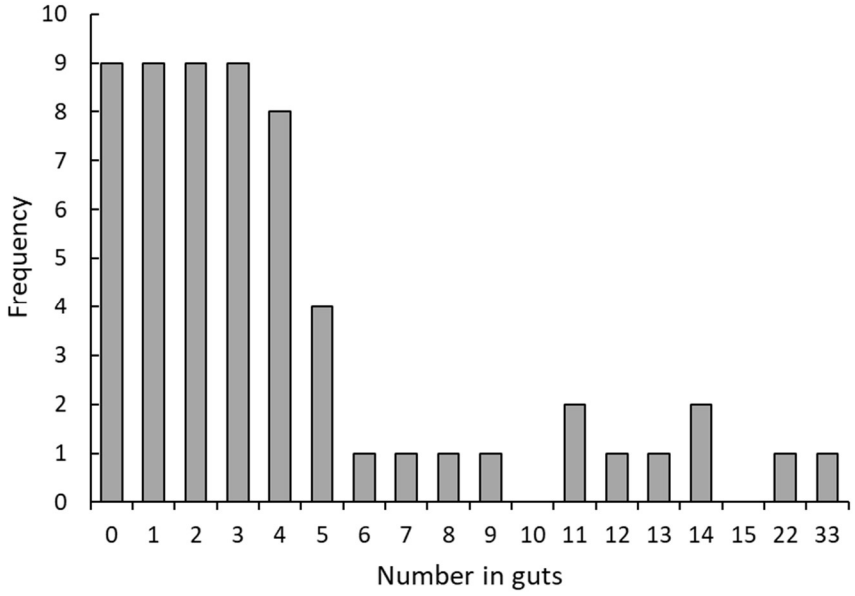

Figure S4. Consumption of polystyrene spheres (200-300 µm) by juvenile *Pomacentrus amboinensis* after a 4 h exposure to 50 particles per litre (n = 60 fish).

PART 5. Egestion trials

To quantify the gut passage time of polystyrene spheres, fish were exposed to a high concentration of plastic spheres and then serially sampled to quantify egestion. Light trap caught *Pomacentrus chrysurus* were used for this study as no *P. amboinensis* were available. These congenerics have similar planktonic life-styles as juveniles, so data on microplastics egestion in *P. chrysurus* should inform us on the likely egestion characteristics of *P. amboinensis*. In this experiment, 60 *P. chrysurus* were placed into a 2 L tank of aerated flow-through seawater. After a 1 hour acclimation period, the fish were exposed to ~3000 polystyrene spheres (200-300 µm) for 1 hour with mild aeration, but without flow-through water. The high concentration of beads was used to simply give all fish the opportunity to feed on microplastics so that we could best quantify their gut through-put rates. After this period, fish were removed and transferred to a clean tank with flow-through seawater. Ten individuals were then removed and euthanized by cold shock at 0 h, 6 h, 8 h, 10 h, 12 h and 14 h. Fish were dissected as above and the number of spheres within the digestive tract was counted.

In keeping with Fig. S4, ingestion of polystyrene spheres (time zero) was highly variable, ranging from none to 51 particles. Serial sampling indicates that *P. chrysurus* can pass the particles through their alimentary tracts, taking up to 14 h to achieve egestion of all particles (Fig. S5).

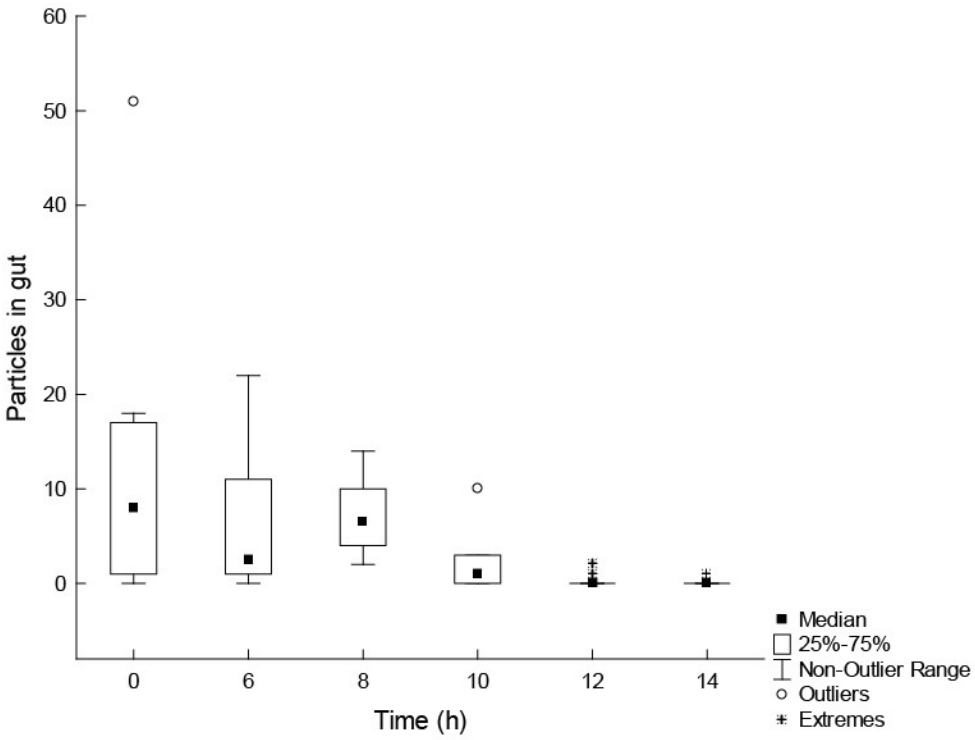

Figure S5. Egestion of polystyrene spheres (200-300 μm) by juvenile *Pomacentrus chrysurus*. Fish were exposed to 3000 particles for 1 hour, then transferred to clean seawater and groups of 10 fish were randomly sampled over a 14 h period.

PART 6. Boldness behavioural assessment

The boldness score follow methodology used in previous studies on small fishes [21, 22] and was categorized as: 0 if the fish was positioned within a small hole and seldom emerged; 1 if it retreated to a hole when approached by the pencil tip and took more than 5 sec to re-emerge,

with weak or tentative strikes at food; 2 if fish shied to shelter of the patch when approached by the pencil tip, but emerged quickly and purposefully struck at food; and 3 if the fish did not retreat to shelter when approached, but rather explored around the coral patch, and struck aggressively at food [22]. Previous research showed that this boldness measure is repeatable across different time scales (e.g., repeatability values of ~ 0.5 over a 2 h period;[23, 24]). Three-minute behavioural assessments have previously been found to be sufficiently long to obtain a representative estimate of an individual's behaviour [24, 25]. Video cameras could not be used to create a reliable record of these assessments because fish move around their topographically complex habitat patches.

PART 7. GLM results on the principle component.

Table S1. Comparison of the effects of pre-exposure to polystyrene spheres and the habitat on which they were placed (live or dead coral patch) on the behaviour of juvenile *Pomacentrus amboinensis*. Pre-exposure of fish to plastics required fish to be placed in tanks in groups, yielding a nested tank term. Behaviour was analysed as a composite variable, represented as PC1 of a principal component analysis on bite rate, total distance moved in 3 min, maximum distance ventured from shelter and a boldness index (see text). The effect sizes are partial eta squares.

Source of variation	df	MS	F	p	Effect size
Plastic treatment	1	36.08	27.11	<0.0001	0.61
Habitat	1	5.67	11.17	0.004	0.41
Plastic * Habitat	1	6.82	13.42	0.002	0.46
Tank(Plastic)	1	1.26	2.78	0.03	0.80
Habitat x Tank(Plastic)	19	0.47	0.83	0.67	0.14
Error	93	0.57			

PART 8. Trends in behavioural measures

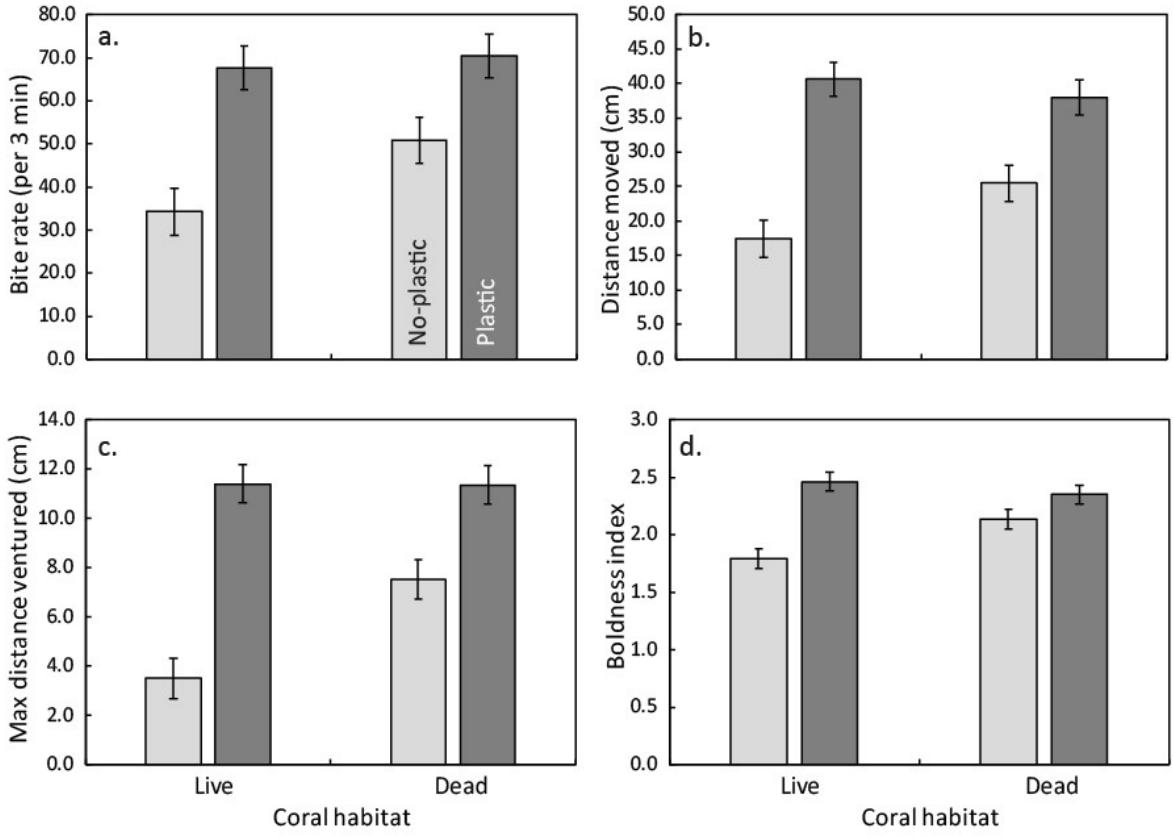

Figure S6. Mean (a) number of bites, (b) distance travelled (in cm), (c) maximum distance ventured from shelter (in cm), and (d) boldness (continuous scale from 0 to 3, see text) for juvenile *Pomacentrus amboinensis* observed during a 3-min period. Fish were maintained for 4 days in glass tanks of aerated seawater and fed *Artemia* only (light grey bars) or *Artemia* and microplastics (dark grey bars), and subsequently released in the field and placed in live or dead-degraded coral habitat. Errors are standard errors. N (left to right) = 28, 32, 32, 31.

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