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Microplastic consumption elevates fish oxidative stress but does not affect predator-driven mortality

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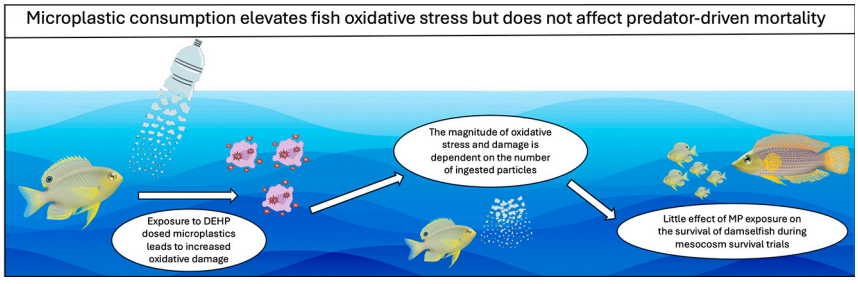
HIGHLIGHTS

- Oxidative damage occurs after microplastic (MP) exposure.
- DEHP dosed MPs yielded the greatest levels of oxidative stress and damage.
- The magnitude of oxidative damage is dependent on the number of ingested particles.
- Little effect of MP exposure on the survival of damselfish during survival trials.
- This research provides a significant advance to predict the impact of MP on fish.

GRAPHICAL ABSTRACT



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ABSTRACT

The ubiquity and abundance of plastic debris is one of the most significant challenges facing marine environments. Recent research has demonstrated that microplastics are consumed by marine organisms from a wide variety of trophic levels. However, little is known about the sub-lethal effects of microplastic exposure on the health of coral reef fishes or their impacts on predator-prey interactions. To examine this, we exposed juvenile Ambon damselfish (*Pomacentrus amboinensis*) to one of two types of polystyrene plastic particles (virgin, or with the plasticizer di(2-ethylhexyl) phthalate - DEHP), and a control (no plastic). After 2 days (6 exposures), the initiation of antioxidant metabolism and oxidative defence was quantified. We also exposed the treated juvenile fish to a piscivorous fish (*Pseudochromis fuscus*) in mesocosms over a 22-h period to investigate whether microplastic exposure affected prey survival. Biomarkers associated with oxidative damage and antioxidant metabolism indicated that microplastic exposure had a negative effect on the health of *P. amboinensis*. Additionally, *P. amboinensis* exposed to DEHP microplastics showed the greatest levels of oxidative stress and damage, however the magnitude of this was dependent on the number of ingested particles. Interestingly, survival of *P. amboinensis* did not differ among plastic treatments during mesocosm survival trials. These results highlight

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that while studies may not find immediate lethal consequences to plastic ingestion, there may be more subtle sub-lethal costs that may have ecologically important consequences at later life stages, through energetic impacts on growth and energy allocation.

1. Introduction

The magnitude and extent of plastic pollution in marine environments underscores the need to investigate the effects of microplastic exposure on ecosystem function (Barnes et al., 2009; Jambeck et al., 2015). Without improvement of waste management systems, plastic discharged into the marine environment is predicted to increase by an order of magnitude within the decade (Jambeck et al., 2015; Meijer et al., 2021). While weathering and fragmentation of plastic waste has resulted in a global distribution of microplastics (Gove et al., 2019; Oberbeckmann and Labrenz, 2020), threats appear to be more severe in tropical regions, due to the compounding effects of other factors, such as increasing human populations, rapid economic development and expanding manufacturing sectors (Eriksen et al., 2014; Ivar do Sul and Costa, 2014). Notably, coral reef ecosystems in the Asia-Pacific region are particularly vulnerable due to the current high levels of mismanaged plastic waste and the forecasted rapid increase in litter outflow to coastal areas (Lamb et al., 2018; Kako et al., 2014). This directly compromises the health of coral reef ecosystems, with flow-on effects to fisheries and tourism (Lamb et al., 2018).

Evidence of plastic ingestion throughout the marine food web is documented across a range of taxa (Kühn et al., 2020). Physical impacts of plastic ingestion on marine organisms include internal abrasion, digestion tract blockages and ulcers (Wright et al., 2013). Additionally, plastic debris can act as a source and a transport medium for toxic chemicals, due to the incorporation of chemical additives during plastic manufacturing or the adsorption and accumulation of chemicals present within the environment (Mato et al., 2001; Lithner et al., 2011; Gulizia et al., 2023). For instance, di(2-ethylhexyl) phthalate (DEHP) - a commonly used plasticizer introduced to improve product flexibility – is known to have endocrine disrupting and immunotoxic properties (Halden, 2010; Huang et al., 2015). The toxicological effects of microplastic exposure on marine organisms are largely unknown due to their complex and variable nature, however the potential to interfere with organism health is clear (Rochman et al., 2013). One known physiological consequence of exposure to potentially toxic compounds is oxidative stress. Oxidative stress is caused when the level of reactive oxygen species (ROS), including stable non-radical oxidants (e.g., hydrogen peroxide [H2O2]) and highly reactive oxygen radicals (e.g., superoxide anion [O^{2-}]) exceeds the capacity of the organism to eliminate them (Storz and Imlayt 1999, Gough and Cotter, 2011). Accumulation of ROS can damage proteins, nucleic acids and cell membranes, and ultimately cause disease and/or death (Janssens et al., 2000, Monaghan et al., 2009). Defenses against oxidative stress include antioxidant scavenging enzymes and non-enzymatic antioxidants that detoxify ROS, and mechanisms to repair oxidative damage (Sies et al., 2017).

Few studies have examined the physiological and behavioural effects of microplastic exposure on marine taxa (Andrady, 2011, Wright et al., 2013, Rivers-Auty et al., 2023; Hawke et al., 2024). There is limited research on their effects on ecological processes, fitness or survival. Predation rates on young coral reef fishes are affected by many environmental stressors, such as ocean acidification, temperature changes and habitat degradation (Allan et al., 2015; Ferrari et al., 2015), but only two studies have examined whether microplastic consumption affects mortality through predation. Jacob et al. (2019) exposed juvenile surgeonfish (*Acanthurus triostegus*) to very small (90 μm) polystyrene beads and found no effect on survival rates when exposed to a lionfish predator (*Pterois radiata)*. However, this outcome may be the consequence of a low consumption rate of the small beads (< 2 particles per fish), or the prey's inability to detect the predator rather than a lack of treatment effect (McCormick and Allan, 2016). By contrast, McCormick et al. (2020) found that survival of polystyrene-exposed damselfish (*Pomacentrus amboinensis*) released onto live coral habitat was six times lower than for fish not exposed to microplastics. However, the mechanisms underlying this mortality pattern are unknown. One possible explanation is that microplastic exposure alters escape performance (Hawke et al., 2024). Indeed, if the consumption of plastic particles even slightly alters the way these vulnerable young fish interact with predators, it has the potential to affect fish recruitment at a particularly sensitive life stage, with possible alterations to population dynamics (McCormick et al., 2020).

The present study experimentally investigated whether exposure to polystyrene particles had a physiological and ecological impact on juveniles of a common tropical damselfish (*Pomacentrus amboinensis*). Juveniles were exposed to one of three plastic treatments for 2 days (polystyrene particles, polystyrene with DEHP, and a control (no plastic)) and then sampled to determine whether there had been oxidative damage or repair, as indicated by the levels of protein carbonyls, lipid peroxides and antioxidant enzyme activity. Survival trials were also undertaken to determine whether exposure to microplastics altered fish survival during predator-prey encounters. Our prediction based on previous research was that exposure to microplastics would result in oxidative damage, the upregulation of antioxidant metabolism, which may impose an energetic stress on the fish (Kelly et al., 2024). Fish under energetic stress are more active and take more risk to obtain food (Ferrari et al., 2009) and should be more commonly caught by predators.

2. Methods

2.1. Ethics statement

Research was undertaken with a James Cook University ethics approval permit (permit: A2408) and fish collected under the Great Barrier Reef Marine Park Authority permit G12/35117.1.

2.2. Study species

The study species, the Ambon damselfish *Pomacentrus amboinensis*, is a common omnivorous damselfish within coral reef fish communities of the Indo-Pacific. *P. amboinensis* are 10.3–15.1 mm long and 15–23 days old at settlement (Kerrigan, 1996). They are commonly preyed upon by a variety of predatory fishes, including the dusky dottyback (*Pseudochromis fuscus*) (Feeney et al., 2012). *Ps. fuscus* are highly active and territorial mesopredators, regarded to be an important predator of newly settled coral reef fish (Feeney et al., 2012). They are commonly found throughout the Indo-Pacific and due their rapid acclimation to laboratory conditions are a frequently used model predator in studies examining predator-prey interactions (e.g., Allan et al., 2013).

Newly metamorphosed *P. amboinensis* individuals were collected using light traps moored over an 8-16 m water column in the backreef of Lizard Island (14°40′S, 145°28′E), northern Great Barrier Reef, Australia, during November 2019. Traps were recovered just after dawn and fish were immediately transported to the Lizard Island Research Station laboratory and held in the environmentally controlled aquarium facility in 32-l flow-through tanks (432 × 324 × 305 mm) that contained a small coral habitat. Fish were fed newly hatched *Artemia* sp. twice daily to satiation. Tanks were maintained at ambient conditions (28.5–29 °C) with a fresh supply of seawater continuously delivered to

each aquarium. Biological waste material was removed daily via suction cleaning. To allow recovery from stress during capture, juveniles were held for a minimum of 24 h prior to treatment allocation. Adult *Ps. fuscus* were captured from the surrounding reefs, with hand nets and dilute clove oil, while on SCUBA, and maintained individually in mesh baskets placed in a large flow through tank and fed daily with four euthanised juvenile damselfish.

2.3. Microplastic preparation and exposure

Microplastics were produced at James Cook University, Australia using Polystyrene (PS) (Sigma Aldrich, Mw = 192 KDaltons), dioctyl phthalate (DEHP) (Sigma-Aldrich, ≥99.5 %) and Dichloromethane (DCM) (Univar, ACS Grade). Pure PS beads (10.30 g) were dissolved in DCM (50 ml) under ambient conditions with constant stirring, after which, a DEHP (2.5 ml) was added (81:19 PS:DEHP) with constant stirring until the solution was uniform. This homogenous solution was then drop cast on watch glasses (50 mm in diameter) and allowed to evaporate in a fume hood to form a thick white membrane. The membranes were dried further in vacuo overnight to form brittle plates of PS containing DEHP (19 *w*/w%). The dried membranes were ground into microplastic particles using a food processor (NutriBullet, 900 Series) and sieved in the range of 200 to 300 μm (Geo-Con). Size distribution was confirmed using a scanning electron microscope (SEM) (Jeol Superprobe JXA-8200). Plasticizer incorporation was confirmed using Fourier Transform-Infrared Spectroscopy (FT-IR; Nicolet 6700) equipped with an attenuated total reflectance (ATR) diamond head attachment. The composition of the samples were measured by thermal gravimetric analysis (TGA; TA Instruments SDT 650 Instrument) at a heating rate of 10 °C/min up to 500 °C under constant flow of nitrogen (50 ml/min). The maximum concentration of the DEHP in the water is $<1.025 \times 10^{-5}$ g/l. This estimation is based on the assumption that (a) 200 particles would weigh 1.23 mg, (b) 5 % of the DEHP leaches (upper limit in stagnant water), (c) the particles were spherical in shape, (d) the density of the particles containing DEHP and without is the same, (e) DEHP is dispersed uniformly within the water (Gulizia et al., 2023).

Juvenile *P. amboinensis* were exposed to microplastic treatments 48 h prior to the start of the predator-prey mesocosm trials. Juvenile fish (n = 90) were randomly allocated to nine, 1.2-l glass tanks (10 fish per tank) in a static system, with air provided via an air-stone. The tanks were placed in a flow-through water bath to maintain and control the temperature across tanks. Tanks were randomly distributed throughout the water bath and rotated between replicate exposures to control for any potential spatio-temporal effects (e.g., light, or disturbance). Fish were exposed to one of three treatments: virgin polystyrene particles, polystyrene particles with DEHP plasticizer, or no particles (control). Fish were simultaneously exposed to microplastics and food 3 times per day (09:00, 12:00 and 15:00 h) for 2 days. Each feeding event consisted of 200 microplastic particles (200–300 μm) added simultaneously with newly hatched *Artemia* sp. ad libitum. Particles were hand counted into small glass vials before delivery. Vials were flushed ten time to ensure all particles were removed. All treatments were delivered with the same technique, with empty vials used for control tanks. Using this design, we produced 30 fish in each of our 3 treatments every 48 h, making a total of 90 fish per treatment. To remove excess food and any microplastics that were negatively buoyant, 1/3 of the water was removed from the bottom on the tank using a siphon halfway through the exposure period (e.g., after 24 h). Fresh seawater was used to replace the removed water. Tanks were fully cleaned between each replicate exposure event using fresh water and UV sterilised before being reset with the next round of experimental fish.

2.4. Oxidative stress protocols

To measure the effects of plastic exposure on oxidative stress, and using the same treatment protocol as described above, a further 36 *P. amboinensis* individuals per treatment were euthanised in an ice slurry (in accordance with James Cook University animal ethics guidelines, permit: A2408). Individuals were blotted dry to remove excess water, and then weighed to the nearest 0.0001 g. The digestive tract was removed and the number of microplastic particles were quantified for each individual using a microscope. White muscle tissue was excised from the ventral side of the fish, stored in 0.6 ml Eppendorf tubes, snap frozen in liquid nitrogen and transported to the University of Otago in charged dry shippers for oxidative stress analysis.

Due to low tissue mass available for laboratory analysis, three muscle samples of a similar weight, taken from three individual fish, within each treatment, were pooled to create one sample with sufficient tissue for analysis, yielding 12 replicates from each treatment. These replicates were further split into groups based on the number of microplastics present in their digestive tract: low (0–2 particles), medium (3–12 particles) and high (>12 particles) (See Fig. 2). Oxidative damage to proteins and lipids, and the activities of antioxidant enzymes were determined using established methods (Lister et al., 2015) and were used as biomarkers of oxidative stress. Total protein was extracted for analysis of protein carbonyls (PC) and antioxidant enzyme activities by homogenizing muscle tissue samples in 400 μl of 50 mM potassium phosphate buffer (pH 7.0) containing 0.2 mM Na_2 EDTA, 1 % PVP-44, 1 mM PMSF and 0.5 % *v*/v Triton X-100. Three zirconia beads to each tube containing the sample and buffer, and the tissue was homogenized using a bead beater (BioSpec mini-beater) set to speed 48 for 15 s. Each homogenate was centrifuged at 14,000 x*g* for 15 min at 4 °C (Eppendorff 5425R) and the supernatant was collected. Ultra-filtration was used to semi-purify the proteins contained in 400 μl of the supernatant using Amicon™ ultracentrifuge units (10kD; MWCO) by centrifugation at 10,000 x*g* for 15 min at 4 °C. The protein sample was then reconstituted with 400 μl of 50 mM phosphate buffer (pH 7.0) and divided into 50 μl aliquots that were then stored at −80 °C until biochemical analysis.

Biochemical assays were conducted following the methodology outlined by Lister et al. (2015). A Lowry protein assay (Fryer et al. 1986), with a bovine serum albumin (BSA) as the standard was used to determine soluble protein contents. The spectrophotometric method for analysing protein carbonyl levels in semi-purified protein samples via reaction with 2.4-dinitrophenylhydrazine (DNPH) was conducted as described by Reznick and Packer (1994) and adapted for microplates as per Lister et al. (2015). Lipid peroxides were extracted and measured as per Lister et al. (2015). Superoxide dismutase (SOD) activity was determined using the microplate quantification method described by Banowetz et al. (2004), with minor modifications. Catalase (CAT) activity was determined using the chemiluminescent methodology outlined by Maral et al. (1977) and adapted for 96-well microplates by Janssens et al. (2000). Glutathione reductase (GR) activity was assayed using the method of Cribb et al. (1989) with minor modifications. Glutathione peroxidase (GPx) activity was measured according to the spectrophotometric method described by Paglia and Valentine (1967). Glutathione-S-transferase activity (GST) was measured using the method of Habig et al. (1974), modified by Brogdon and Barber (1990) for use in a microplate reader.

All assays were conducted using a PerkinElmer (Wallac) 1420 multilabel counter (PerkinElmer, San Jose, California, U.S.A), controlled by a computer and fitted with a temperature control cell and an autodispenser. The WorkOut 2.0 software package (PerkinElmer) was used to acquire and process the data.

2.5. Mesocosm survival trials

To assess the impact of microplastic exposure on survival, groups of juvenile P. amboinensis from each treatment were exposed to a mesopredator, Ps. fuscus, for 22 h and their survival compared across groups (as per Ferrari et al., 2011). Trials were conducted in nine outdoor, flow-through mesocosm tanks (111 cm diameter, 45 cm high, 368 l) that contained 1 cm deep sand substrate and a coral rubble patch in the

centre, approximately 20 cm in height and 30 cm in diameter and pieces of PVC pipe to provide habitat complexity. P. amboinensis are a generalist damselfish that will associate with both live and dead coral (McCormick and Weaver, 2012). Ten individual P. amboinensis, from the same treatment, were placed within each of the mesocosm one hour prior to the release of the predator. After this acclimation period, a single Ps. fuscus was carefully introduced into the centre of the tank. Mesocosms were left undisturbed, except for a singular feeding event (1600 h) during which a 60 ml solution of freshly hatched Artemia sp. nauplii was added 15 cm from the coral patch. Trials lasted 22 h, after which we captured and removed all fish from the tank. The number of surviving P. amboinensis was recorded. Mesocosms were drained and refilled after each trial. Coral structures were rebuilt and tank treatment was re-allocated after every trial. When the mesocosms were reset, we switched treatment and tank combinations to control for any spatio-temporal effects. In total, we ran 24 control, 23 virgin plastic and 24 DEHP plastic mesocosm replicates. Predators were fed euthanised juvenile damselfish daily, but not fed 48 h prior to the start of trials to standardise for satiation. Predators (n = 37) were randomly allocated to each treatment group to control for differences between individual predator ability. After each trial, predators were fed euthanized juvenile damselfish and starved for a 48 h period before use in subsequent trials.

2.6. Statistical analysis

2.6.1. Pooled oxidative stress

A principal component analysis (PCA) followed by an analysis of variance was used to determine whether exposure to microplastics (polystyrene particles, polystyrene treated with DEHP, and no plastics (control)) affected oxidative stress biomarkers. A PCA on the correlation matrix was used to summarise the most important trends in the variability in oxidative stress markers throughout the dataset. The first principal component (PC1) was then used in an ANOVA to test the equality of the stress markers among treatments. Residual analysis was used examine the assumptions of normality and homogeneity of variance. Tukey's (HSD) tests for non-equal sample sizes were undertaken to find the nature of significant differences found. To further understand how oxidative stress markers differed among markers and with treatments, ANOVAs were undertaken on the individual markers, with effect size represented by eta-squared (Richardson (2011). Trends were plotted to enhance interpretation.

2.6.2. Dosage dependent oxidative stress

Residual analysis indicated that not all of the data met the assumptions of normality and homogeneity of variance (R software package 'car': Fox and Weisberg, 2019). Therefore, a non-parametric, one-way Welch's test was conducted on lipid peroxide and protein carbonyl concentrations and GPX and GST activity levels to determine statistical significance between treatments. This was followed by a non-parametric post hoc analysis using a Games-Howell function (R software package 'rstatix: R Core Team, R, 2013). One-way ANOVA's and Tukey post-hoc tests were used to analyse activity levels of SOD (after log transformation) CAT and GR.

2.6.3. Survival

Survival rate of *P. amboinensis* from each treatment group as well as predator size, trial day and mesocosm number were recorded with Microsoft Excel version 16.3 (Excel 2019). All statistical analysis were conducted in R software Version 1.1.463 (R Core Team, R, 2013). A generalised linear model (glm) with a Gaussian family was used to analyse the survival rates of *P. amboinensis* between treatments and ensure no confounding effect of experimental variables.

3. Results

3.1. Oxidative stress

Oxidative stress biomarkers had strong positive correlations with one another (r ranges 0.74–0.96). The first axis of the PCA accounted for 85.9 % of the variance in oxidative markers, with the second axis representing a further 5.3 %. All markers had a similar loading and sign on the first axis, suggesting that the magnitude of oxidative stress effects were well represented by all stress markers. The position of the centroids for each treatment in the PCA biplot indicates that treatments were separated along the first principle component (Fig. 1). An ANOVA on PC1 found strong differences among treatments ($F_{6,29} = 50.80$, $p < 0.001$, $\eta^2 = 0.91$), with significantly higher scores of PC1 (i.e., oxidative stress) for those fish found to consume medium and high levels of DEHP, or high levels of virgin PS.

Oxidative damage.

Interestingly, there was an effect of the number of microplastics retrieved from the digestive tract on the two oxidative damage biomarkers ($F_{6, 8.79} = 15.26$, $p = 0.0003$, Fig. 2 A,B). A Games-Howell non-parametric post-hoc test revealed that *P. amboinensis* exposed to DEHP or virgin microplastics and classified as high dosage (12+ microplastic particles) had significantly higher levels of lipid peroxide compared to fish containing low levels of microplastic (0–2 particles) in their digestive tract (Fig. 2 A). Similarly, *P. amboinensis* individuals in the DEHP treatment group had higher protein carbonyl levels in the high dosage group compared to the low dosage group (Fig. 2 B). Regardless of plastic type, there was no difference between lipid peroxide or protein carbonyl levels in fish found with low doses of microplastics (0–2 particles) compared to fish exposed to no microplastics (Fig. 2 A,B).

3.2. Oxidative defence

In addition, there was an effect of the number of microplastics retrieved from the digestive tract on activity levels of SOD ($F_{6, 29} = 29.54$, $p < 0.0001$), CAT ($F_{6, 29} = 22.46$, $p < 0.0001$), GPOX ($F_{6,9.47} = 9.05$, $p < 0.01$) and GR ($F_{6, 29} = 17.77$, $p < 0.001$) enzymes was apparent (Fig. 2 C,D,E,F). Specifically, *P. amboinensis* who consumed more microplastics (12+) had significantly higher activity levels of SOD and GR compared to fish with low levels of microplastic (0–2) for both virgin and DEHP treatments (Fig. 2 D,E). *P. amboinensis* found to have high amounts of DEHP microplastics (12+) had greater activity levels of CAT compared to the low dose group (0–2) (Fig. 2 C). Similarly, *P. amboinensis* from the high dosage group of virgin microplastics (12+) had greater activity levels of CAT compared to both the low and medium dose group (0–2 and 2–12 particles respectively) (Fig. 2 C). Similar to lipid peroxide and protein carbonyls, there was little difference between activity levels of SOD, CAT, GPOX or GR enzymes in fish with a low amount of virgin or DEHP microplastic found in their digestive tract (0–2 particles) compared to fish that were not exposed to microplastics (Fig. 2 C,D,E,F).

3.3. Antioxidant metabolism

There was a significant treatment effect on GST activity on *P. amboinensis* exposed to microplastics or no microplastics ($F_{6,9.33} = 20.84$, $p < 0.0001$). Fish exposed to DEHP microplastics had the greatest activity of GST, followed by fish exposed to virgin microplastics with lowest GST activity found in the control group (Fig. 3.). Although a microplastic dose effect was seen with elevated GST activity in high compared to low microplastic groups (0–2 and 12+ particles respectively), there was no difference between low microplastic (0–2 particles) and control groups (Fig. 3.).

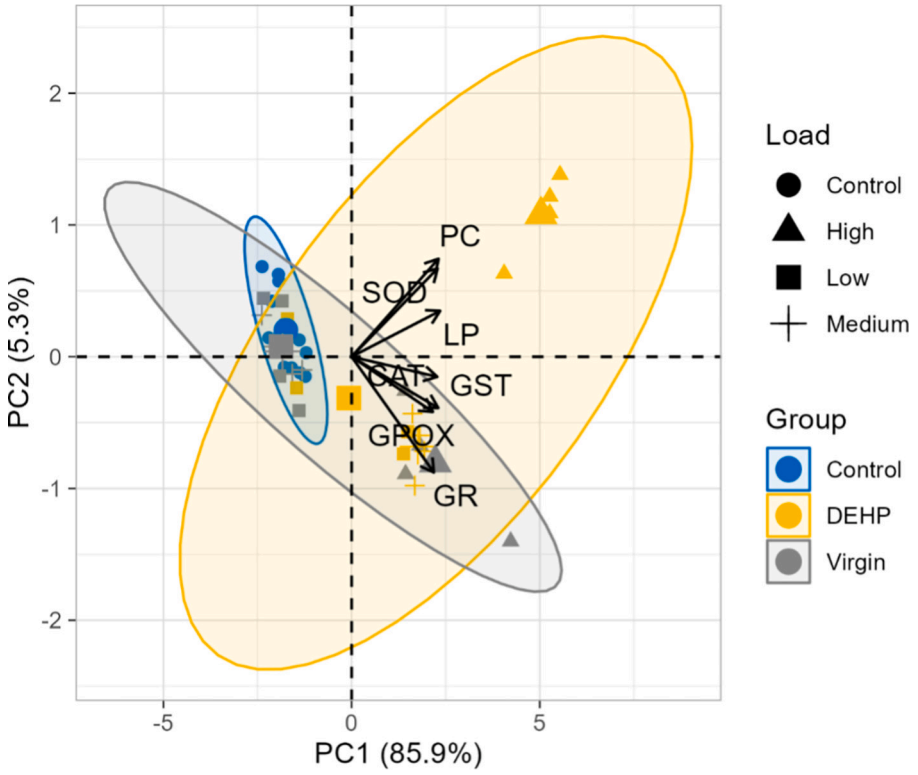

Fig. 1. Principal component (PC) biplot for PC1 and PC2 in oxidative stress biomarkers (abbreviations as per the methods text). Arrows are vectors that represent the correlation coefficients of oxidative stress biomarkers with principal components. Load refers to the number of plastics found in the gut at the end of the experiment.

3.4. Predation rate

Predation rate by *Ps. fuscus* on *P. amboinensis* juveniles was not influenced by microplastic treatment ($p > 0.05$). There were no temporal-spatial effects of trial day or mesocosm number on the survival rate. Additionally, there was no effect of predator size on the survival rate of *P. amboinensis*.

4. Discussion

The effects of microplastic exposure on the health and survival of coral reef fish recruits is a new and developing research field, with differences in findings apparent between studies (Jacob et al., 2020). We found a clear physiological cost of microplastic exposure on juvenile coral reef fish due to oxidative damage and the activation of enzymatic defence mechanisms, both of which were microplastic dose dependent. Specifically, we found individuals exposed to DEHP microplastic particles had higher levels of oxidative damage biomarkers (lipid peroxide and protein carbonyl) as well as higher activities of antioxidant enzymes (CAT, SOD, GR, GPx and GST) involved in oxidative defence and detoxification. These results suggest a clear physiological cost of short term DEHP microplastic exposure in juvenile *P. amboinensis*.

Exposure to DEHP has been observed to induce cellular oxidative stress across multiple taxa, including fish, rats and humans (Li et al., 2014; Molino et al., 2019; Pérez-Albaladejo et al., 2020). However, differences are apparent across taxa in the activation processes of antioxidant systems, suggesting that activation of the antioxidant system is complex, and caution is urged when generalising results. For example, Mo et al. (2019) found that CAT and SOD enzymes were downregulated after exposure to DEHP microplastics in juvenile yellow catfish, *Pelteoagrus fulviraco*. Furthermore, antioxidant enzyme activity levels decreased with exposure to DEHP in grass carp hepatocytes (epithelial liver cells) (Cui et al., 2020), mouse neural stem cells (Liu et al., 2018) and medaka fish larvae (Yang et al., 2018). Excessive ROS production can exceed the upper limit of physiological tolerance and subsequently reduce antioxidant enzyme activity (Mo et al., 2019). Additionally, accumulation of the lipid peroxidation by-product malondialdehyde, in body tissues may result in an inhibition of antioxidative processes (Mo et al., 2019; Shen et al., 2019). Differences between experimental designs and plasticizer concentration exposure may result in the initiation or suppression of individual stress responses and therefore explain differences among research findings.

Interestingly, there was a distinctive dose-dependent effect on oxidative damage and antioxidative processes between low (0–2 particles), medium (2–12 particles) and high (12+ particles) quantities of virgin and DEHP microplastics in the digestive tract of *P. amboinensis* (Fig. 2). Although, this was not an initial research objective, it was evident that activities of enzymes involved in oxidative defence, including SOD, CAT, GR and GST, were significantly elevated in individuals that had a high particle count in the gastrointestinal tract, compared to low particle counts for both virgin and DEHP microplastic treatments. Additionally, the activity of enzymes involved in oxidative defence processes, did not differ between *P. amboinensis* from low category microplastic treatments compared to controls. There was also no difference in GST activity of individuals containing low levels of microplastic particles and individuals from control treatments. Information on microplastic particle abundance in the digestive tract of *P. amboinensis* individuals during muscle tissue excision, provides insight into variability between microplastic exposure across individuals. Despite controlled microplastic particle delivery during the treatment phase, ingestion rates and gut retention naturally varied among *P. amboinensis* individuals, potentially attributed to different levels of boldness (Nanninga et al., 2020) or position in the social hierarchy within the group (McCormick, 2016). Moreover, it is unknown how long the particles are retained in the gut. Using a closely related species (*Pomacentrus chrysurus*), McCormick et al. (2020) demonstrated that spherical particles are egested after 12 h. In the present study, rough, irregular shaped particles were used and so it is possible that

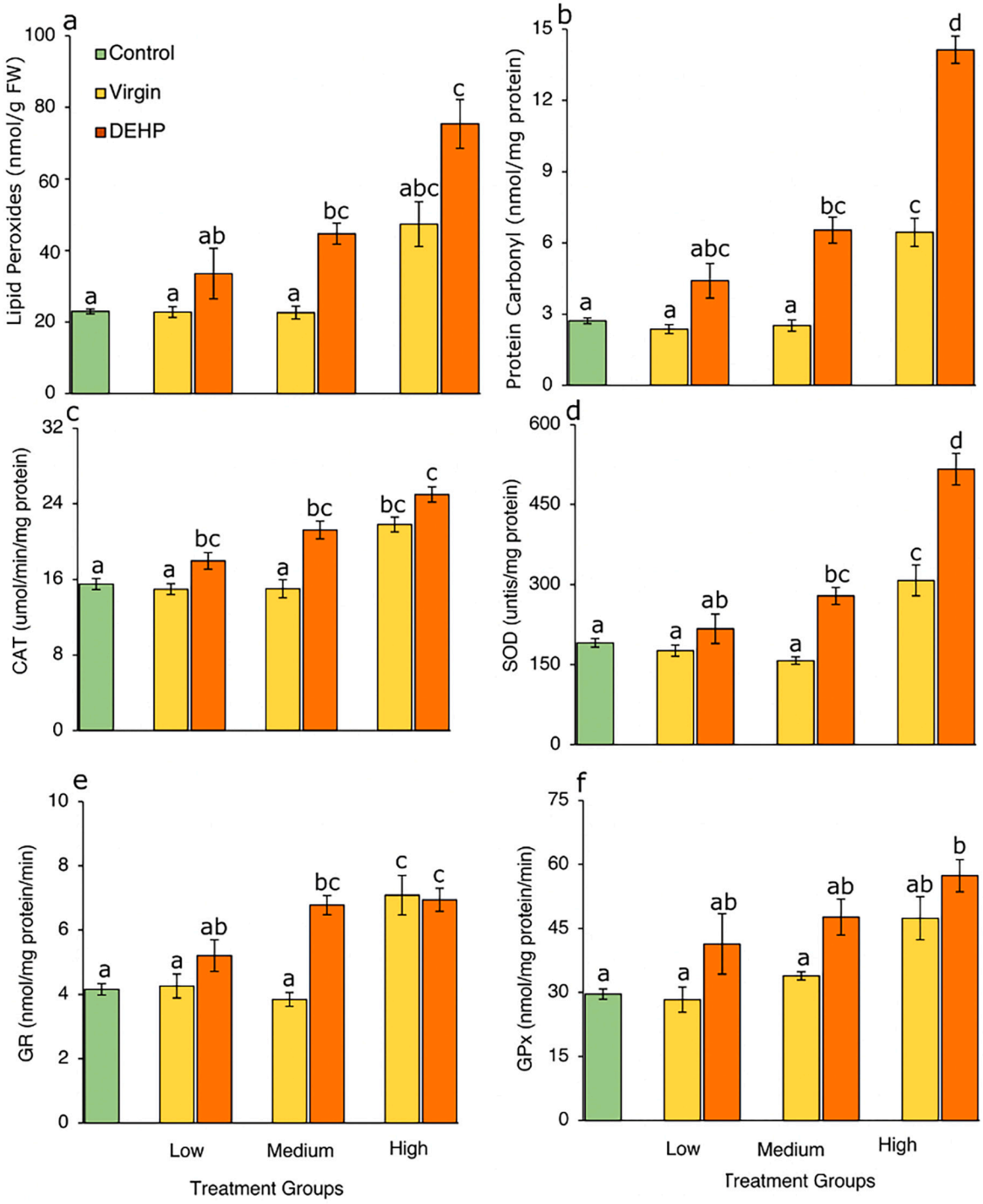

Fig. 2. Changes in the mean (**a**) lipid peroxide concentration, (**b**) protein carbonyl concentration, (**c**) catalase (CAT), (**d**) superoxide dismutase (SOD), (**e**) glutathione reductase (GR) and (**f**) glutathione peroxidase (GPX) in white muscle tissue of *P. amboinensis* individuals exposed to virgin and DEHP microplastics with low (0–2 particles), medium (2–12 particles) or high (12+ particles) levels of microplastic in their digestive tract ($n = 4$ for each category) as well as no microplastic (control, $n = 12$). Error bars display standard error. Significance between treatment groups indicated by lettering ($p < 0.05$).

these were retained in the gut for longer, although to the best of our knowledge this has not been empirically tested. Future work should focus on this aspect of microplastic ingestion.

Our hypothesis that exposure to microplastics would result in oxidative damage was partially supported, but was dependent on plastic type. For example, although there was no significant difference in oxidative damage or defence processes between virgin microplastic and control treatment groups, there was a confounding effect of microplastic particle quantity. *P. amboinensis* individuals exposed to virgin microplastics and categorized with a high (12+ particles) quantity of microplastic at the end of the experimental period, had significantly elevated activities of antioxidant enzymes (SOD, CAT, GR) (see Fig. 2) as well as

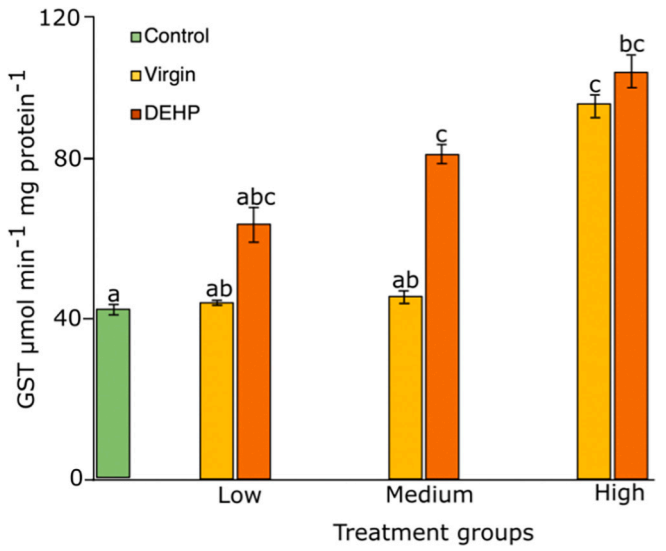

Fig. 3. Changes in the mean mean activities of glutathione-S-transferase (GST) in white muscle tissue of *P. amboinensis* individuals exposed to virgin and DEHP microplastics with low (0–2 particles), medium (2–12 particles) or high (12+ particles) levels of microplastic in their digestive tract (n = 4 for each category) as well as no microplastic (control, n = 12). Error bars display standard error. Significance between treatment groups indicated by lettering ($p < 0.05$).

elevated GST activity (see Fig. 3). Quantifying plastic particles retained in the gut is evidently an important variable to be included in future experimental studies as it enables examination of particle retention rates of microplastic exposure, dosage analysis and may avoid potential misinterpretation of results.

Despite a clear physiological cost of microplastic ingestion on *P. amboinensis*, our hypothesis that microplastic treatment will affect survival rate of prey fish in mesocosm predation trials was not supported. This is in contrast to McCormick et al. (2020), who reported increased mortality rates of newly settled *P. amboinensis* after exposure to polystyrene microbeads. However, there are some fundamental differences between McCormick et al. (2020) and the present study. For example, the present study used a mesocosm approach where any recorded mortality could be attributed to the presence of a single predator (*Ps. fuscus*). By contrast, McCormick et al. (2020) placed microplastic exposed individual fish onto small coral patch reefs in situ and any loss in prey fish were assumed to be due to predation. However, the predator-prey interactions were not recorded, and it is unknown whether mortality was due to attacks from predators, or whether prey, potentially weakened by the effects of plastic ingestion, were swept off the patch reef by currents. It is also possible that differences in mortality effects between the two studies are due to McCormick et al. (2020) using a more sensitive method of tracking mortality, whereby the fates of individual fish rather than groups were measured. It is possible that due the use of a low power method (e.g., group mortality trajectories), we were unable to detect differences in mortality due to microplastic exposure and that the results of McCormick et al. (2020) may actually be due in part to elevated ROS activity causing an energetic cost, rather than the nutritional deficit hypothesis argued by McCormick et al. (2020).

With a growing number of microplastic particles entering and persisting in marine systems, understanding how plastic exposure affects ecological interactions can provide evidence on the functioning of these ecosystems. Although we found no difference in survival rates from mesocosm trials, there were clear physiological costs of microplastic exposure on *P. amboinensis* individuals, including both a plasticizer effect and dose dependent effect, which was most marked when plastics had a DEHP plasticizer incorporated. The duration of this effect, and whether the effect may be cumulative is currently unknown and warrants further study. These results highlight that while studies may not find immediate mortal consequences to plastic ingestion, there may be more subtle sub-lethal cost that may manifest to have ecologically important consequences at later life stages, through energetic impacts on growth and energy allocation.

Authors' contribution

AMM, MCOF, and BJMA, conceived and designed the study. AMM and BJMA undertook the laboratory conditioning of fish, dissected fish and counted particles. GV and AMG undertook chemical assays of the microplastics. DJB and AMM undertook biochemical analysis on fish tissue. AMM, VK and BJMA analysed and visualised the data. All authors contributed to the writing of the final manuscript.

CRediT authorship contribution statement

Amelia M. Mannering: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **David J. Burritt:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Valeriya Komyakova:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Maud C.O. Ferrari:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **George Vamvounis:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Conceptualization. **Alexandra M. Gulizia:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Conceptualization. **Adam Staples:** Investigation. **Mark I. McCormick:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Bridie J.M. Allan:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available upon request.

References

Allan, B.J.M., Domenici, P., McCormick, M.I., Watson, S.-A., Munday, P.L., 2013. Elevated CO_2 affects predator-prey interactions through altered performance. PLoS One 8, e58520.

Allan, B.J.M., Domenici, P., Munday, P.L., McCormick, M.I., 2015. Feeling the heat: the effect of acute temperature changes on predator–prey interactions in coral reef fish. Conserv. Physiol. 3.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605.
Banowetz, G.M., Dierksen, K.P., Azevedo, M.D., Stout, R., 2004. Microplate quantification of plant leaf superoxide dismutases. Anal. Biochem. 332, 314–320.
Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans.: Biol. Sci. 364, 1985–1998.
Brogdon, W.G., Barber, A.M., 1990. Microplate assay of glutathione s-transferase activity for resistance detection in single-mosquito triturates. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 96, 339–342.
Cribb, A.E., Leeder, J.S., Spielberg, S.P., 1989. Use of a microplate reader in an assay of glutathione reductase using 5,5′-dithiobis(2-nitrobenzoic acid). Anal. Biochem. 183, 195–196.
Cui, Y., Zhang, Q., Yin, K., Song, N., Wang, B., Lin, H., 2020. DEHP-induce damage in grass carp hepatocytes and the remedy of eucalyptol. Ecotoxicol. Environ. Saf. 206, 111151.
Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9, e111913.
Feeney, W.E., Lönnstedt, O.M., Bosiger, Y., Martin, J., Jones, G.P., Rowe, R.J., McCormick, M.I., 2012. High rate of prey consumption in a small predatory fish on coral reefs. Coral Reefs 31, 909–918.
Ferrari, M.C.O., Dixson, D.L., Munday, P.L., McCormick, M.I., Meekan, M.G., Sih, A., Chivers, D.P., 2011. Intrageneric variation in antipredator responses of coral reef fishes affected by ocean acidification: implications for climate change projections on marine communities. Glob. Chang. Biol. 17, 2980–2986.
Ferrari, M.C.O., Munday, P.L., Rummer, J.L., McCormick, M.I., Corkill, K., Watson, S.-A., Allan, B.J.M., Meekan, M.G., Chivers, D.P., 2015. Interactive effects of ocean acidification and rising sea temperatures alter predation rate and predator selectivity in reef fish communities. Glob. Chang. Biol. 21, 1848–1855.
Ferrari, M.C.O., Sih, A., Chivers, D.P., 2009. The paradox of risk allocation: a review and prospectus. Anim. Behav. 78, 579–585.
Fox, J., Weisberg, S., 2019. Using Car Functions in Other Functions. CRAN R.
Fryer, A.A., Hume, R., Strange, R.C., 1986. The development of glutathione S-transferase and glutathione peroxidase activities in human lung. Biochimica et Biophysica Acta (BBA)-General Subjects 883 (3), 448–453.
Gough, D.R., Cotter, T.G., 2011. Hydrogen peroxide: a Jekyll and Hyde signalling molecule. Cell Death Dis. 2 (10), e213.
Gove, J.M., Whitney, J.L., McManus, M.A., Lecky, J., Carvalho, F.C., Lynch, J.M., Li, J., Neubauer, P., Smith, K.A., Phipps, J.E., Kobayashi, D.R., Balagso, K.B., Contreras, E. A., Manuel, M.E., Merrifield, M.A., Polovina, J.J., Asner, G.P., Maynard, J.A., Williams, G.J., 2019. Prey-size plastics are invading larval fish nurseries. Proc. Natl. Acad. Sci. 116, 24143–24149.
Gulizia, A.M., Patel, K., Philippa, B., Motti, C.A., van Herwerden, L., Vamvounis, G., 2023. Understanding plasticiser leaching from polystyrene microplastics. Sci. Total Environ. 857, 159099.
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione s-transferases. the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.
Halden, R.U., 2010. Plastics and health risks. Annu. Rev. Public Health 31, 179–194.
Hawke, A.M., Trujillo, J.E., Oey, I., Giteru, S.G., Allan, B.J., 2024. Exposure to petroleum-derived and biopolymer microplastics affect fast start escape performance and aerobic metabolism in a marine fish. Sci. Total Environ. 906, 167423.
Huang, Q., Chen, Y., Chi, Y., Lin, Y., Zhang, H., Fang, C., Dong, S., 2015. Immunotoxic effects of perfluorooctane sulfonate and di(2-ethylhexyl) phthalate on the marine fish *Oryzias melastigma*. Fish Shellfish Immunol. 44, 302–306.
Ivar do Sul, J.A., Costa, M.F., 2014. The present and future of microplastic pollution in the marine environment. Environ. Pollut. 185, 352–364. https://doi.org/10.1016/j.envpol.2013.10.036.
Jacob, H., Besson, M., Swarzenski, P.W., Lecchini, D., Metian, M., 2020. Effects of virgin micro- and nanoplastics on fish: trends, meta-analysis, and perspectives. Environ. Sci. Technol. 54, 4733–4745.
Jacob, H., Gilson, A., Lanctôt, C., Besson, M., Metian, M., Lecchini, D., 2019. No effect of polystyrene microplastics on foraging activity and survival in a post-larvae coral-reef fish, *Acanthurus triostegus*. Bull. Environ. Contam. Toxicol. 102, 457–461.
Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771.
Janssens, B.J., Childress, J.J., Baguet, F., Rees, J.F., 2000. Reduced enzymatic antioxidative defense in deep-sea fish. J. Exp. Biol. 203, 3717–3725.
Kako, S., Isobe, A., Kataoka, T., Hinata, H., 2014. A decadal prediction of the quantity of plastic marine debris littered on beaches of the east Asian marginal seas. Mar. Pollut. Bull. 81, 174–184.
Kelly, E.R., Trujillo, J.E., Setiawan, A., Pether, S., Burritt, D., Allan, B.J.M., 2024. Investigating the metabolic and oxidative stress induced by biofouled microplastics exposure in *Seriola lalandi* (yellowtail kingfish). Mar. Pollut. Bull. 203, 116438.
Kerrigan, B., 1996. Temporal patterns in size and condition at settlement in two tropical reef fishes (Pomacentridae: *Pomacentrus amboinensis* and *P. Nagasakiensis*). Mar. Ecol. Prog. Ser. 135, 27–41.
Kühn, S., van Franeker, J.A., O'Donoghue, A.M., Swiers, A., Starkenburg, M., van Werven, B., Foekema, E., Hermsen, E., Egelkraut-Holtus, M., Lindeboom, H., 2020. Details of plastic ingestion and fibre contamination in North Sea fishes. Environ. Pollut. 257, 113569. https://doi.org/10.1016/j.envpol.2019.113569.
Lamb, J.B., Willis, B.L., Fiorenza, E.A., Couch, C.S., Howard, R., Rader, D.N., True, J.D., Kelly, L.A., Ahmad, A., Jompa, J., Harvell, C.D., 2018. Plastic waste associated with disease on coral reefs. Science 359, 460–462.
Li, X., Fang, E.F., Scheibye-Knudsen, M., Cui, H., Qiu, L., Li, J., He, Y., Huang, J., Bohr, V. A., Ng, T.B., Guo, H., 2014. Di-(2-ethylhexyl) phthalate inhibits DNA replication leading to hyperPARylation, SIRT1 attenuation and mitochondrial dysfunction in the testis. Sci. Rep. 4, 6434.
Liu, W., Zhang, L., Wu, R., 2018. Enteric neural stem cells expressing insulin-like growth factor 1: a novel cellular therapy for Hirschsprung's disease in mouse model. DNA Cell Biol. 37 (7), 642–648.
Lister, K.N., Lamare, M.D., Burritt, D.J., 2015. Pollutant resilience in embryos of the Antarctic Sea urchin *Sterechinus neumayeri* reflects maternal antioxidant status. Aquat. Toxicol. 161, 61–72.
Lithner, D., Larsson, Å., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409, 3309–3324.
Maral, J., Puget, K., Michelson, A.M., 1977. Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. Biochem. Biophys. Res. Commun. 77, 1525–1535.
Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ. Sci. Technol. 35, 318–324.
McCormick, M.I., 2016. Protogyny in a tropical damselfish: females queue for future benefits. PeerJ 4, e2198.
McCormick, M.I., Allan, B.J.M., 2016. Lionfish misidentification circumvents an optimized escape response by prey. Conserv. Physiol. 4, cow064.
McCormick, M.I., Chivers, D.P., Ferrari, M.C.O., Blandford, M.I., Nanninga, G.B., Richardson, C., Fakan, E.P., Vamvounis, G., Gulizia, A.M., Allan, B.J.M., 2020. Microplastic exposure interacts with habitat degradation to affect behaviour and survival of juvenile fish in the field. Proc. R. Soc. B Biol. Sci. 287 (1937), 20201947. https://doi.org/10.1098/rspb.2020.1947.
McCormick, M.I., Weaver, C.J., 2012. It pays to be pushy: intracohort interference competition between two reef fishes. PLoS One 7 (8), e42590. https://doi.org/10.1371/journal.pone.0042590.
Meijer, L.J., Van Emmerik, T., Van Der Ent, R., Schmidt, C., Lebreton, L., 2021. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. Sci. Adv. 7 (18), eaaz5803.
Mo, N., Zhang, M., Wang, R., Xia, S., Meng, F., Qian, Y., Li, M., 2019. Effects of α-ethinyl estradiol (EE2) and diethylhexyl phthalate (DEHP) on growth performance, antioxidant status and immune response of juvenile yellow catfish *Pelteobagrus fulvidraco*. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 226, 108615.
Molino, C., Filippi, S., Stoppiello, G.A., Meschini, R., Angeletti, D., 2019. In vitro evaluation of cytotoxic and genotoxic effects of Di(2-ethylhexyl)-phthalate (DEHP) on European sea bass (*Dicentrarchus labrax*) embryonic cell line. Toxicol. in Vitro 56, 118–125.
Monaghan, P., Metcalfe, N.B., Torres, R., 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol. Lett. 12 (1), 75–92.
Nanninga, G.B., Scott, A., Manica, A., 2020. Microplastic ingestion rates are phenotype-dependent in juvenile anemonefish. Environ. Pollut. 259, 113855.
Oberbeckmann, S., Labrenz, M., 2020. Marine microbial assemblages on microplastics: diversity, adaptation, and role in degradation. Annu. Rev. Mar. Sci. 12 (1), 209–232.
Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70, 158–169.
Pérez-Albaladejo, E., Solé, M., Porte, C., 2020. Plastics and plastic additives as inducers of oxidative stress. Curr. Opin. Toxicol. 69–76, 20–21.
R Core Team, R, 2013. R: A Language and Environment for Statistical Computing.
Reznick, A.Z., Packer, L., 1994. Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. In: Methods in Enzymology (Ed.), Oxygen radicals in biological systems part C. Academic Press, pp. 357–363.
Richardson, J.T., 2011. Eta squared and partial eta squared as measures of effect size in educational research. Educ. Res. Rev. 6, 135–147.
Rivers-Auty, J., Bond, A.L., Grant, M.L., Lavers, J.L., 2023. The one-two punch of plastic exposure: macro-and micro-plastics induce multi-organ damage in seabirds. J. Hazard. Mater. 442, 130117.
Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263.
Shen, C., Wei, J., Wang, T., Wang, Y., 2019. Acute toxicity and responses of antioxidant systems to dibutyl phthalate in neonate and adult *Daphnia magna*. PeerJ 7, e6584.
Sies, H., Berndt, C., Jones, D.P., 2017. Oxidative stress. Annu. Rev. Biochem. 86 (1), 715–748.
Storz, G., Imlayt, J.A., 1999. Oxidative stress. Curr. Opin. Microbiol. 2 (2), 188–194.
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.
Yang, W.-K., Chiang, L.-F., Tan, S.-W., Chen, P.-J., 2018. Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval growth and locomotion in medaka.