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Trophic transfer of microplastics does not affect fish personality

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Microplastics are ubiquitous in the marine environment. Their small size makes them bioavailable to a range of organisms and studies have reported ingestion across the food chain. Few studies have demonstrated physical transfer of microplastics between organisms, and no research has assessed the ecological impacts of transferred microplastics and contaminants over different trophic levels. Contaminants associated with plastics can alter animal behaviour; thus, exploring changes in behaviour may be fundamental in understanding ecosystem effects of microplastics. This study explored the effects of microplastics and associated contaminants through the food chain in the marine intertidal zone. We exposed beach hoppers, *Platorchestia smithi*, to environmentally relevant concentrations of microplastics and then fed them to Krefft's frillgobies, *Bathygobius krefftii*, ray-finned fish that inhabit shallow coastal ecosystems. We tested fish personality to see whether there were any changes that could be attributed to trophic transfer of microplastics, as even subtle changes in behaviour can have cascading effects on other organisms and the wider ecosystem. Exploring behavioural changes in response to contaminant exposure is a developing area in ecotoxicology due to its increased sensitivity compared with the traditional LD50 approach. While gobies readily ingested contaminated beach hoppers, we detected no effect of microplastic trophic transfer on fish personality relative to control groups. While chronic exposure studies assessing a suite of behaviours are required, it is possible that the transfer of microplastics via trophic interactions does not provide an additional exposure pathway for contaminants through the food web.

Food webs and trophic interactions are essential for the transfer of energy and nutrients throughout ecosystems. Urbanization, development and industry have influenced pollutants in aquatic systems, with chemicals, metals, pesticides and pharmaceuticals all presenting risks to ecosystems and associated biota (von Glasow et al., 2013; Islam & Tanaka, 2004). Contaminants can accumulate and concentrate in biota (bioaccumulation) and then subsequently transfer through the food web (Borgå, Fisk, Hoekstra, & Muir, 2004; Nfon, Cousins, & Broman, 2008). These pollutants can impact animal physiology (Hontela, 1998) and can also alter behaviours important for foraging, reproduction, social interactions and anti-predator behaviour (Clotfelter, Bell, & Levering, 2004; Söffker & Tyler, 2012).

Plastic is a contemporary pollutant in our marine environment. While the deleterious effects of large plastic debris on marine life have received much publicity (Barnes, Galgani, Thompson, & Barlaz, 2009), microplastics are of increasing concern (Cole, Lindeque, Halsband, & Galloway, 2011). Their small size and variable densities mean they occur throughout marine (Law & Thompson, 2014) and freshwater (Eerkes-Medrano, Thompson, & Aldridge, 2015) environments and are bioavailable to a broad range of organisms (Thompson et al., 2004). Microplastics present a physical and chemical risk to organisms. (Rochman, 2013). Potentially toxic additives such as phthalates, bisphenol A (BPA) and flame-retardants are added to many plastics at manufacture to increase functionality and extend their life (Browne, Galloway, & Thompson, 2008; Rochman, 2013). Furthermore, plastics are porous and accumulate and concentrate contaminants including polychlorinated biphenyls (PCBs), pesticides and fertilizers at high intensities from the surrounding sea water (Mato et al., 2001). Many of the additives and the absorbed contaminants are known endocrine disruptors, carcinogens and mutagens (Lithner, Damberg, Dave, & Larsson, 2009) and it is thought that contaminants may transfer from plastics to organisms following ingestion (Teuten, Rowland, Galloway, & Thompson, 2007; Teuten et al., 2009).

Many of the contaminants associated with plastics such as BPA and PCBs can affect foraging efficiency, alter schooling behaviour or

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increase agonistic encounters in fish (Clotfelter et al., 2004; Sloman & Wilson, 2006). Complex behaviours such as foraging, predator avoidance and social interactions are all fundamental to individual fitness and an animal's functioning in an ecosystem (Wolf & Weissing, 2012). Changes in behaviour may take effect even with small quantities of pollutants (Bae & Park, 2014), and while changes in animal health may not be apparent, these behavioural alterations may affect how the animal performs in an ecological context (Scott & Sloman, 2004). Behavioural responses provide useful markers of pollution effects on individuals, potentially performing as reliable and economical indicators of sublethal effects of pollutants (Weis, 2014). Accordingly, there is growing emphasis in ecotoxicology on examining changes in behaviour in response to exposure to contaminants (Oulton, Taylor, Hose, & Brown, 2014).

Microplastics can be ingested by organisms across the marine environment (Fig. 1). A range of studies have assessed effects of plastic consumption at the individual organism level but few have assessed the capacity for microplastics and associated contaminants to move through the food chain. Our understanding of how pollutants can bioaccumulate (Borgå et al., 2004) suggest microplastics provide an additional exposure pathway for contaminants to transfer through the food web. The physical transfer of microplastic fragments between organisms has been demonstrated from mussels, *Mytilus edulis*, to crabs, *Carcinus maenas* (Farrell & Nelson, 2013) and mesozooplankton to macrozooplankton (Setälä, Fleming-Lehtinen, & Lehtiniemi, 2014; Fig. 1). Recently, the transfer of microplastic fragments as well as contaminants was demonstrated in an artificial food chain from *Artemia* nauplii to the intestinal tract of laboratory-raised zebrafish, *Danio rerio*. The study used high concentrations of microplastics spiked with large amounts of benzo(a)pyrene (BaP) and there was no assessment of biological consequences for either *Artemia* or fish (Batel, Linti, Scherer, Erdinger, & Braunbeck, 2016). No studies have assessed the biological effects of a microplastic-contaminated diet on higher trophic levels in an ecologically relevant setting. Exposing animals to more environmentally relevant concentrations and evaluating behaviours in relation to an animal's habitat is important in providing realistic insights to the risks our marine ecosystems face.

We established a model food web to assess the effects of contaminated microplastics via trophic transfer. As microplastics accumulate on shorelines (Setälä, Norkko, & Lehtiniemi, 2016), coastal biota are exposed to them. Coastal ecosystems are often dominated by small crustaceans such as talitrid amphipods. In this study, coastal talitrids (beach hoppers, *Platorchestia smithi*), primary consumers that inhabit the sediment, were exposed to environmentally relevant concentrations of naturally contaminated microplastics and then fed to Krefft's frillgobies, *Bathygobius krefftii*, teleost fish that inhabit shallow coastal ecosystems. Beach hoppers

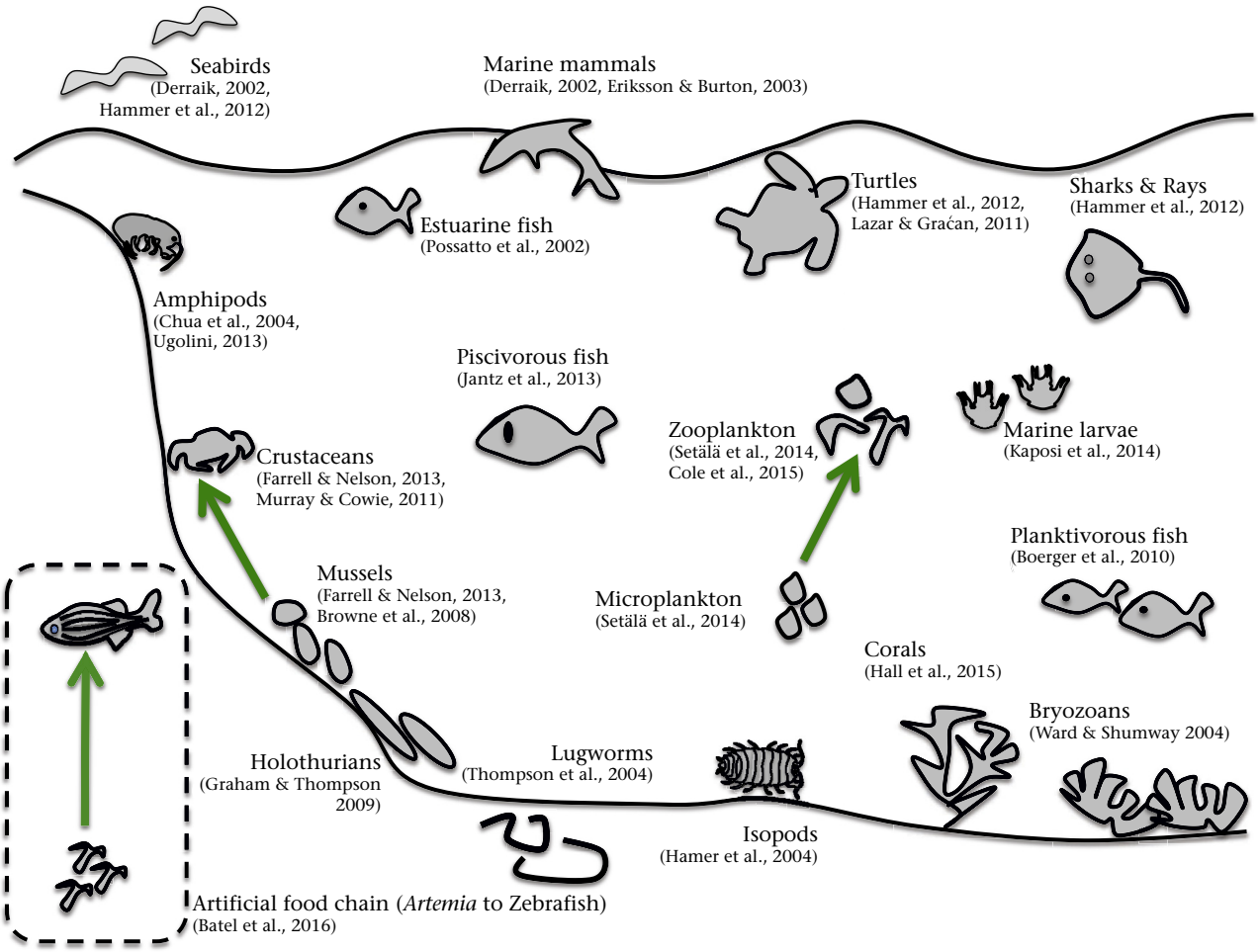

Figure 1. The range of marine biota that have been shown to ingest microplastics. The green arrows indicate where physical transfer of microplastic fragments has been demonstrated. The box in the dotted lines illustrates a recent study where transfer of microplastics and associated contaminants was demonstrated via BaP spiked microplastics in an artificial food chain study Hall et al. 2015.

are an important food source for birds (Dugan, Hubbard, McCrary, & Pierson, 2003), insects (Poore & Gallagher, 2013) and fish (Fanini & Lowry, 2014) and therefore important in the transfer of energy between the different trophic levels (Griffiths, Stenton-Dozey, & Koop, 1983). Gobies are intermediate predators that include amphipods in their diet (Souza, Dias, Marques, Antunes, & Martins, 2014), thus making the model suitable to assess trophic transfer of microplastics. This study used wild-caught fish rather than laboratory-raised animals to recreate a more realistic setting in assessing impacts of contamination. Fish came from an urban bay and so it is likely that some background levels of contaminants were present in them.

We assessed how transfer of a microplastic-contaminated diet affected fish personality. We used personality tests as a sensitive assay for assessing the impact of a microplastic-contaminated diet on fish behaviour. Personality is a major driver in population dynamics that can influence foraging success, reproduction and predator interactions (Budaev & Brown, 2011). Variation in personality traits such as activity or boldness can affect food acquisition and social encounters (Stamps, 2007), with bolder individuals benefiting in a foraging and social context (Smith, Miner, Wiegmann, & Newman, 2009). Some studies have found alterations in personality traits following exposure to pollutants. These studies include modifications to boldness in three-spined sticklebacks, *Gasterosteus aculeatus*, in response to increased carbon dioxide (Jutfelt, de Souza, Vuylsteke, & Sturve, 2013), increased activity and decreased sociality in European perch, *Perca fluviatilis*, following exposure to pharmaceuticals (Brodin, Fick, Jonsson, & Klaminder, 2013), changes to boldness and activity levels in female Siamese fighting fish, *Betta splendens*, after exposure to the hormone 17α-ethinylestradiol (Dzieweczynski, Campbell, Marks, & Logan, 2014) and changes in key male behaviours in Siamese fighting fish following treatment with the contraceptive *Ruta graveolens* (Forsatkar, Nematollahi, & Brown, 2016). A shift in the personality distribution within a population can have consequences for the local community; thus, changes to personality on an individual level may be an indicator of wider ecosystem effects (Weis, Smith, Zhou, Santiago-Bass, & Weis, 2001). Moreover, it is well established that hormone levels can influence boldness in fish (Chang, Li, Earley, & Hsu, 2012; King, Fürtbauer, Mamuneas, James, & Manica, 2013), and contaminants can alter endocrine systems, with many having a feminizing effect (Vos et al., 2000); thus, we predicted a shift in the personality of affected fish in response to ingestion of a microplastic-contaminated diet.

METHODS

Preparation of Microplastics

Commercial polyethylene (PE) microspheres (Cospheric UVPMS-BG, 1.004 g/ml density, nominal 38–45 μm diameter, colour green, Cospheric, Santa Barbara, CA, U.S.A.) were used as proxies for microplastics in marine environments (Kaposi, Mos, Kelaher, & Dworjanyn, 2014). To replicate microplastics in the marine environment, the microplastics were deployed in an urban bay in Port Jackson, Australia (33°50′24″, 151°15′13″) for 2 months. Microplastics were assessed post deployment in Port Jackson for polycyclic aromatic hydrocarbons (PAHs) as PAHs are one of the most widespread organic pollutants in the aquatic environment (Gonçalves, Scholze, Ferreira, Martins, & Correia, 2008). Following exposure in sea water, the microplastics contained 0.007 μg/g of PAHs, suggesting that the microplastics had absorbed a small amount of contaminants (Tosetto, Brown, & Williamson, 2016).

Study Animals

Platorchestia smithi were collected from March to July 2015 from the supralittoral zone of Forrester's Beach, NSW (33°24′37.06″, 151°28′05.39″). Details of transport and husbandry are provided in Tosetto et al. (2016). Beach hoppers in treatment groups were exposed to sediment that had an addition of contaminated PE microplastics at 3.8% (dry weight) while the beach hoppers in control groups were exposed to sediment only. Previous studies reporting negative effects on biota have used microplastics of over 5% wet weight (Besseling, Wegner, Foekema, van den Heuvel-Greve, & Koelmans, 2013; Graham & Thompson, 2009; Wright, Rowe, Thompson, & Galloway, 2013). The average distribution of microplastics on polluted beaches in Hawaii has been recorded as 3.3% by weight (Carson, Colbert, Kaylor, & McDermid, 2011). As microplastics continue to accumulate in coastal environments (Turra et al., 2014), the concentration of 3.8% microplastics in the current study was deemed an environmentally relevant concentration. Beach hoppers were exposed to treatments for 72 h (exposure period based on previous studies with amphipods (Chua, Shimeta, Nugegoda, Morrison, & Clarke, 2014; Hämer, Gutow, Köhler & Saborowski, 2014)). They readily ingested microplastics, which were found to accumulate in the beach hoppers. Following 72 h exposure there was a 30% increase in PAHs in the treatment group (3.09 μg/g) compared with the control group (2.34 μg/g; Tosetto et al., 2016).

Krefft's frillgobies are small, ray-finned marine fish that grow to a maximum length of 90 mm (Kuiter, 1993). Fish were caught using hand nets from Chowder Bay, Sydney, Australia (33°50′24″, 151°15′13″) in March and April 2015. A total of 33 fish were collected and acclimated in the laboratory for 2 months prior to testing. Individuals were housed in groups of eight in four flow-through sea water aquaria (640 × 420 mm and 260 mm deep: 70 litres), held in the flowthrough sea water facility at the Sydney Institute of Marine Science (SIMS), Australia. All aquaria were maintained at the same sea water flow rate (1 litre/min), temperature (16–22 °C), with a gravel substrate and aeration from an air stone. Fish were fed live black worms, *Lumbriculus variegatus*, during the 1-week settling period and then a mixture of frozen commercial 'Hikari Bio-Pure' Mysis shrimp, brine shrimp and black worms every other day as per White and Brown (2014). Tanks were physically enriched with terracotta pots and were lit from overhead fluorescent tubes for 12 h/day. Four weeks before testing, fish were lightly anaesthetized using a solution of 50 mg of MS222 buffered with sodium bicarbonate (fish were placed in a bucket containing 1.5 litres of solution until subdued). While anaesthetized their total length was measured and individuals were tagged using visible implant fluorescent elastomer tags (Northwest Marine Technology, Inc., Shaw Island, WA, U.S.A.) to assist identification. Gobies recover from tagging almost immediately and no long-term effects on behaviour are apparent (White & Brown, 2013). Tanks were cleaned weekly and all excess food removed after feeding.

Fish Behaviour Assays

Fish behaviour was tested using combined emergence (Brown & Braithwaite, 2004) and open-field trial tests (Archard & Braithwaite, 2011). These assays quantify a range of boldness and exploration variables that are used as proxies for overall personality in individual fish (Budaev & Brown, 2011). To maintain novelty across behavioural trials two slightly different arenas were established in 70-litre aquariums identical in size to the housing tanks for the boldness-exploration tests: (1) the bottom of the aquarium was covered with 5 cm of sand from a nearby beach and the sides lined with sand colour shade cloth (Coolaroo 1.83 m Sandstone 70%

UV Shade Cloth, Gale Pacific Australia, Braeside, VIC, Australia); and (2) shell grit (3 cm) covered the bottom of the tank and the sides lined with green turf (1.8 m wide Natural Synthetic Turf, Tuff Turf, Mentone, VIC, Australia). Fish were tested individually in both arenas. The aquarium was filled with 150 mm sea water. A start box was placed at one end of the arena. The start box (140 × 140 mm and 190 mm high) was made of dark plastic, had a lid to cover the top and a doorway (30 mm × 60 mm) on one side that could be covered with a sliding piece of black plastic. An air stone was placed behind the start box for aeration. After capture with a hand net from the housing tank, the test fish was placed into the start box, and the lid placed on top. The fish was left for a 2 min settling period after which a remote pulley lifted the door, allowing the individual to emerge from the doorway and explore the test arena. To eliminate impacts of human observers on fish behaviour, observers stood behind a screen and fish were observed remotely via a computer monitor connected by firewire (Belkin FireWire cable IEEE 1394) to a digital camera mounted over the test arena. At the end of the test period (600 s) fish were netted and returned to their housing tank.

Experimental Treatments

Six individuals died prior to microplastic exposure and so were excluded from the treatment groups; 28 fish remained for analysis of personality. All fish were put through behavioural trials before exposure to beach hopper treatments. The test was repeated three times prior to microplastic exposure: trials 1 and 3 were in arena 1 (sandy) and trial 2 in arena 2 (turf). There was at least a 7-day interval between each trial. Following initial tests, gobies were randomly assigned to two treatment groups and housed accordingly. We placed around 25 beach hoppers in each tank every 2 days (two–three beach hoppers per fish). We fed the fish with a pipette so that we could ensure that they all ate the beach hoppers. Tanks were observed until all fish had fed. The second round of testing commenced at the end of the first week of the beach hopper diet. Three trials were undertaken after microplastic exposure. Trials 4 and 6 were again undertaken in arena 1 and trial 5 in arena 2. There was at least 7 days between trials 4, 5 and 6 (Fig. 2).

Scoring Behaviour

Videos were scored for emergence, exploration and activity levels using Etholog (Ottoni, 2000). Emergence time was recorded when the entire fish (i.e. the last of the caudal fin) had emerged out of the start box. Fish that failed to emerge were given the maximum score of 600 s. To quantify exploration, i.e. the amount of time fish spent in various parts of the arena, a grid was placed over the tank that divided the arena into 10 sections (Fig. 3). The various behaviours scored are outlined in Table 1. The start box and cover

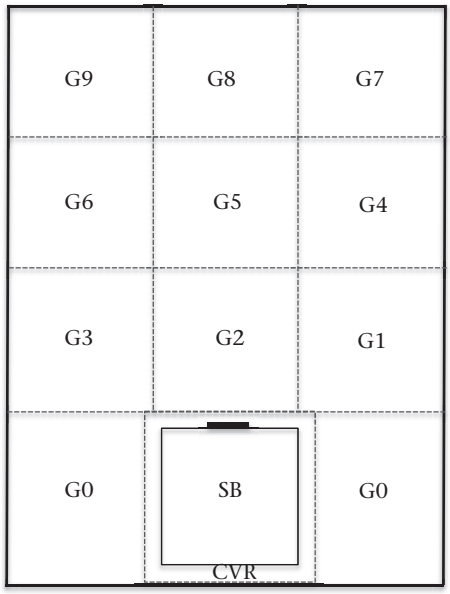

Figure 3. The test arena used for scoring fish boldness and exploration.

were considered safe areas for the fish, the top and middle rows were identified as risky, and the bottom row and centre were deemed dangerous as they were furthest from any cover. The total time spent in each quadrat was recorded, and a fish was not deemed to be in a new quadrat until the entire fish had moved into the area. The number of movements, each time the fish swam forwards or turned left or right, made by the fish over the test period was a proxy for activity.

Statistical Analysis

To test for the presence of personality, incorporating boldness and exploration, individual traits were collapsed into principal component scores using principal components analysis (PCA). PCA was undertaken using the princomp function in R (R Core Team, 2013). Eigenvectors with a value greater than one were used to describe the variance in the data set. Each retained principal component was then used as a composite behavioural variable in future analyses.

Agreement repeatability (R; the intraclass correlation coefficient) of component scores was calculated between trials using the within- and between-variance components in a linear mixed-effects model (LMM). The restricted maximum likelihood method (REML) was used in the package rptR, with individual fish identity as the grouping factor. Adjusted repeatability (R_A) was assessed

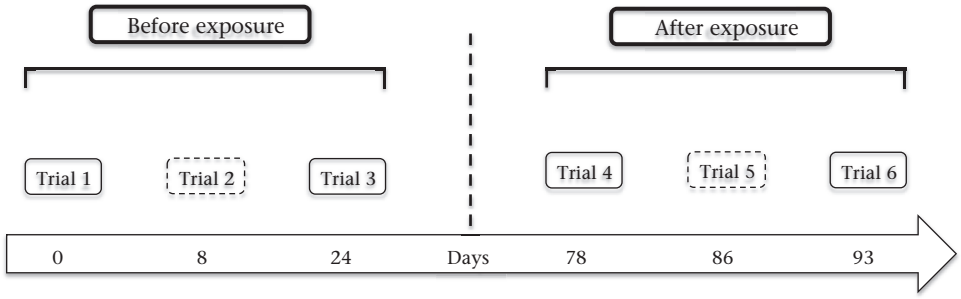

Figure 2. Schematic diagram of behavioural trials. The dashed line indicates where diet changed from commercial fish food to beach hoppers. Trials with solid lines indicate arena 1 (Sand) and dashed lines indicate arena 2 (Turf).

Table 1
The 10 variables used in the fish personality testing

Code	Variable	Description
FE	Emergence	The time for fish to emerge in full from the start box, recorded when the caudal fin was out of the box
SB	Start box	The total time (initial time + return time) is indicated here. The total time spent in the start box. The start box was a safe area and fish would return to the start box after emergence
TR	Top row	Total time fish spent in grids 1, 2 and 3
MR	Middle row	Total time fish spent in grids 4, 5 and 6
G5	Grid 5	Although related to MR, the centre of the arena was deemed one of the most dangerous areas for the fish to move into
CV	Cover	The total time spent exploring the area around the start box. Cover was defined as 2 cm around the start box
BR	Bottom row	Total time fish spent exploring the bottom of the arena, a combination of time spent in grids 7, 8 and 9
G0	Grid 0	Total time spent the top area of the arena once outside the cover of the start box
G8	Grid 8	Related to BR but like G5, a little riskier given it was in the centre of the arena
AC	Activity	The total movements made by the fish over the course of the test

across trials for each treatment group before and after exposure where size and days between trial were included as fixed effects and individual fish identity as the grouping factor. Confidence intervals and standard errors for both R and R_A were calculated from parametric bootstraps that created the distributions of likelihood ratios (1000 times). P values for repeatability estimates were derived from permutation tests (Nakagawa & Schielzeth, 2010). See the Supplementary Material for the R code for R and R_A.

We analysed data in line with their longitudinal nature (repeated measures over time) using linear mixed models (LMM; see the Supplementary Material for the R code). The PC1 scores were not normally distributed but because both positive and negative values were present in the data set the negative signs were removed before performing a log transformation on the PC1 values. The negative sign was replaced following transformation. As the PC2 scores were normally distributed, no transformation was carried out.

In the analysis, we first assessed whether there was a trend over time within each exposure (before and after exposure to the beach hopper diet) for PC1 and PC2. We built a model using days since the first observation as the principal variable of interest to quantify any changes in behaviour over time within each of the exposure periods. The variables treatment (control/treatment) and fish size were also included as fixed effects. To account for individual fish variability we included individual fish identity as a random effect. There was no trend over time before exposure to the contaminated diet or either PC1 ($P = 0.727$) or PC2 ($P = 0.621$). There was also no effect of fish size for either PC1 ($P = 0.738$) or PC2 ($P = 0.141$). Similarly, when we assessed any effects after exposure to a contaminated diet there was no effect of time for PC1 ($P = 0.812$) or PC2 ($P = 0.359$), nor was there an effect of fish size in either PC1 ($P = 0.999$) or PC2 ($P = 0.479$; Fig. 4).

As there was no effect of days or size within each exposure period we ran a final model that assessed changes in PC scores before and after exposure. Fixed effects were treatment and exposure with an interaction term between them. Random effects were individual fish identity.

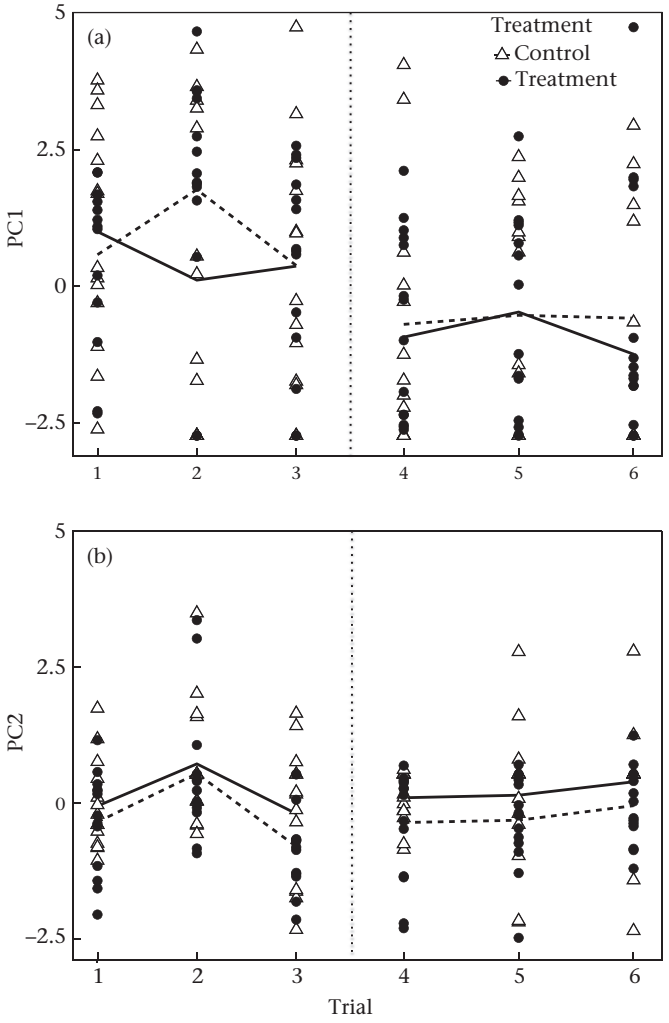

Figure 4. Effect of microplastic exposure on PC scores of gobies monitored in the six behavioural trials. The dotted line between trials 3 and 4 indicates where the diet changed to beach hoppers. (a) PC1 (boldness) scores. (b) PC2 (exploration) scores. White triangles indicate control individuals and black circles indicate treatment individuals. The solid lines indicate the population mean with the solid line representing control fish and broken lines representing treatment fish.

Ethical Note

Our experimental methods conformed to the standards set by Macquarie University Animal Ethics committee (ARA 2014/003-7). Fish and beach hopper collections were conducted under NSW Fisheries Scientific Collection Permit numbers P08/0010-4.2 and P14/0032-1.1, respectively. Fish were observed at least every second day to ensure their health and wellbeing and environmental housing conditions (water temperature, salinity and pH) were also regularly monitored. At completion of the study the fish were released to their original environment.

RESULTS

PCA condensed the variables to just two components that accounted for 56% of the total variance. Component score coefficients showed that boldness variables, such as emergence time, total start box time and time spent in more risky parts of the arena,

were the largest contributors to PC1. Exploratory variables, such as time spent exploring around the start box (cover) and the more exposed and furthest areas from the start box (bottom row), contributed most to PC2. Boldness contributed to almost half of the variance compared with exploratory variables that explained a relatively small proportion of the variance, suggesting the experiment was largely a boldness assay (Table 2).

Both repeatability (R) and adjusted repeatability (R_A) results are provided in Table 3. As fish size and number of days were not included in the final mixed model we used the repeatability (R) values in all further results. All trials were repeatable for PC1 before exposure ($R = 0.476$ (0.116), 95% CI 0.220 to 0.664, $P < 0.001$) and after exposure ($R = 0.684$ (0.085), 95% CI 0.474 to 0.816, $P < 0.001$) suggesting that the personality traits were robust. Only trials after exposure were repeatable for PC2 ($R = 0.401$ (0.119), 95% CI 0.129 to 0.603, $P < 0.001$). When repeatability was assessed for treatment and control groups separately, there was no significant difference in repeatability values for PC1 before or after exposure to treatments suggesting that consumption of prey items contaminated with microplastics did not affect repeatability of boldness traits. There was, however, an increase in repeatability values for PC2 in both treatments following exposure. This implies an effect of familiarization to the test arena rather than the exposure to a contaminated diet (Table 3).

Exposure to a microplastic-contaminated diet did not affect boldness or exploration in fish. No significant interactions occurred between exposure (before and after exposure to beach hoppers) and treatment (contaminated or not) for either PC1 (boldness: $t_{137} = -0.035$, $P = 0.891$) or PC2 (exploration: $t_{137} = 0.197$, $P = 0.557$). There was a significant effect of exposure to beach hoppers on the PC1 scores ($t_{27} = -0.418$, $P < 0.05$) with fish in both treatment and control groups decreasing in boldness following exposure to the beach hopper diet (Fig. 4). In scores indicating exploratory behaviour (PC2), there was a trend for fish in the control group to show greater exploratory tendencies across both exposures; however, this was not significant ($t_{27} = -0.594$, $P = 0.058$; Fig. 4).

DISCUSSION

This is the first study to assess possible behavioural consequences of trophic transfer of microplastics and associated contaminants, in this case through consumption of contaminated prey by a fish. As toxic contaminants found in plastics have been associated with endocrine disruption (Söffker & Tyler, 2012), we expected a shift in the behaviour and personality of affected fish. Here we have shown that, contrary to expectations, transfer of contaminated microplastics from beach hoppers to fish did not affect fish personality. There was, however, an overall shift in boldness with fish becoming shyer after exposure to the beach hopper diet irrespective of treatment. This was perhaps due to fish habituating to the experimental tasks, or possibly the alteration in diet. Exploration remained relatively constant before and after exposure possibly due to the variation in individuals between treatments, with control fish being more exploratory throughout. Repeatability values for boldness were not significantly different before or after exposure to treatments. Exploration repeatability values increased across both control and treatment groups after exposure suggesting acclimatization with the test arena.

Concern surrounding microplastics is largely due to the associated contaminants and their capacity to desorb into animals (Bakir, Rowland, & Thompson, 2014), and subsequently accumulate through the food web in different trophic levels (Teuten et al., 2009). The assumption that pollutants biomagnify at all levels of the marine food chain has been disputed (Gray, 2002) but the biomagnification of organic pollutants from lower trophic levels to fish has been demonstrated (Kelly, Ikonomou, Blair, Morin, & Gobas, 2007; Nfon et al., 2008). Whether microplastics are a viable pathway for contaminant exposure is currently under debate. Conceptual models assessing the rates of desorption in organisms suggest that microplastics do not provide a relevant exposure pathway (Gouin, 2011; Koelmans, 2013, 2014). However, positive relationships between microplastic consumption and increased contamination concentration in animal tissue have been demonstrated in seabirds (Ryan, Connell, & Gardner, 1988), amphipods (Chua et al., 2014) and bivalves (Avio et al., 2015). Furthermore, recent studies suggest that desorption rates of contaminants in gut surfactants are 30 times faster than in sea water for some contaminants, with pH and body temperature also influencing the rate (Bakir et al., 2014). Polyethylene microplastics deployed in the marine environment have the capacity to absorb contaminants, which can accumulate in beach hoppers following consumption, increasing the contaminant load of the organism (Tosetto et al., 2016). Given our current understanding of microplastics and contaminants we expected to see a change in fish personality following consumption of contaminated beach hoppers.

Using personality to assess anthropogenic impacts on individuals is a relatively new area of research. Personality is a major driver in population dynamics that can influence competitive interactions and how animals deal with changes in their environment (Montiglio & Royauté, 2014), particularly those attributed to anthropogenic impacts such as environmental contamination (Wong & Candolin, 2015). Assessing how contaminants alter personality traits provides a far subtler way of establishing effects in comparison to the standard LD50 approach commonly used in ecotoxicology (Oulton et al., 2014). Recent studies have observed sublethal effects of pollutants on behaviour (Brodin et al., 2013, 2014; Dzieweczynski et al., 2014; Forsatkar et al., 2016; Jutfelt et al., 2013); however, when assessing the effects of hormone 17α-ethinylestradiol on female Siamese fighting fish (Dzieweczynski et al., 2014) and increased carbon dioxide on three-spined sticklebacks (Jutfelt et al., 2013) some habituation to boldness assays over time was reported.

The current study did not observe significant effects of treatment on fish personality or the repeatability of these behaviours. There was, however, an alteration in fish behaviour following exposure to the beach hopper diet with all fish becoming shyer. The longer fish are exposed to a similar task the more inflexible behaviour comes, with less motivation to emerge and subsequently less time given to exploring an area (Kieffer & Colgan, 1992). Moreover, fish will decrease activity as they become familiar with a

Table 2
PCA loadings for each of the behaviour variables in the personality tests

Behaviour	Code	Boldness (PC1)	Exploration (PC2)
Full emergence	FE	**−0.372**	0.188
Start box	SB	**−0.423**	0.161
Cover	CV	0.280	**−0.514**
Bottom row	BR	0.246	**0.513**
Middle row	MR	**0.379**	0.260
Top row	TR	**0.365**	−0.146
Grids adjacent to start box	G0	0.213	−0.119
Centre grid	G5	0.285	0.091
Bottom centre grid	G8	0.287	**0.523**
Activity	AC	0.241	−0.164
Eigenvalue		4.99	1.44
Percentage variance explained		49.4	6.4

The corresponding code for each behaviour is provided. Variables in bold contribute the highest amounts to each behavioural variable.

Table 3
Repeatability estimates across trials for both Control and Treatment groups

Treatment	Exposure	PC	R (SE)	CI	P	R_A (SE)	CI	P
Control	Before	PC1	0.462 (0.163)	[0.089, 0.729]	**0.005**	0.493 (0.162)	[0.124, 0.735]	**0.003**
		PC2	0.072 (0.128)	[0.000, 0.047]	0.294	0.155 (0.146)	[0.000, 0.495]	0.184
	After	PC1	0.690 (0.126)	[0.361, 0.860]	**0.001**	0.686 (0.133)	[0.353, 0.855]	**0.001**
		PC2	0.328 (0.171)	[0.000, 0.642]	**0.017**	0.343 (0.175)	[0.000, 0.649]	**0.024**
Treatment	Before	PC1	0.498 (0.161)	[0.138, 0.746]	**0.004**	0.620 (0.145)	[0.254, 0.818]	**0.002**
		PC2	0.116 (0.131)	[0.000, 0.430]	0.210	0.293 (0.165)	[0.000, 0.590]	**0.041**
	After	PC1	0.688 (0.129)	[0.344, 0.853]	**0.001**	0.691 (0.146)	[0.282, 0.856]	**0.001**
		PC2	0.488 (0.166)	[0.096, 0.732]	**0.001**	0.470 (0.171)	[0.066, 0.731]	**0.008**

The table shows repeatability (R) and adjusted repeatability (R_A), standard errors (SE), the 95% confidence interval (CI) and associated P value of each estimate ($N = 14$ individual fish per treatment). Significant repeatability estimates are shown in bold.

tank environment (Brown, 2001; Sharma, Coombs, Patton, & de Perera, 2009). By varying the test arenas in trials 2 and 5 we attempted to reduce habituation to the behavioural assays. While some variation in boldness traits was observed in the trials before exposure, we did not see corresponding variation after exposure, suggesting fish had become familiarized to the task in the later trials. It is possible that the change in behaviour could be due to increased fear (Carter, Feeney, Marshall, Cowlishaw, & Heinsohn, 2013) but this is unlikely as the open-field trial was designed to mirror the ecological setting of *B. krefftii* which seek shelter in rock pools and crevices (White & Brown, 2015), and there was no novel object or risk of predation included in the assay. Alternatively, the alteration in diet may have affected the behaviour of the fish after exposure. Hunger, or perceived hunger, has been shown to increase activity and emergence times in fish (Brown & Braithwaite, 2004; Thomson, Watts, Pottinger, & Sneddon, 2012); thus, it is possible the gobies became satiated on beach hoppers in this study. Note that while there was a shift in fish boldness after exposure to the beach hopper diet, there was no difference across the treatment and control groups that could indicate an effect of our treatments on these processes.

It is possible that a longer exposure timeframe is required to observe an effect of microplastic exposure. When directly exposed to marine contaminated microplastics, Japanese meduka, *Oryzias latipes*, suffered hepatic distress but this was not obvious until 2 months after initial exposure, suggesting that a certain contamination threshold may be needed before a physiological effect is observed (Rochman, Hoh, Kurobe, & Teh, 2013). It is possible that the short duration of this study may not have been long enough to reach the contamination threshold for either *P. smithi* or *B. kreftii*. As these animals live up to 8 months and several years, respectively, it is possible they are exposed to far greater amounts of plastics and associated pollutants in their lifetime.

Alternatively, it is possible that background contamination levels in the wild-caught fish masked any environmental effects of microplastic exposure. Studies assessing contaminant transfer from microplastics to fish have fed microplastics directly to laboratory-raised fish that have not had prior exposure to environmental pollutants (Rochman et al., 2013). Moreover, a recent laboratory-based study assessing the capacity for microplastics and associated contaminants to transfer through an artificial food chain used concentrations of microplastics and contaminants at much higher levels than environmentally relevant concentrations and used animals raised in the laboratory environment (Batel et al., 2016). Theoretical models predict that the consumption of microplastics does not increase the burden of pollutants in fish given the background levels already present in the environment (Gouin, 2011). Recently Koelmans, Bakir, Burton, and Janssen (2016), following an assessment of the scientific literature, determined that the uptake of contaminants through natural pathways possibly exceeds accumulation via microplastics in most habitats. This implies that ingestion of microplastics is unlikely to increase an animal's exposure to contaminants in the marine environment. Using wild-caught fish is more realistic than using laboratory-raised animals in assessing ecological impacts of pollutants. As we used fish from an urbanized area in Sydney, Australia and fed a diet of commercial frozen food during acclimation, it is plausible that the experimental effects of plastic consumption were not discernible from the background levels in our wild-caught fish.

Gobies collected from highly polluted areas have shown differences in behavioural responses to stress than counterparts collected from less contaminated sites (Marentette, Tong, & Balshine, 2013). It is possible that fish with lower levels of background pollution may elicit a different response when exposed to contaminated microplastics. As we expect the distribution of microplastics to increase and expand through our marine ecosystems (Iñiguez, Conesa, & Fullana, 2016), the effects of microplastic exposure to wildlife from areas of low contamination should be explored. Studies have demonstrated that assessment of complex behaviours provides us with effective tools to assess sublethal levels of pollutants; however, future studies should also incorporate analysis of animal digestive tracts and tissues to establish whether microplastics are changing the contaminant load of animals through the food web.

Overall, this study found that short-term trophic transfer of microplastics via prey does not affect the personality of fish. (Brodin et al., 2014; Dzieweczynski et al., 2014; Jutfelt et al., 2013). We did observe a shift in boldness after exposure to the beach hoppers that may be attributed to familiarization, or a change in diet; however, there was no significant effect between the treatment groups after exposure. The problem of animals learning and habituating to an experiment will persist across any longitudinal study. To understand the broad impacts of plastic exposure we need to take an all-inclusive approach to behavioural experiments that assess the impacts of microplastics on behaviours applicable to foraging, sexual activity, sociality and learning. Future long-term, ecologically relevant studies should directly examine, via tissue analysis, whether microplastics and contaminants are accumulating through the food web. A suite of personalities and behaviours should also be assessed to get a more comprehensive picture of changes in behaviour in response to microplastics through different trophic levels. It is possible, however, given the background levels of contamination already present in coastal environments, that trophic transfer of microplastics does not provide an additional exposure pathway for contaminants to move through the food web.

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Supplementary Material

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