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Animal behaviour

Microplastic leachates impair behavioural vigilance and predator avoidance in a temperate intertidal gastropod

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Microplastics are a ubiquitous source of contaminations in marine ecosystems, and have major implications for marine life. Much effort has been devoted to assessing the various effects of microplastics on marine life. No evidence exists, however, on the effects of microplastic leachates on chemically mediated predator–prey interactions and the ability of prey to detect and avoid its predator. This study shows that microplastic leachates have direct biological effects by disturbing the behavioural response of the intertidal gastropod *Littorina littorea* to the presence of *Carcinus maenas* chemical cues, hence increasing their vulnerability to predation. Leachates from virgin and beached pellets respectively impaired and inhibited *L. littorea* vigilance and anti-predator behaviours. These results suggest that the biological effects from microplastic leachates may have major implications for marine ecosystems on taxa that rely on chemosensory cues to escape predation.

1. Introduction

Plastics are a major source of global marine pollution [1]. They have conspicuous effects such as the deposition of beached debris [2] and the entanglement of marine fauna [3]. Microplastics (i.e. plastic particles less than 5 mm) are a more recent and pernicious source of pollution, persistent and nearly ubiquitous in marine systems [1]. One of the greatest concerns about microplastics in marine environments is their effect on marine organisms [3]. Microplastics are a vector of chemical pollutants adsorbed onto their surface. Their ingestion by organisms as small as zooplankton prompts the desorption of these chemicals which cause adverse effects [4]. They also accumulate into the tissues and organs, hence cascade through the food chain [5]. The effects of microplastics through the release in the environment of additives used in their manufacture [6] and the contaminants that adsorb and accumulate onto their surface [7] have been far less studied. They were essentially inferred through experiments assessing the toxicity of leachates from new plastic consumer products to aquatic invertebrates [8]. The potential effect of microplastic leachates is critical as most plastic contamination in the ocean is made of microplastics, which tend to accumulate more persistent pollutants than large debris [9].

A key component of microplastic pollution is raw resin pellets used in the manufacture of plastic products [10]. These pellets are found on beaches worldwide, and accumulate various types of persistent pollutants [11]. However, only a few studies have assessed the toxicity of leachate from plastic pellets, and found high toxicity to the embryonic development of marine invertebrates [12,13]. So far, no evidence exists on the potential effect of plastic pellet leachate on predator–prey interactions, in particular on the ability of prey to detect and avoid its predator. These species-specific behaviours are typically mediated by chemical cues [14], whose detection is compromised by chemical pollution [15]. This study assessed experimentally whether prey vigilance and

Table 1. Statistical assessment of the differences between experimental treatments for each behavioural variable.

behavioural variable	treatment	statistic	d.f.	*p*
righting time	overall (Kruskal–Wallis)	$H = 196.7$	5	<0.001
	SW versus SW_{CC}	$U = 0$	2	<0.001
	SW versus SW_{VP}	$U = 1088$	2	>0.05
	SW versus SW_{BP}	$U = 830.5$	2	>0.05
	SW versus $SW_{CC\text{-}VP}$	$U = 126.5$	2	<0.001
	SW versus $SW_{CC\text{-}BP}$	$U = 966.5$	2	>0.05
	SW_{CC} versus SW_{VP}	$U = 0$	2	<0.001
	SW_{CC} versus SW_{BP}	$U = 0$	2	<0.001
	SW_{CC} versus $SW_{CC\text{-}VP}$	$U = 133.5$	2	<0.001
	SW_{CC} versus $SW_{CC\text{-}VP}$	$U = 0$	2	<0.001
	SW_{VP} versus SW_{BP}	$U = 1012$	2	>0.05
	SW_{VP} versus $SW_{CC\text{-}VP}$	$U = 139$	2	<0.001
	SW_{VP} versus $SW_{CC\text{-}BP}$	$U = 1191$	2	>0.05
	SW_{BP} versus $SW_{CC\text{-}VP}$	$U = 76$	2	<0.001
	SW_{BP} versus $SW_{CC\text{-}BP}$	$U = 1048$	2	>0.05
	$SW_{CC\text{-}VP}$ versus $SW_{CC\text{-}BP}$	$U = 0$	2	<0.001
time to explore	overall (Kruskal–Wallis)	$H = 209.2$	5	<0.001
	SW versus SW_{CC}	$U = 0$	2	<0.001
	SW versus SW_{VP}	$U = 1073$	2	>0.05
	SW versus SW_{BP}	$U = 1240$	2	>0.05
	SW versus $SW_{CC\text{-}VP}$	$U = 10$	2	<0.001
	SW versus $SW_{CC\text{-}BP}$	$U = 988.5$	2	>0.05
	SW_{CC} versus SW_{VP}	$U = 0$	2	<0.001
	SW_{CC} versus SW_{BP}	$U = 0$	2	<0.001
	SW_{CC} versus $SW_{CC\text{-}VP}$	$U = 7$	2	<0.001
	SW_{CC} versus $SW_{CC\text{-}VP}$	$U = 0$	2	<0.001
	SW_{VP} versus SW_{BP}	$U = 1043$	2	>0.05
	SW_{VP} versus $SW_{CC\text{-}VP}$	$U = 10$	2	<0.001
	SW_{VP} versus $SW_{CC\text{-}BP}$	$U = 1177$	2	>0.05
	SW_{BP} versus $SW_{CC\text{-}VP}$	$U = 0$	2	<0.001
	SW_{BP} versus $SW_{CC\text{-}BP}$	$U = 982$	2	>0.05
	$SW_{CC\text{-}VP}$ versus $SW_{CC\text{-}BP}$	$U = 0$	2	<0.001
skioptic withdrawal	overall (Kruskal–Wallis)	$H = 203.3$	5	<0.0001
	SW versus SW_{CC}	$U = 0$	2	<0.0001
	SW versus SW_{VP}	$U = 1046$	2	>0.05
	SW versus SW_{BP}	$U = 1208$	2	>0.05
	SW versus $SW_{CC\text{-}VP}$	$U = 129$	2	<0.0001
	SW versus $SW_{CC\text{-}BP}$	$U = 1196$	2	>0.05
	SW_{CC} versus SW_{VP}	$U = 0$	2	<0.0001
	SW_{CC} versus SW_{BP}	$U = 0$	2	<0.0001
	SW_{CC} versus $SW_{CC\text{-}VP}$	$U = 359.5$	2	<0.0001
	SW_{CC} versus $SW_{CC\text{-}VP}$	$U = 0$	2	<0.0001
	SW_{VP} versus SW_{BP}	$U = 1007$	2	<0.0001
	SW_{VP} versus $SW_{CC\text{-}VP}$	$U = 182.5$	2	<0.0001
	SW_{VP} versus $SW_{CC\text{-}BP}$	$U = 1124$	2	>0.05
	SW_{BP} versus $SW_{CC\text{-}VP}$	$U = 121$	2	<0.0001

(*Continued*.)

Table 1. (*Continued.*)

behavioural variable	treatment	statistic	d.f.	p
	SW_{BP} versus $SW_{CC\text{-}BP}$	$U = 1160$	2	>0.05
	$SW_{CC\text{-}VP}$ versus $SW_{CC\text{-}BP}$	$U = 171.5$	2	<0.0001
avoidance response	overall (Kruskal–Wallis)	$H = 201.3$	5	<0.001
	SW versus SW_{CC}	$U = 0$	2	<0.001
	SW versus SW_{VP}	$U = 1127$	2	>0.05
	SW versus SW_{BP}	$U = 747.5$	2	>0.05
	SW versus $SW_{CC\text{-}VP}$	$U = 0$	2	<0.001
	SW versus $SW_{CC\text{-}BP}$	$U = 1019$	2	>0.05
	SW_{CC} versus SW_{VP}	$U = 0$	2	<0.001
	SW_{CC} versus SW_{BP}	$U = 0$	2	<0.001
	SW_{CC} versus $SW_{CC\text{-}VP}$	$U = 64.5$	2	<0.001
	SW_{CC} versus $SW_{CC\text{-}VP}$	$U = 0$	2	<0.001
	SW_{VP} versus SW_{BP}	$U = 976$	2	>0.05
	SW_{VP} versus $SW_{CC\text{-}VP}$	$U = 4.5$	2	<0.001
	SW_{VP} versus $SW_{CC\text{-}BP}$	$U = 1170$	2	>0.05
	SW_{BP} versus $SW_{CC\text{-}VP}$	$U = 10$	2	<0.001
	SW_{BP} versus $SW_{CC\text{-}BP}$	$U = 1146$	2	>0.05
	$SW_{CC\text{-}VP}$ versus $SW_{CC\text{-}BP}$	$U = 9$	2	<0.001

antipredator behaviour of the intertidal gastropod *Littorina littorea* is impaired by leachates from virgin and beached plastic pellets.

2. Material and methods

Littorina littorea collected in August 2016 from mid-intertidal rockpools at the Pointe du Noirda (50°49′45.15 N, 1°35′19.366 E)—a reef typical of the French coast of the eastern English Channel—were exposed to a factorial treatment of normal versus microplastic leachates seawater and the presence of chemical cues from the predatory crab *Carcinus maenas*. Snails (mean shell length 10.1 ± 1.2 mm) were acclimated without food for 24 h in the laboratory in 120 l tanks of running natural seawater at 18°C, and each individual was only used once.

Microplastic leachate seawater was prepared from commercially available virgin polypropylene pellets (SW_{VP}), and from beached pellets (SW_{BP}) haphazardly collected from the high-tide mark sediment surface of beaches surrounding the reef. Pellets (diameter 3.3–4.7 mm) were mixed with seawater at a concentration of 20 ml of pellets per litre—a concentration one order of magnitude lower than those that impaired the embryonic development of mussels and sea urchins [12,13], which is not uncommon in the sampling area—and left for 24 h before the beginning of the behavioural assays. Crab cue seawater (SW_{CC}) was prepared through the addition of an individual *C. maenas* (carapace width 80.3 ± 3.3 mm) into 5 l of aerated seawater for a 48 h period prior to the trial. Crabs were previously fed *L. littorea* for a period of 10 days in the laboratory to stimulate predator-induced alarm responses [16]. Mixtures of SW_{CC} and SW_{VP} ($SW_{CC\text{-}VP}$) and SW_{CC} and SW_{BP} ($SW_{CC\text{-}BP}$) were prepared at a 1 : 1 ratio using crab cues and microplastic leachates that were twice as concentrated as in SW_{CC} and SW_{VP} to ensure they were present at the same concentrations than in other experiments.

Individual snails were placed in 500 ml borosilicate beakers filled with 300 ml of control and cue seawater for a 30 min acclimation period. Three measures of prey vigilance and predator detection were investigated [17]. First, snails were dislodged using tissue forceps, placed onto their dorsal surface, and the righting time needed to regain contact with the substrate was taken in each treatment ($N = 50$). Second, snails were dislodged, immediately placed into a beaker filled with the same water treatment, and the time to explore determined as the time taken by snails to re-emerge from their shell, extend their tentacles and began to actively crawl along the substrate ($N = 50$). Finally, the skioptic (i.e. shadow-induced) withdrawal response was measured by passing a shadow over a snail actively crawling along the substrate. A 35 W halogen light was placed 50 cm above 500 ml borosilicate beakers filled with 300 ml of control and cue seawater. A 20 cm diameter black PVC piece was placed above experimental beakers for 5 s. Withdrawal was successful if the snail completely retracted into their shell within the 5 s interval. Each snail was assessed 10 times at a 1 min interval and withdrawal quantified as the percentage of individuals that successfully withdrew ($N = 50$).

The percentage of time spent in a refuge or either above or at the water surface was used as a measure of avoidance [18]. These trials were performed in glass aquaria (15 × 15 cm, filled with 1100 ml of control and cue seawater) where PVC tubes (length 8 cm, diameter 5 cm) cut in half and glued down onto the centre of the aquarium created a refuge. Snails were placed next to the refuge, and left to acclimatize for 30 min. Their position was subsequently noted every 5 s for an hour after the addition of 100 ml of cue or non-cue water.

Individual snails were randomly exposed to control seawater (SW) and cue seawater (SW_{VP}, SW_{BP}, SW_{CC}, $SW_{CC\text{-}VP}$ and $SW_{CC\text{-}BP}$) to avoid the effect of trial order on responses. Behavioural traits were also investigated randomly. As circatidal rhythms could be a confounding factor, all experiments were conducted within a 2-h window before and after high-tide. As none of the four behavioural measures conformed to the normality assumption (Kolmogorov–Smirnov test, $p < 0.05$), multiple comparisons between treatments were conducted using the Kruskal–Wallis

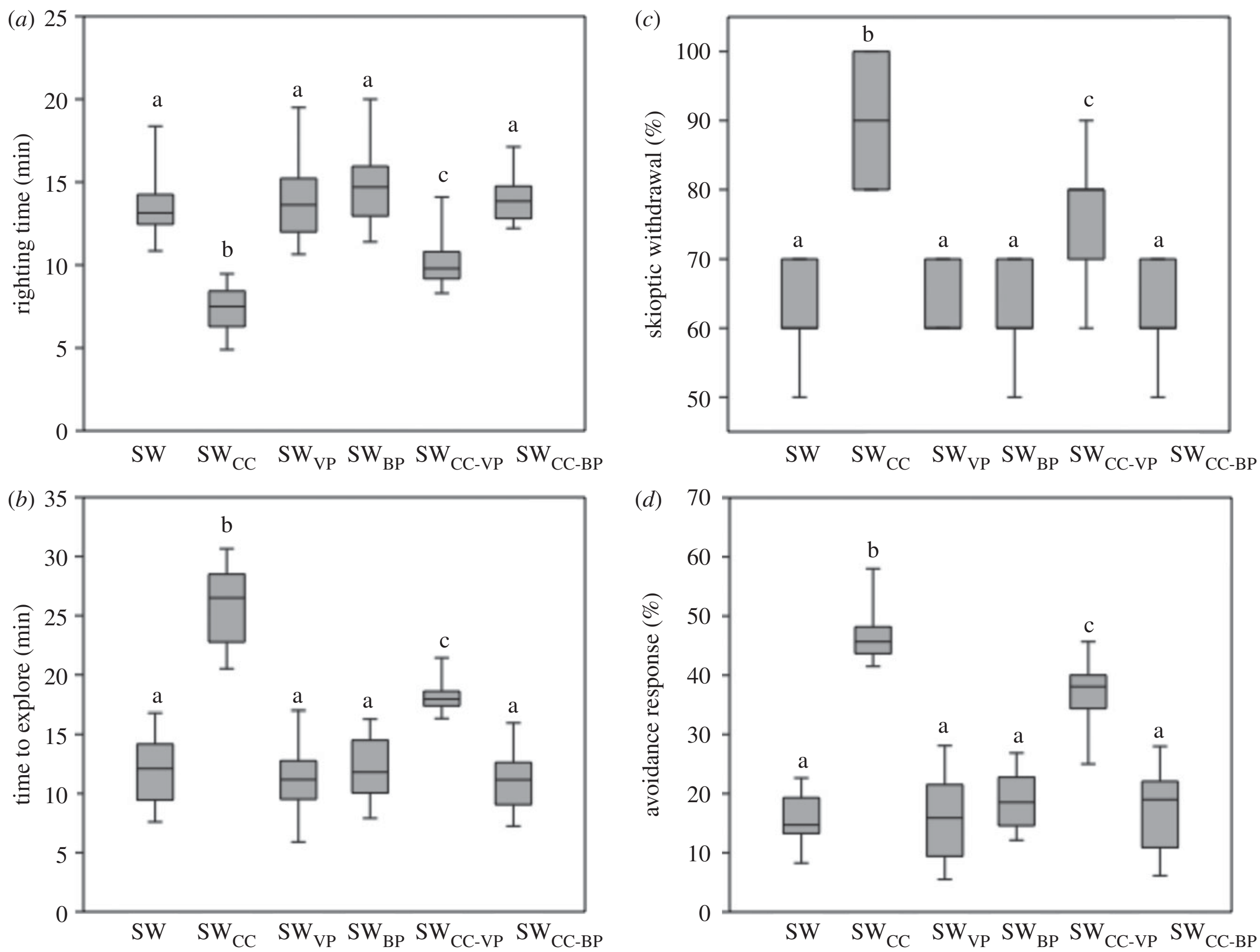

Figure 1. Behavioural responses of *Littorina littorea* to control seawater (SW), seawater conditioned with *Carcinus maenas* cues (SW_{CC}), leachates from virgin (SW_{VP}) and beached (SW_{BP}) polypropylene pellets, and a 1 : 1 mixture of SW_{CC} and SW_{VP} ($SW_{CC\text{-}VP}$) and SW_{CC} and SW_{BP} ($SW_{CC\text{-}BP}$). The letters 'a', 'b' and 'c' indicate significantly distinct groups of measurements. The box represents the 25–75 quartiles, the median is shown with a horizontal line inside the box and the vertical lines indicate the minimal and maximal values.

test, followed by a Mann–Whitney *U*-test to check for significant pairwise differences.

3. Results and discussion

Carcinus maenas cue had a significant effect (table 1) on vigilance and antipredator responses of *L. littorea*. Snails exposed to crab cue (SW_{CC}) significantly decreased their righting time compared with snails in control seawater (SW, figure 1*a*). Similarly, snails exposed to SW_{CC} took significantly longer to emerge from their shells than following SW exposure (figure 1*b*) and elicited a full body withdrawal more often when presented a passing shadow that did snails in SW (figure 1*c*). *Littorina littorea* exhibited antipredator behaviour significantly more frequently in SW_{CC} than in SW (figure 1*d*). These data demonstrate that *L. littorea* significantly increased their vigilance and antipredator responses following exposure to SW_{CC} [19,20].

Leachates from both virgin (SW_{VP}) and beached pellets (SW_{BP}) did not elicit any significant change in snail behaviour in SW (figure 1). In turn, snails exposed to $SW_{CC\text{-}VP}$ significantly decreased vigilance and antipredator responses compared to those exposed to SW, SW_{VP} and SW_{BP} (table 1). These responses were, however, less pronounced than following an exposure to crab cue (table 1). Finally, *L. littorea* did not significantly modify their behavioural responses when exposed to $SW_{CC\text{-}BP}$ compared with SW, SW_{VP} and SW_{BP} (table 1, figure 1). These results suggest that leachates from virgin and beached microplastic pellets did not affect *L. littorea* neuromuscular performance, while they respectively impaired and inhibited their ability to respond to predator cues through a decrease in their chemosensory abilities.

The alteration observed in *L. littorea* responses likely results from different contaminants and concentrations in the leachates from virgin and beached pellets. Virgin pellets essentially contain plastic additives (e.g. ultraviolet stabilizers and antimicrobials) that are adsorbed with the polymer, hence are very likely to leach to the water and cause a toxic effect [8]. In turn, beached pellets adsorbed persistent organic pollutants, polycyclic aromatic hydrocarbons and heavy metals onto their surface at concentrations higher than those found in the environment [7]. As a result the leachate from beached pellets is likely to contain a more complex mixture of contaminants, and at higher concentrations, than the leachate from virgin pellets. This hypothesis is consistent with the observed more pronounced effects of leachates from beached than from virgin pellets. The relatively high (though locally realistic) pellet concentration considered here and the lack of information on the actual pollutants at play stress the need to assess *L. littorea* behavioural traits in response to various leachates concentrations and specific pollutants that are both likely to be seasonal and site-dependent [12]. Note, however, that a 24-h incubation time is extremely short compared to the residence times of microplastic pellets in the ocean [1,2], which may indicate that the pollutant concentrations assessed here are not irreconcilable with *in situ* concentrations. Microplastics are also known to accumulate in intertidal gastropods [21]. Potential leachates from ingested microplastics hence warrant the need for further studies to decipher the effect of extrinsic versus intrinsic microplastic leachates. These results suggest that microplastic pellet leachates only impact the behaviour of *L. littorea* when predator cues are present. Microplastic pollution may have major implications for marine ecosystems through direct effects on taxa that rely on chemosensory cues to escape predation. This is crucial as the effects of anthropogenic stressors on species interactions could cascade up and/or down foodwebs. *Littorina littorea* being a commercial species of gastropod across much of Europe, these findings also have potential industrial relevance.

Data accessibility. The dataset supporting this article has been uploaded as part of the electronic supplementary material.
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