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Impact of microplastic leachates on the anti-predator behaviour of the intertidal limpet *Cellana nigrolineata*

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Abstract The anti-predator behaviour of *Cellana nigrolineata* was assessed in laboratory-controlled conditions in response to the presence of the whelks *Reishia clavigera* and *Tenguella musiva* in natural seawater and in leachate solutions prepared from commercially available polypropylene (PP), polyethylene (PE), polyamide (PA) and polylactic acid (PLA) pellets. Our results first showed that *C. nigrolineata* used both chemosensory and contact cues to react to the presence of *R. clavigera* and *T. musiva*. The anti-predator response of *C. nigrolineata* was much stronger towards *R. clavigera* than *T. musiva*. We also identified a new escape behaviour of *C. nigrolineata*, which was able to climb on top of the predator shells before flying away. We subsequently showed that leachate solutions of three conventional polymers (PP, PE and PA) and one biosourced and biodegradable polymer (PLA) respectively inhibited and impaired the ability of *C. nigrolineata* to react to *R. clavigera* and *T. musiva* chemical cues. The anti-predator responses elicited by contact between the whelk foot and the limpet epipodial tentacles were both polymer- and species-dependent, with a similar effect of polymers and a stronger effect of PP and PE leachate solutions. Taken together, our results indicate that microplastic pollution is likely to affect non-lethal predator-prey interactions in a species- and polymer-specific fashion.

Keywords: behaviour, predator-prey interaction, *Reishia clavigera*, *Tenguella musiva*, plastic pollution, leachates

Introduction

Predators impact prey species not just through lethal (consumptive) effects but also through non-lethal (non-consumptive) effects. The latter occur when prey survives an encounter by modifying one or more traits following the detection of a predator (Werner and Peacor, 2003; Miner et al., 2005; Peckarsky et al., 2008; Hawlena and Schmitz, 2010). These lethal and non-lethal interactions fundamentally induce short- and long-term effects on physiological, reproductive and overall performance of individuals. They also impact the demography and distribution of prey population, the structure, diversity and dynamics of communities and the functioning of entire ecosystems; see, e.g., Sih et al. (1985, 1990), Chase et al. (2002), Hawlena and Schmitz (2010) and Taylor (2013). The influence of predation has been particularly well documented for rocky shores globally and has long been acknowledged as a key factor determining the structure of intertidal assemblages (Paine, 1974, 1994; Connell, 1972; Chilton and Bull, 1984; Paine, 1974; Sih et al., 1985; Little and Kitching, 1996; Yamada and Boulding, 1996; Raffaelli and Hawkins, 1996; Connolly and Roughgarden, 1999; Robles and Desharnais, 2002; Little et al., 2009; Hawkins et al., 2019). In the marine realm in general and intertidal ecosystems in particular, prey species have noticeably developed a range of strategies to minimize and eventually avoid predation. These include escaping predators in space or time or by differences in size, using morphological and structural deterrents, or using chemical deterrents (Duffy and Hay, 2001).

Specifically, limpets have developed a range of anti-predator responses. These include the use of chemical deterrents (Branch and Cherry, 1985; McQuaid et al., 1999) and mucus capable of stunning predators (Rice, 1985), as well as aggressive behaviour clamping the shell down on the predator's foot (Stimson, 1970; Branch, 1979). They also exhibit fast escape response that allow to outrun their predators (Iwasaki, 1993; Escobar and Navarrete 2011; Aguilera et al., 2019), and clamping onto the rock eventually using home scars which further improves the efficiency of the clamp mechanism (Garrity and Levings 1983; Iwasaki 1993; Williams and Morritt 1995; Espoz and Castilla 2000). Limpets also form aggregates (Coleman et al., 2004) and engaging in various shell movements such as ‘mushrooming’, ‘shell rocking’ and ‘stomping’ which respectively involve (i) a limpet to extend its epipodial tentacles and raise its shell above the substrate, a behaviour often followed by mantle folding (i.e., the extension of the mantle edge by rolling it over the edge of the shell to cover all or part of the shell surface), (ii) spin its shell from side to side and (iii) suddenly smashing the edge of the shell down upon the predator (Espoz and Castilla, 2000; Mahon et al., 2002; Markowska and Kidawa, 2007). Mushrooming, rocking and stomping may noticeably lead to dislodge predator such as starfish, shake a predator off limpet’s shell and seriously damage and deter the predator (Hawkins and Jones, 1992; Little et al., 2009). Some species even use the abovementioned processes serially or simultaneously. This is noticeably the case for the limpet *Cellana nigrolineata* (Reeve, 1854), a species characteristic of the northwestern coasts of the Pacific Ocean (Sasaki, 2017) and commonly found in the mid-intertidal zone of the rocky shores of the Wakayama Prefecture (Asakura et al., 2018), which typically respond to the predatory whelks *Reishia clavigera* and *Reishia bronni* by sequentially mushrooming, shell rocking, mantle folding and escaping (Sogame et al., 2009).

The ability of an organism to assess and react to predator cues strongly influences the decision of when and how long/far to escape from predators (Lima and Dill, 1990; Lima, 1998; Ferrari et al., 2010), hence impacts predator-prey interactions, both predator and prey populations, and can ultimately propagate through the entire food web (Trussell et al. 2003; Dee et al. 2012; Manzur et al. 2014; Weissburg et al. 2014). In this context, there is a growing awareness of the ubiquity and toxicity of plastic compounds and their leachates (i.e., the cocktail of chemical compounds released by plastic items in the environment; see Delaeter et al. (2022) for a review) in marine systems (Gall and Thompson, 2015; Jamieson et al., 2017; Gunaalan et al., 2020; Delaeter et al., 2022; Seuront et al., 2022). Recent evidence indicates that an exposure to microplastic leachates impairs the ability of the common periwinkle *Littorina littorea* (Seuront, 2018) and the blue mussel *Mytilus edulis* (Uguen et al., 2022) to recognize the chemical cues of their predators. Despite these, we are still critically lacking information on how these chemicals may affect anti-predator behaviour of intertidal invertebrates and limpets in particular.

In this context, the rationale behind the present work is two-fold:

First, we assess the defensive behaviour of the intertidal limpet *C. nigrolineata* in response to the presence of the whelks *R. clavigera* and *Tenguella musiva*. These species typically co-occur on the intertidal rocky shores of the Wakayama Prefecture (Asakura et al., 2018). The muricid gastropods *R. clavigera* and *T. musiva* are often described as essentially feeding on sessile invertebrates such as barnacles and bivalves; see, e.g., Abe (1980, 1989), Blackmore and Morton (2002), Tomatsuri and Kon (2015), Astudillo et al. (2018) and Li et al. (2020). There is nevertheless a growing body of evidence that both *R. clavigera* and *T. musiva* are also preying on the motile true limpets *Patelloida pygmaea* (Taylor and Morton, 1996), *Cellana grata* and *Cellana toreuma* (Abe, 1983; Iwasaki, 1993), the false limpets *Siphonaria japonica* and *Siphonaria sirius* (Taylor and Morton, 1996), *Siphonaria atra* (Lam, 2002; Chim and Ong, 2012), *Siphonaria guamensis* and *Siphonaria javanica* (Chim and Ong, 2012) as well as other highly motile organisms such as the gastropods *Monodonta labio*, *Nerita albicilla*, *Nerita undata*, *Nodillitorina radiata* and *Nodillitorina trochoides* (Lam, 2002; Chim and Ong, 2012; Lai et al., 2018), and even the isopod *Ligia* sp. (Chim and Ong, 2012). To the best of our knowledge, the defensive behaviour of *C. nigrolineata* has only been described in response to *R. clavigera* and *R. bronni* (Sogame et al., 2009), and no information is available on the potential predatory interactions between *C. nigrolineata* and *T. musiva*. Observations conducted on the rocky shores of the Wakayama Prefecture nevertheless indicate that *T. musiva* occasionally preys on *C. nigrolineata* (Fig. 1), although this is typically observed far less commonly than for *R. clavigera* (Tomoyuki Nakano, personal observations).

Second, in an era of ever-growing anthropogenic pressure on both terrestrial and aquatic ecosystems (see e.g., Häder et al. (2020, 2021) and Rillig et al. (2021) for reviews), plastic pollution has become one of the most ubiquitous sources of both contamination and pollution of the Anthropocene, threatening both terrestrial and aquatic environments, the economy and human well-being on a global scale (Marks et al., 2020; Frias et al., 2021; Kumar et al., 2021). Beyond the extent and conspicuousness of plastic pollution, the effect of plastic leachates, i.e., the cocktail of potentially harmful molecules that are released from the surface of a polymer and/or absorbed into the polymer matrix, is still a relatively untapped area of research in particular when it comes to interspecific interactions (see Delaeter et al. (2022) for a review). In this context, we assess how an exposure to microplastic leachates from conventional polymers (polypropylene, polyethylene and polyamide) based on their prevalence in intertidal environments (Delaeter et al., 2022) and a biosourced and biodegradable polymer (polylactic acid) putatively considered as an ecofriendly alternative may impact the observed behavioural response.

Figure 1. Field observation of the gastropod *Tenguella musiva* preying on *Cellana nigrolineata*. Source: Tomoyuki Nakano.

Materials and Methods

Sampling

Cellana nigrolineata (16.5 ± 2.8 mm, shell length; mean ± SD), *R. clavigera* (32.3 ± 3.2 mm) and *T. musiva* (21.5 ± 2.2 mm, shell length) were sampled from an intertidal rocky platform located north of Shirahama Beach, Wakayama Prefecture (33°41’03N, 135°20’26E) and acclimatized for 1 h in natural seawater until the behavioural experiments took place following Sogame et al. (2009).

Microplastic leachate treatments

The behavioural response of *C. nigrolineata* to the presence of *R. clavigera* and *T. musiva* was assessed either in control seawater or microplastic leachate seawater. Microplastic leachate seawater solutions were prepared using commercially available virgin pellets from (i) three conventional polymers, i.e., polypropylene (PP; Pemmiproducts, Aachen, Germany), low-density polyethylene (PE; Materialix Ltd, London, UK), and polyamide (PA; Akulon F136-C) and (ii) one biobased and biodegradable polymer, i.e., polylactic acid (PLA; NatureWorks LLC, IngeoTM 4043D). Microplastic pellets were consistently mixed with control seawater at a concentration of 20 mL of pellets per litre and aerated for 24 h before the beginning of the behavioural assays (Seuront 2018; Seuront et al., 2020). The key driver of the desorption of the molecules that are adsorbed onto the surface of a polymer and/or absorbed into the polymer matrix (hence their release in seawater) is the surface area (Seuront et al., 2022). In this context, it is key to ensure that the four different types of pellets used has comparable surface areas, which was the case given the similarity of their spherical shape and size (typically 3 to 4 mm in diameter). The polypropylene, polyethylene, polyamide and polylactic acid leachate solutions were respectively referred to as SW_{PP}, SW_{PE}, SW_{PA} and SW_{PLA} hereafter.

Chemical assessment of the additive composition of microplastic pellets

The identification of the additives content of the plastic pellets was assessed using a CDS Pyroprobe 6150 pyrolyzer (CDS Analytical) in association with a GC-HRMS instrument (GC Trace 1310-MS Orbitrap Q Exactive, Thermo Fisher Scientific). Thermal desorption was performed (350 °C) to remove the potential additives from the samples. The samples were then separated using a Restek Rxi-5-MS capillary column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) with a cross-linked poly 5 % diphenyl-95 % dimethylsiloxane stationary phase (slip ratio: 1:5), and the acquisition was conducted on full-scan (FS) mode (m/z = 30.00000 – 600.00000). The resulting chromatograms were analyzed using Xcalibur and TraceFinder software for the identification of organic plastic additives among a selection of 57 additives (i.e., plasticizers, flame retardant, antioxidants and UVs stabilizers). The subsequent identification of the additives was based on their retention times, m/z values, and specific ions, which were compared with the chromatograms obtained from standard solutions of each additive.

Behavioural observations

All behavioural experiments were run in 12 cm diameter glass Petri dishes with smooth, featureless surfaces under static conditions to avoid passive movement of limpets by water currents (Seuront et al., 2020). In each arena, 75 ml of either control seawater or microplastic leachate was used. To assess the presence of (i) a leachate effect on the behavioural response of *C. nigrolineata* to either *R. clavigera* or *T. musiva* and (ii) differences in the response between leachate treatments, we ran a series of trials, pairing control vs. leachate treatments. One *C. nigrolineata* was placed in the middle of a Petri dish and a whelk (either *R. clavigera* or *T. musiva*) immediately placed next to it (typically within one centimetre) with its anterior part oriented toward the limpet. For each trial, two control and four leachate treatments were run simultaneously and replicated 10 times on the same day, which led to n = 20 replicates for the experiments conducted in control seawater and n = 10 for each leachate treatment. Behavioural observations were conducted for 15 minutes. An additional experiment was run in control seawater to assess the behavioural response of *C. nigrolineata* to several predator where one *C. nigrolineata* was positioned in a Petri dish and surrounded by either five *R. clavigera* or five *T. musiva* in an attempt to prevent the occurrence of any escape reaction. This experiment was replicated 5 times on the same day (n = 5). Different organisms were used for each behavioural experiment.

Behavioural analyses

Based on previous description of the anti-predator responses of various limpet species and *C. nigrolineata* in particular, we classified the observed behavioural response to the presence of either *R. clavigera* or *T. musiva* before and after the whelks got in contact with the limpet epipodial tentacle. More specifically, the behavioural responses considered were in both cases (i) no response, (ii) mushrooming, (iii) shell rocking, (iv) stomping, (v) mantle folding, and (vi) escape.

Statistical analyses

The proportions of limpets exhibiting any behaviour indicative of predator detection before and after the whelks got in contact with their tentacles were first compared to a theoretical equipartition (i.e., 50:50) using a χ^2 test within each treatment. These proportions were subsequently compared between treatment using a χ^2 test.

Results

***The defensive behaviour of* Cellana nigrolineata *towards* Reishia clavigera *and* Tenguella musiva**

In control seawater, the proportion of *C. nigrolineata* that detected either *R. clavigera* or *T. musiva* before and after they got in contact with their epipodial tentacles were not evenly distributed ($p < 0.05$). Specifically, a significant majority of *C. nigrolineata* (i.e., 70%; $p < 0.05$) detected *R. clavigera* before they got in contact with their epipodial tentacles (Fig. 2A). This was perceptible through a rotation of the shell that consistently occurred when a whelk was within 5 mm from the epipodial tentacles, and a subsequent escape reaction in the direction opposite to the whelk. This behavioural response was, however, significantly less frequently observed in the presence of *T. musiva* (i.e., 30%; Fig. 2B; $p < 0.05$) which were essentially detected once they got in contact with *C. nigrolineata* epipodial tentacles. The 30% and 70% of *C. nigrolineata* that respectively did not remotely detect *R. clavigera* and *T. musiva* consistently reacted to the presence of *R. clavigera* and *T. musiva* when their foot touched their epipodial tentacles by sequentially mushrooming, rocking, mantle folding and escaping (Fig. 3).

When surrounded by either five *R. clavigera* or five *T. musiva*, *C. nigrolineata* consistently first exhibited a mushrooming behaviour, followed by a shell rotation and a subsequent escape from the whelk aggregate that was consistently achieved by climbing on top of the whelk shell (Fig. 2).

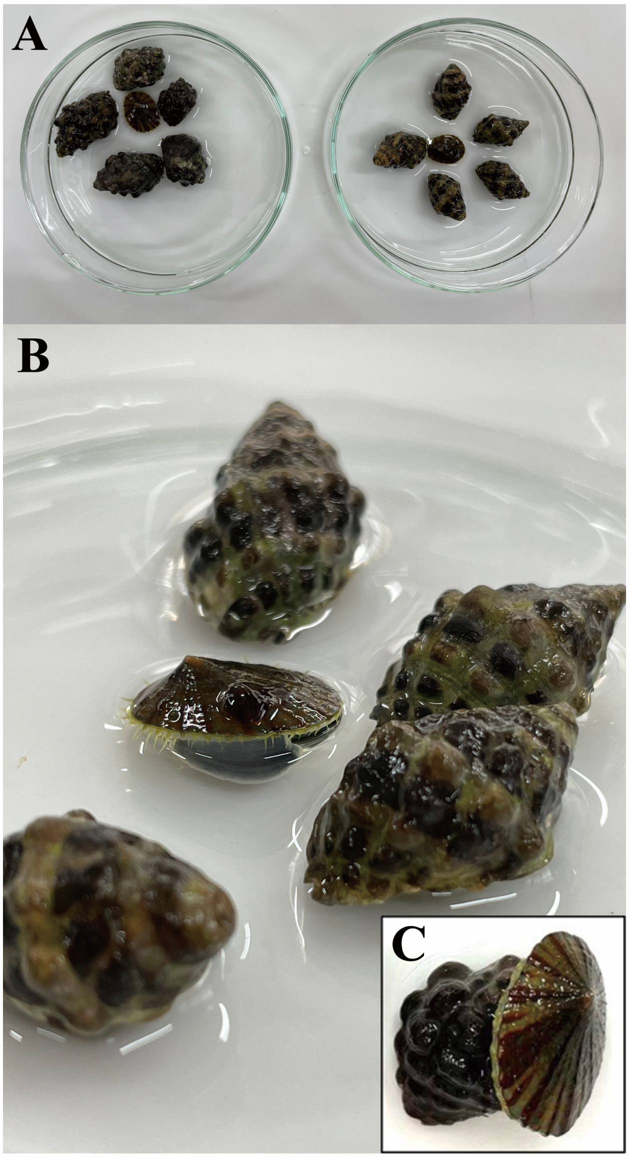

Figure 2. Experimental set-up used to assess the anti-predator response of *Cellana* nigrolineata when direct escape is prevented by the presence of *Reishia clavigera* (left) and five *Tenguella musiva* (right) (A), and illustration of the subsequent mushrooming behaviour (B) and escape typically achieved by climbing onto the whelk shell (C). Source: L. Seuront.

***Effect of plastic leachates on the defensive behaviour of* C. nigrolineata**

The analysis of additives in PE pellets revealed the presence of 11 plasticizers, 6 antioxidants and 7 flame retardants. In contrast, PP pellets contained 6 plasticizers and 2 antioxidants, while PA and PLA pellets only contained respectively 4 and 3 plasticizers (Table 1). In the presence of plastic leachates, no behavioural response was ever observed before the whelk foot actually touched *C. nigrolineata* epipodial tentacles in leachate solutions from conventional plastics, i.e., polypropylene, polyethylene and polyamide. In contrast, *C. nigrolineata* remotely detected the presence of both *R. clavigera* and *T. musiva* in leachate from polylactic pellets (Fig. 3A, B). The observed responses to *R. clavigera* (40%) and *T. musiva* (10%) were, however, 1.75- to 3-fold less pronounced than in control seawater (Fig. 3).

More specifically, and in sharp contrast with observations conducted in control seawater, the defensive response of *C. nigrolineata* in plastic leachate solutions was clearly species- and polymer-specific. In the presence of *R. clavigera*, *C. nigrolineata* consistently exhibited a mantle folding behaviour, followed by an escape reaction in the direction opposite to the whelk in all leachate solutions (Fig. 4A). In contrast, when exposed to *T. musiva*, *C. nigrolineata* consistently exhibited a mushrooming behaviour, followed by shell rocking (which only occurred when a whelk managed to climb on the limpet shell), mantle folding and escaping. In control seawater, *R. clavigera* and *T. musiva* climbed on all *C. nigrolineata* individuals that did not detect them before getting in contact with their foot. Noticeably, this frequency was 5-, 2-, 1.4- and 1.1-fold lower (i.e., 20, 50, 70 and 89%) respectively in PP, PE, PA and PLA leachate solutions, hence results in the observed decrease in shell rocking behaviour (Fig. 4B). Note that the observed decrease in climbing frequency was not a by-product of a putative decrease in the contact frequency of whelks with *C. nigrolineata*, which further suggests that plastic leachates did not impair the ability of *R. clavigera* and *T. musiva* to detect their prey. It is finally stressed that the epipodial tentacles were consistently observed moving back and forth in the archetypical tentacular scanning mode in control seawater, in sharp contrast with observations conducted in plastic leachate solutions where these tentacles were not moving.

Table 1. List of the additives found in the pellets of the four different polymers used in the present work, i.e., polypropylene (PP), polyethylene (PE), polyamide (PA) and polylactic acid (PLA), shown together with their function. Abbreviations: 4-ter-octylphenol (4tOP), tributyl Acetyl Citrate (ATBC), benzyl butyl phthalate (BBP), 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE153), 2,2',4,4',5,6'-hexabromodiphenyl ether (BDE154), 2,2',3,4,4',5',6-heptabromodiphenyl ether (BDE183), butylated hydroxytoluene (BHT), bisphenol A (BPA), bisphenol F (BPF), bisphenol S (BPS), diallyl phthalate (DAIP), phthalates dibutyl phthalate (DBP), bis-2-ethylhexyl adipate (DEHA), di(2-ethyhexyl)phthalate (DEHP), diethyl phthalate (DEP), di-isobutyl phthalate (DIBP), diisodecyl phthalate (DIDP), disoheptyl phthalate (DIHP), DiisononylPhthalate (DINP), Di-n-octyl phthalate (DIOP), dimethyl phthalate (DMP), nonylphenol monoethoxylate (NP1EO), nonylphenol (NPs), tributyl phosphate (TBP), tris(2-chloroethyl)phosphate (TCEP), tris(2-chloroisopropyl)phosphate (TCPP), tris(1,3-dichloro-2-propyl)phosphate (TDCPP).

Polymer type	**Additive function**	**Additives found in pellets**
PP	Plasticizers	DEP, DIBP, DBP, DEHP, DIOP, DINP
	Antioxidants	4tOP, BHT
	Flame retardants	-
PE	Plasticizers	ATBC, BBP, DAIP, DBP, DEHA, DEHP, DEP, DIBP, DIDP, DIHP, DMP
	Antioxidants	BPA, BHT, BPF, BPS, NP1EO, NPs
	Flame retardants	BDE153, BDE154, BDE 183, TBP, TCEP, TCPP, TDCPP
PA	Plasticizers	DBP, DEP, DIBP, DMP
	Antioxidants	-
	Flame retardants	-
PLA	Plasticizers	DEP, DIBP, DMP
	Antioxidants	-
	Flame retardants	-

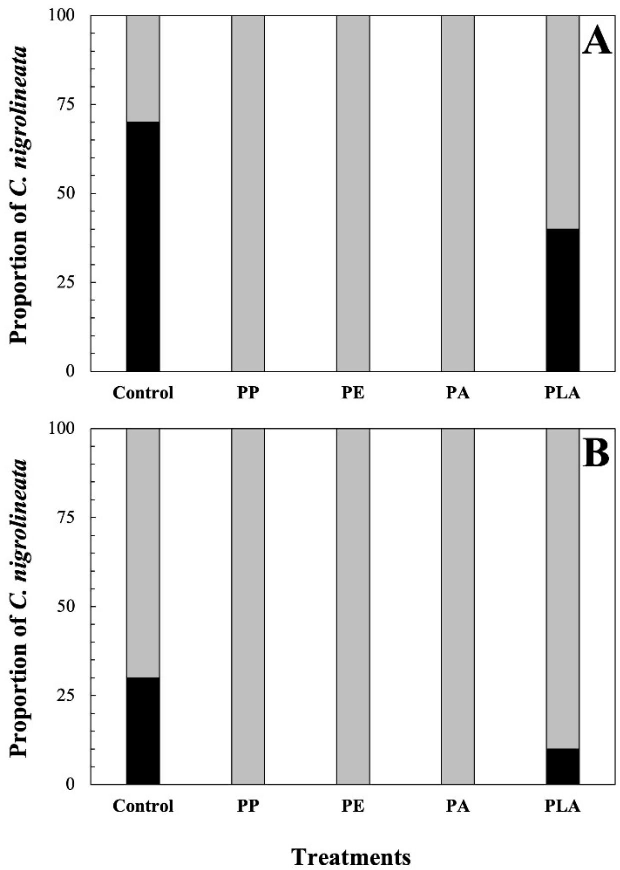

Figure 3. Proportion of *Cellana nigrolineata* exhibiting a behavioural response before (black) and after (grey) the foot of *Reishia clavigera* (A) and *Tenguella musiva* (B) got in contact with their epipodial tentacles. Control: control seawater (n = 20); PP, PE, PA and PLA are respectively polypropylene, polyethylene, polyamide and polylactic acid leachate solutions (n = 10).

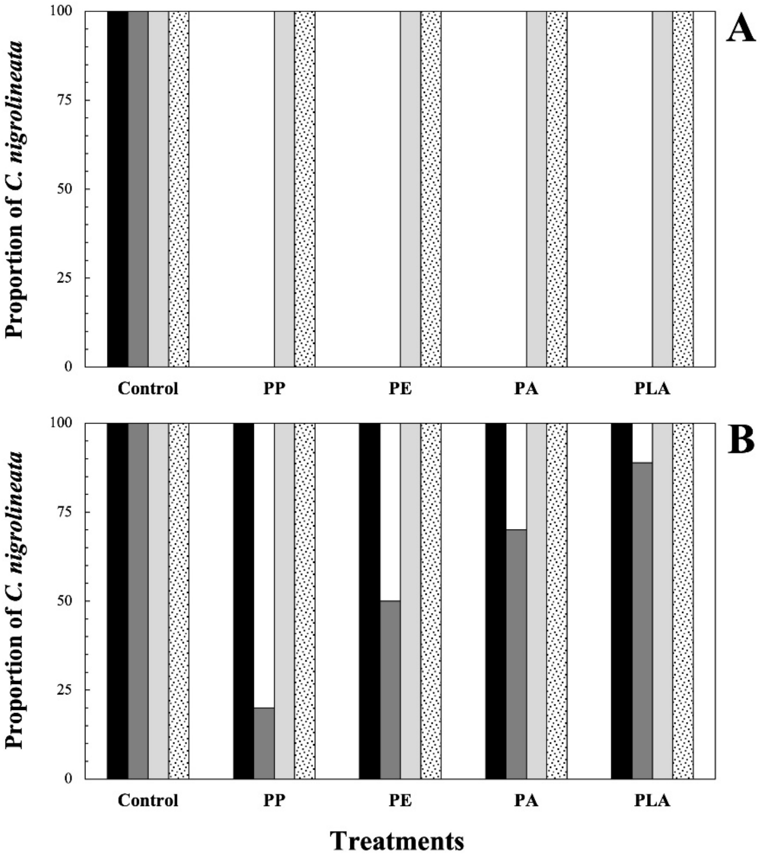

Figure 4. Proportion of *Cellana nigrolineata* mushrooming (black bars), shell rocking (dark grey bars), mantle folding (light grey bars) and escape (dotted bars) behavioural response following a contact of the foot of *Reishia clavigera* (A) and *Tenguella musiva* (B) with their epipodial tentacles. Control: control seawater (n = 20); PP, PE, PA and PLA are respectively polypropylene, polyethylene, polyamide and polylactic acid leachate solutions (n = 10).

Discussion

Our results show that *Cellana nigrolineata* use both chemosensory and contact cues to react to the presence of *Reishia clavigera* and *Tenguella musiva*. In addition, the observed stronger response to chemical cues of *R. clavigera* may point towards a higher sensitivity of *C. nigrolineata* towards this species. This result is consistent with field observations showing that *C. nigrolineata* is preyed upon far more frequently by *R. clavigera* than by *T. musiva* (Tomoyuki Nakano, personal observations). The ability to remotely detect the presence of *R. clavigera* (Fig. 3A) and *T. musiva* (Fig. 3B) was noticeably impaired in leachate solution of polylactic acid pellets, and inhibited in leachate solutions of polypropylene, polyethylene and polyamid pellets. These results are consistent with previous observations showing that an exposure to polypropylene leachates impair the ability of the common periwinkle *Littorina littorea* (Seuront, 2018) and the blue mussel *Mytilus edulis* (Uguen et al., 2022) to recognize the chemical cues of their predators. More specifically, the observed absence of response to *R. clavigera* and *T. musiva* in SW_{PP}, SW_{PE} and SW_{PA} (Fig. 3) is consistent with more pronounced effects of leachates from polypropylene, polyethylene and polyamid pellets than from polylactic acid pellets. These observations indicate that, under conditions of plastic leachate contamination, *C. nigrolineata* essentially rely on contact cues to trigger their anti-predator behaviour, which likely considerably increase their vulnerability. It is also noticeable that the strongest effects were observed for leachate solutions of polymers that contain more additives (Table 1).

Once the foot of the predatory whelks got into contact with the epipodial tentacles of *C. nigrolineata*, the observed behavioural response did not differ in response to *R. clavigera* and *T. musiva* (Fig. 4) in control seawater, where all individuals consistently responded sequentially by mushrooming, rocking, mantle folding and escaping. In microplastic leachate solutions, the behavioural responses exhibited by *C. nigrolineata* to the presence of *R. clavigera* and *T. musiva* were, however, very distinct. The response to *R. clavigera* was limited to a sequence involving mantle folding followed by a rotation of the shell and an escape reaction in the direction opposite to the whelk anterior part. This behavioural response may indicate that, under conditions of microplastic leachate contamination, *C. nigrolineata* may not be able to sustain the energy expenditure associated to shell mushrooming, hence suffer from an alteration of their neuromuscular performance. In contrast, when exposed to *T. musiva*, *C. nigrolineata* responded through mushrooming, mantle folding and escaping, but only a smaller proportion of them exhibited a shell rocking behaviour, i.e., 20% in SW_{PP}, 50% in SW_{PE}, 70% in SW_{PA} and 89% in SW_{PLA}. As shell rocking behaviour only occurred when a whelk climbed onto the shell of *C. nigrolineata*, these figures also indicate that *T. musiva* climbed significantly less frequently (especially in polyethylene and polypropylene leachate solutions where they were observed on *C. nigrolineata* shell 2 and 5 times less frequently than in control seawater). As hypothesized from the observed defensive response of *C. nigrolineata* to *R. clavigera* in leachate solutions, these observations may indicate that an exposure to microplastic leachate might lead to a form of neuromuscular impairment in *T. musiva*, which prevent them to climb onto the shell of their prey.

Taken together, our results illustrate that the presence of plastic additives in seawater are very likely to negatively affect non-lethal predator-prey interactions through (i) a decrease in the ability of prey to remotely identify the presence of their predator, (ii) a drastic change in the anti-predator responses once the predator was identified through contact cues and (iii) a noticeable lack of impact of leachates on the ability of whelks to locate and identify their prey. It is nevertheless noticeable that the biosourced and biodegradable polymer considered here (i.e., polylactic acid) had less impact on the chemosensory abilities of *C. nigrolineata* than conventional polymers (i.e., polypropylene, polyethylene and polyamide). The impact of microplastic leachates increases with the number of additives present in the polymer (see Figs. 3 and 4; Table 1). This suggests that the observed behavioural effects may be more dependent to the additive content of the microplastic pellets than to the intrinsic nature of their polymeric matrix. Our results further point towards an alteration of the neuromuscular performance or a metabolic depression in both *C. nigrolineata* and *T. musiva* following an exposure to plastic leachates. These results warrant the need for further work to determine the relative contribution of the behavioural changes observed in both predator and prey following an exposure to plastic leachates in the functioning of the food web of intertidal rocky shore of the Wakayama Prefecture in particular and intertidal rocky ecosystems in general.

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