

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

Cellana nigrolineata

LAURENT SEURONT^{a, b, c, *}, TOMOYUKI NAKANO^d

^a CNRS, Univ. Lille, Univ. Littoral Côte d'Opale, IRD, UMR 8187 LOG, Station Marine de Wimereux,
F - 59000 Lille, France.

^b Department of Marine Resources and Energy, Tokyo University of Marine Science and Technology,
4-5-7 Konan, Minato-ku, Tokyo, 108-8477, Japan.

^c Department of Zoology and Entomology, Rhodes University,
Grahamstown 6140, South Africa.

^d Seto Marine Biological Laboratory, Field Science Education and Research Center, Kyoto University,
459 Shirahama, Wakayama, 649-2211, Japan.

* Corresponding author. E-mail: laurent.seuront@cnrs.fr

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 $\pm$ 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 ($33^{\circ}41'03\text{N}$, $135^{\circ}20'26\text{E}$) 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; Pemmiproducs, 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 μ m 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).

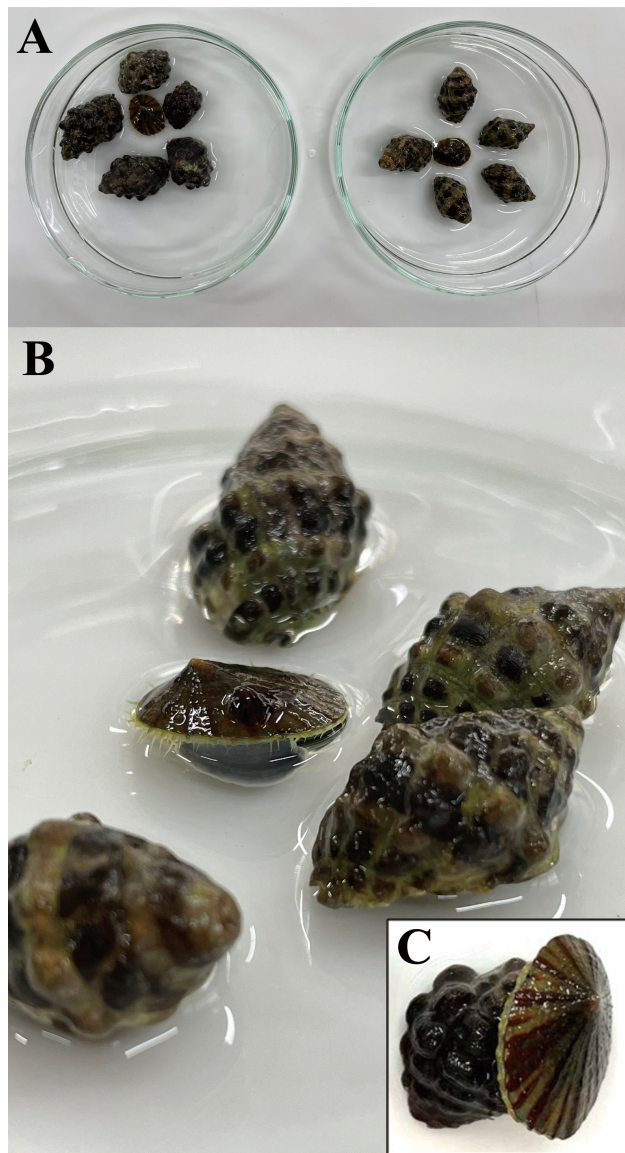


Figure 2. Experimental set-up used to assess the anti-predator response of *Cellana nigrolineata* when direct escape is prevented by the presence of *Reishia clavigera* (left) and five *Tenguella musiva* (right) (A), and illustration of the subsequent mushrooming behaviour (B) and escape typically achieved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

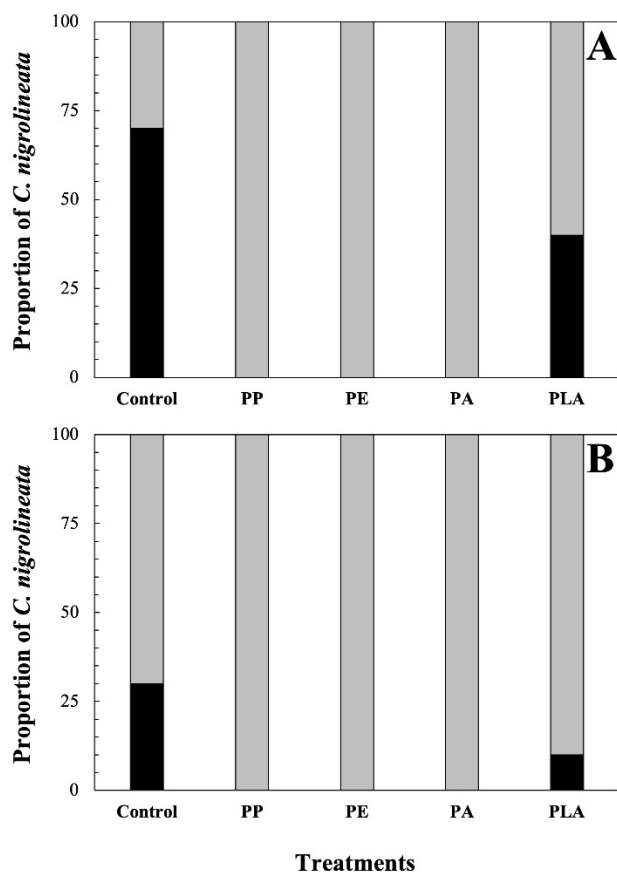


Figure 3. Proportion of *Cellana nigrolineata* exhibiting a behavioural response before (black) and after (grey) the foot of *Reishia clavigera* (A) and *Tenguella musiva* (B) got in contact with their epipodial tentacles. Control: control seawater (n = 20); PP, PE, PA and PLA are respectively polypropylene,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

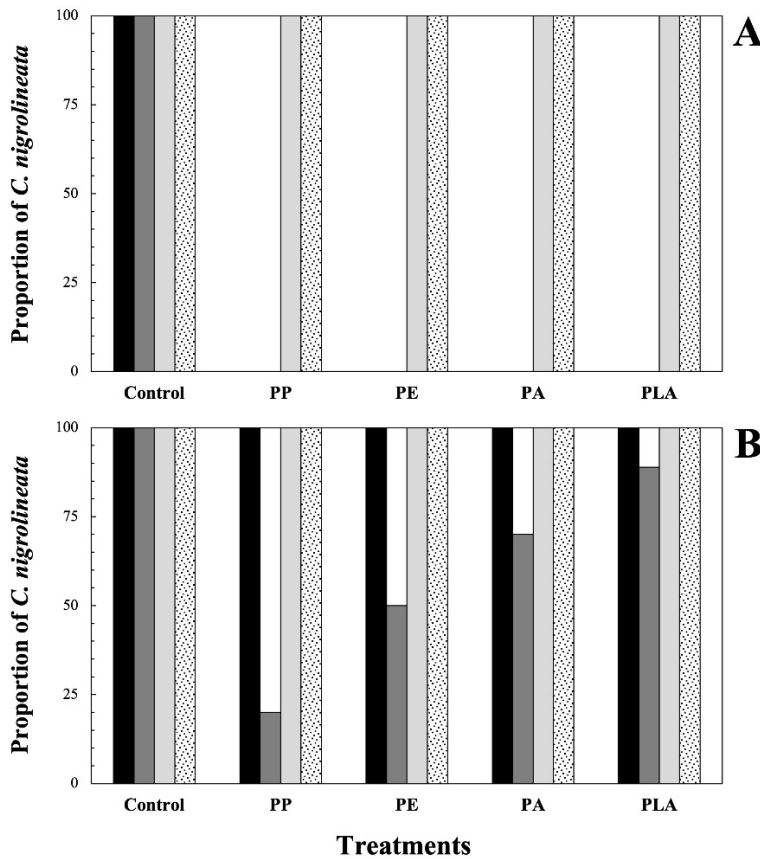


Figure 4. Proportion of *Cellana nigrolineata* mushrooming (black bars), shell rocking (dark grey bars), mantle folding (light grey bars) and escape (dotted bars) behavioural response following a contact of the foot of *Reishia clavigera* (A) and *Tenguella musiva* (B) with their epipodial tentacles. 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.

Acknowledgements

We thank two anonymous reviewers and Prof. Akira Asakura for their criticisms, comments and suggestions on a previous version of the present work. This work is a contribution to the research project SOLACE funded by the European Maritime and Fisheries Fund and France Filière Pêche. The authors are grateful to Fleurine Akoueson and Guillaume Duflos for conducting the additives identification, which has been partially financially supported by the European Union (ERDF), the French Government, the Région Hauts-de-France and IFREMER, in the framework of the project CPER IDEAL 2021–2027.

Literature cited

- Abe, N. 1980. Food and feeding habit of some carnivorous gastropods (preliminary report). Benthos Research, 19/20, 39–47.
- Abe, N. 1983. Escape responses of patellid limpets to carnivorous gastropods. Nanki Seibutu, 25, 193–194.
- Abe, N. 1989. Prey value to the carnivorous gastropods *Morula musiva* (Kiener) and the two forms of *Thais clavigera* (Küster): effect of foraging duration and abandonment of prey. Malacologia, 30, 373–395.
- Asakura, T., Kawamura, M., Nakano, T., Kubota, S., Miyazaki, K., Yamato, S., Goto, R., Kato, T., Sato, T. 2018. A Guide Book of Marine Organisms in Shirahama. Seto Marine Biological Laboratory, Shirahama Aquarium, Field Science Education and Research Center, Kyoto University, 94 pp.
- Astudillo, J. C., Bonebrake, T. C. and Leung, K. M. Y. 2018. Deterred but not preferred: predation by native whelk *Reishia clavigera* on invasive bivalves. PLoS ONE, 13: e0196578.
- Aguilera, M. A., Weib, M and Thiel, M. 2019. Similarity in predator-specific anti-predator behavior in ecologically distinct limpet species, *Scurria viridula* (Lottiidae) and *Fissurella latimarginata* (Fissurellidae). Marine Biology, 166, 41.
- Blackmore, G. and Morton, B. 2002. The influence of diet on comparative trace metal cadmium, copper and zinc accumulation in *Thais clavigera* (Gastropoda: Muricidae) preying on intertidal barnacles or mussels. Marine Pollution Bulletin, 44, 870–876.

- Branch, G. M. 1979. Aggression by limpets against invertebrate predators. *Animal Behaviour*, 27, 408–410.
- Branch, G. M. and Cherry, M. I. 1985. Activity rhythms of the pulmonate limpet *Siphonaria capensis* Q. & G. as an adaptation to osmotic stress, predation and wave action. *Journal of Experimental Marine Biology and Ecology*, 87, 153–168.
- Chase, J. M., Abrams, P. A., Grover, J. P., Diehl, S., Chesson, P., Holt, R. D., Richards, S. A., Nisbet, R. M. and Case, T. J. 2002. The interaction between predation and competition: a review and synthesis. *Ecology Letters*, 5, 302–315.
- Chilton, N. B. and Bull, C. M. 1984. Influence of predation by a crab on the distribution of the size-groups of three intertidal gastropods in South Australia. *Marine Biology*, 83, 163–169.
- Coleman, R. A., Browne, M. A. and Theobalds, T. 2004. Aggregation as a defense: limpet tenacity changes in response to simulated predator attack. *Ecology*, 85, 1153–1159.
- Connell, J. H. 1972. Community interactions on marine rocky intertidal shores. *Annual Review of Ecology and Systematics*, 3, 169–192.
- Connolly, S. R. and Roughgarden, J. 1999. Theory of marine communities: competition, predation, and recruitment-dependent interaction strength. *Ecological Monographs*, 69, 277–296.
- Dee, L. E., Witman, J. D. and Brandt, M. 2012. Refugia and top-down control of the pencil urchin *Eucidaris galapagensis* in the Galapagos marine reserve. *Journal of Experimental Marine Biology and Ecology*, 416/417, 135–143.
- Delaeter, C., Spilmont, N., Bouchet, V. M. P. and Seuront, L. 2022. Plastic leachates: bridging the gap between a conspicuous pollution and its pernicious effects on marine life. *Science of the Total Environment*, 826, 154091.
- Duffy, J. E. and Hay, M. E. 2001. The ecology and evolution of marine consumer–prey interactions. In: Bertness, M. D., Gaines, S. D. and Hay, M. E. (Eds.), *Marine Community Ecology*. Sinauer, Massachusetts, 131–157.
- Escobar, J. B. and Navarrete, S. A. 2011. Risk recognition and variability in escape responses among intertidal molluscan grazers to the sunstar *Heliaster helianthus*. *Marine Ecology Progress Series*, 421, 151–161.
- Espoz, C. and Castilla, J. C. 2000. Escape responses of four Chilean intertidal limpets to seastars. *Marine Biology*, 137, 887–892.
- Ferrari, M. C. O., Wilsenden, B. D., and Chivers, D. P. 2010. Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology*, 88, 698–724.
- Frias, J. P., Ivar do Sul, J., Panti, C., Lima, A. R. A. 2021. Microplastics in the marine environment: sources, distribution, biological effects and socio-economic impacts. *Frontiers in Environmental Science*, 9, 676011.
- Häder, D. P., Banaszak, A. T., Villafañe, V. E., Narvarte, M. A., González, R. A. and Helbling, E. W. 2020. Anthropogenic pollution of aquatic ecosystems: emerging problems with global implications. *Science of the Total Environment*, 713, 136586.
- Häder, D. P., Helbling, E. W. and Villafañe, V. E. 2021. *Pollution of Aquatic Ecosystems*. Springer Nature Switzerland, 426 pp.
- Gall, S. C. and Thompson, R. C. 2015. The impact of debris on marine life. *Marine Pollution Bulletin*, 92, 170–179.
- Garrity, S. D. and Levings, S. C. 1983. Homing to scars as a defense against predators in the pulmonate limpet *Siphonaria gigas* (Gastropoda). *Marine Biology*, 72, 319–324.
- Gunaalan, K., Fabbri, E. and Capolupo, M. 2020. The hidden threat of plastic leachates: a critical review on their impacts on aquatic organisms. *Water Research*, 184, 116170.
- Hawlena, D. and Schmitz, O. J. 2010. Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *The American Naturalist*, 176, 537–556.
- Hawkins, S. J., Bohn, K., Firth, L. B. and Williams, G. A. 2019. *Interactions in the Marine Benthos. Global Pattern and Processes*. Cambridge University Press, Cambridge, 534 pp.
- Hawkins, S. J. and Jones, H. D. 1992. *Rocky Shores. Marine Field Course Guide 1*. Immel Publishing, London, 112 pp.
- Iwasaki, K. 1993. Analyses of limpet defense and predator offense in the field. *Marine Biology*, 116, 277–289.
- Jamieson, A. J., Malkocs, T., Pierny, S. B., Fujii, T. and Zhang, Z. 2017. Bioaccumulation of persistent organic pollutants in the deepest ocean fauna. *Nature Ecology and Evolution*, 1, 1–4.
- Kumar, R., Verma, A., Shome, A., Sinha, R., Sinha, S., Jha, P. K. et al. 2021. Impacts of plastic pollution on ecosystem services, sustainable development goals, and need to focus on circular economy and policy interventions. *Sustainability*, 13, 9963.
- Lai, S., Loke, L. H. L., Bouma, T. J. and Todd, P. A. 2018. Biodiversity surveys and stable isotope analyses reveal key differences in intertidal assemblages between tropical seawalls and rocky shores. *Marine Ecology Progress Series*, 587, 41–53.

- Lam, K. K. Y. 2002. Escape response of intertidal gastropods on a subtropical rocky shore in Hong Kong. *Journal of Molluscan Research*, 68, 297–306.
- Li, F., Mu, F. H., Liu, X. S., Xu, X. Y. and Cheung, S. G. 2020. Predator prey interactions between predatory gastropod *Reishia clavigera*, barnacle *Amphibalanus amphitrite* and mussel *Brachidontes variabilis* under ocean acidification. *Marine Pollution Bulletin*, 152, 110895.
- Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions. *Bioscience*, 48, 25–34.
- Lima, S. L. and Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, 68, 619–640.
- Little, C. and Kitching, J. A. 1996. *The Biology of Rocky Shores*. Oxford University Press, Oxford, 240 pp.
- Little, C., Williams, G. A. and Throwbridge, C. D. 2009. *The Biology of Rocky Shores*. Oxford University Press, Oxford, 376 pp.
- Marks, D., Miller, M. A. and Vassanadumrongdee, S. 2020. The geopolitical economy of Thailand's marine plastic pollution crisis. *Asia Pacific Viewpoints*, 61, 266–282.
- McQuaid, C. M., Cretchley, R. and Rayner, J. L. 1999. Chemical defence of the intertidal pulmonate limpet *Siphonaria capensis* (Quoy & Gaimard) against natural predators. *Journal of Experimental Marine Biology and Ecology*, 237, 141–154.
- Mahon, A. R., Amsler, C. D., McClintock, J. B. and Baker, B. J. 2002. Chemotactile predator avoidance responses of the common Antarctic limpet *Nacella concinna*. *Polar Biology*, 25, 469–473.
- Manzur, T., Vidal, F., Pantoja, J. F., Fernández, M. and Navarrete, S. A. 2014. Behavioural and physiological responses of limpet prey to a seastar predator and their transmission to basal trophic levels. *Journal of Animal Ecology*, 83, 923–933.
- Markowska, M. and Kidawa, A. 2007. Encounters between Antarctic limpets, *Nacella concinna*, and predatory sea stars, *Lysasterias* sp., in laboratory and field experiments. *Marine Biology*, 151, 1959–1966.
- Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K. & Relyea, R. A. 2005. Ecological consequences of phenotypic plasticity. *Trends in Ecology and Evolution*, 20, 685–692.
- Paine, R. T. 1974. Intertidal community structure. Experimental studies on the relationship between a dominant competitor and its principal predator. *Oecologia*, 15, 93–120.
- Paine, R. T. 1994. *Marine Rocky Shores and Community Ecology: an Experimentalist's Perspective*. Ecology Institute, Nordbruite, 152 pp.
- Peckarsky, B. L., Abrams, P. A., Bolnick, D. I., Dill, L. M., Grabowski, J. H., Luttbeg, B. et al. 2008. Revisiting the classics: considering non-consumptive effects in textbook examples of predator-prey interactions. *Ecology*, 89, 2416–2425.
- Raffaelli, D. and Hawkins, S. J. 1996. *Intertidal Ecology*. Chapman and Hall, London, 335 pp.
- Reeve, L. A. 1854. Monograph of the genus *Patella*. In: *Conchologia Iconica, or, illustrations of the shells of molluscan animals*, vol. 8, pls. 1–42 and unpaginated tex.
- Rice, S. H. 1985. An anti-predator chemical defense of the marine pulmonate gastropod *Trimusculus reticulatus* (Sowerby). *Journal of Experimental Marine Biology and Ecology*, 93, 83–89.
- Rillig, M. C., Ryo, M. and Lehmann, A. 2021. Classifying human influences on terrestrial ecosystems. *Global Change Biology*, 27, 2273–2278.
- Robles, C. and Desharnais, R. 2002. History and current development of a paradigm of predation in rocky intertidal communities. *Ecology*, 83, 1521–1536.
- Sasaki, T. 2017. Nacellidae. In: Okutani, T. (ed.) *Marine Mollusks in Japan, Second Edition*, Tokai University Press, Japan, pp. 24–33.
- Schmitz, O. J., Hawlena, D. and Trussell, G. C. 2010. Predator control of ecosystem nutrient dynamics. *Ecology Letters*, 13, 1199–1209.
- Seuront, L. 2018. Microplastic leachates impair behavioural vigilance and predator avoidance in a temperate intertidal gastropod. *Biology Letters*, 14, 20180453.
- Seuront, L., Zardi, G. I., Uguen, M., Bouchet, V. M. P., Delaeter, C., Henry, S., Spilmont, N. and Nicastro, K. R. 2022. A whale of a plastic tale: a plea for interdisciplinary studies to tackle micro- and nanoplastic pollution in the marine realm. *Science of the Total Environment*, 846, 157187.
- Sih, A., Englund, G. and Wooster, D. 1998. Emergent impacts of multiple predators on prey. *Trends in Ecology and Evolution*, 13, 350–355.

- Sih, A., Crowley, P., McPeck, M., Petrtanka, J. and Strohmeir, K. 1985. Predation, competition, and prey communities: a review of field experiments. *Annual Review of Ecology, Evolution, and Systematics*, 16, 269–311.
- Sogame, T., Owada, M. and Kanazawa, K. 2009. Comparison of defensive behaviours in limpets inhabiting an intertidal zone. *Science Journal of Kanagawa University*, 20, 89–92.
- Stimson, J. 1970. Territorial behavior of the owl limpet, *Lottia gigantea*. *Ecology*, 51, 113–118.
- Taylor, R. J. 2013. *Predation*. Springer, New York, 165 pp.
- Taylor, J. D. and Morton, B. 1996. The diet of predatory gastropods in the Cape d'Aguilar Marine Reserve, Hong Kong. *Asian Marine Biology*, 13, 141–166.
- Tomatsuri, M. and Kon, K. 2015. Comparison of three methods for determining the prey preference of the muricid snail *Reishia clavigera*. *Journal of Marine Sciences*, 2015, 484392.
- Trussell, G. C., Ewanchuk, P. J. and Bertness, M. D. 2003. Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. *Ecology*, 84, 629–640.
- Uguen, M., Nicastro, K. R., Zardi, G. I., Gaudron, S. M., Spilmont, N. and Seuront, L. 2022. Microplastic leachates disrupt the chemotactic and chemokinetic behaviours of an ecosystem engineer (*Mytilus edulis*). *Chemosphere*, 306, 135425.
- Weissburg, M., Smee, D. L. and Ferner, M. C. 2014. The sensory ecology of nonconsumptive predator effects. *The American Naturalist*, 184, 141–157.
- Werner, E. E. and Peacor, S. D. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology*, 84, 1083–1100.
- Williams, G. A. and Morritt, D. 1995. Habitat partitioning and thermal tolerance in a tropical limpet, *Cellana grata*. *Marine Ecology Progress Series*, 124, 89–103.
- Yamada, S. and Boulding, E. 1996. The role of highly mobile crab predators in the intertidal zonation of their gastropod prey. *Journal of Experimental Marine Biology and Ecology*, 204, 59–83.

Received: 21 June 2024.

Accepted: 10 Oct 2024.

Published online: 5 Nov 2024.