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Environmentally relevant concentrations of microplastics and phenanthrene show limited effects on the behaviour and physiology of green crab lineages

Lorenzo Cozzolino[a,b,*], Katy R. Nicastro[a,c], Christopher D. McQuaid[c], Halldór P. Halldórsson[d], Fernando P. Lima[e,f], Gerardo I. Zardi[a,c]

[a] Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, IRD, UMR 8187, LOG – Laboratoire d'Océanologie et de Géosciences, Lille F-59000, France
[b] CCMAR – Centro de Ciências do Mar, CIMAR Laboratório Associado, Universidade do Algarve, Campus de Gambelas, Faro 8005-139, Portugal
[c] Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa
[d] University of Iceland's Research Centre in Suðurnes, Garðvegi 1, Sandgerði 245, Iceland
[e] CIBIO, Centro de Investigacao em Biodiversidade e Recursos Geneticos, InBIO Laboratorio Associado, Campus de Vairão, Rua Padre Armando Quintas, no7, Vairão 4485-661, Portugal
[f] BIOPOLIS Program in Genomics, Biodiversity and Land Planning, Campus de Vairão, Rua Padre Armando Quintas, 7, Vairão 4485-661, Portugal

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ABSTRACT

Plastic pollution is a growing environmental concern, with microplastics (MPs) and associated contaminants such as polycyclic aromatic hydrocarbons (PAHs) jeopardising the resilience and long-term survival of marine species. While species-specific effects have been well-documented, the role of intraspecific diversity in modulating species' responses to these contaminants remains largely neglected. This study investigates whether genetically distinct lineages of the green crab (*Carcinus maenas*) differ in their behavioural and physiological responses to environmentally relevant concentrations of polyethylene microplastics (PE) and PE contaminated with phenanthrene (PE+PHE). Juvenile crabs from the Southwest European (SWE) and Icelandic (ICE) populations were exposed to control seawater, PE, or PE+PHE for 72 h, and their behavioural (i.e., food contest) and physiological performances (i.e., heart rate) were assessed. Overall, there were no significant effects of PE or PE+PHE on key behavioural traits related to food competition (e.g., latency to initiate movement, interbout intervals, contest outcome) or on physiological parameters such as heart rate. However, resource possession was significantly reduced in ICE crabs exposed to PE. Our findings suggest that *C. maenas* exhibits short-term resilience to MPs and PAHs, with similar behavioural responses across distinct genotypic lineages (SWE = ICE). However, differences in heart rates between genetically distinct populations, even in control conditions, highlight the potential effect of lineage-specific physiological plasticity. In addition, high inter-individual variability in behavioural responses suggests susceptibility to MPs and PAHs may not be uniform within populations. Long-term assessments incorporating individual-based analyses are needed to fully understand the complex interplay between plastic pollution, genetic diversity, and behavioural plasticity in this marine species.

1. Introduction

Over the past few decades, plastic pollution has emerged as a pervasive threat to terrestrial and aquatic ecosystems, jeopardising biodiversity, the balance and functioning of ecological systems and the services they provide (Borrelle et al., 2020; Thushari and Senevirathna, 2020; Kumar et al., 2021).

Microplastics (MPs; Thompson et al., 2004) are of particular concern due to their ever-growing abundance, persistence, and ability to contaminate nearly every level of the marine environment, from surface waters to sediments (Isobe et al., 2019; Phuong et al., 2021). Due to their ubiquity and small size (< 5 mm), microplastics are ingested by hundreds of species across multiple trophic levels (Guzzetti et al., 2018; Carbery et al., 2018; Ugwu et al., 2021), often inducing adverse effects

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* Corresponding author at: Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, IRD, UMR 8187, LOG – Laboratoire d'Océanologie et de Géosciences, Lille F-59000, France.
E-mail address: Lorenzo_cozzolino@hotmail.it (L. Cozzolino).

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that include physical blockages of the gut, impaired growth, and overall reduced fitness (Wright et al., 2013; Ali et al., 2023; Jeong et al., 2024). Beyond these physical effects, microplastics also serve as carriers for harmful chemicals, either through the leaching of additives added during plastic production (e.g., bisphenol A and phthalates) or by adsorbing pollutants from the surrounding water (e.g., persistent organic pollutants and polycyclic aromatic hydrocarbons; Fries and Zarfl, 2012; Rodrigues et al., 2019).

Among these, polycyclic aromatic hydrocarbons (PAHs) are of particular concern and have been prioritised by the US Environmental Protection Agency due to their persistence, toxicity, and potential for bioaccumulation. These organic compounds, derived from the partial combustion of petrol, gas, coal, and wood, enter aquatic ecosystems through atmospheric deposition, industrial and municipal discharges, and stormwater runoff (Douben, 2003; Mojiri et al., 2019; Anyahara, 2021). Once introduced into the environment, the high lipophilic and hydrophobic nature of PAHs enhances their binding to both microplastic surfaces (Sørensen et al., 2020; Hou et al., 2023; Ali et al., 2024) and particulate matter. While sorption to MPs may enhance the bioaccumulation potential of PAHs (Besseling et al., 2013; Miller et al., 2020; Li et al., 2022), their interaction with particulate matter promotes transport and accumulation in sediments (Means et al., 1980; Maletić et al., 2019; Dai et al., 2022). Once ingested, these chemicals can leach into organisms, triggering subtle toxic effects such as endocrine disruption, oxidative stress, and immune suppression (Wang et al., 2019; Gunaalan et al., 2020).

Phenanthrene (PHE) is one of the most common PAHs in marine and freshwater ecosystems and has been extensively investigated for its acute toxicity and harmful effects on natural ecosystems and human health (Ma et al., 2016; Hong et al., 2017). Marine organisms, particularly benthic species and filter-feeders have been shown to be highly susceptible to exposure to PHE through their direct interactions with sediments and suspended particulate matter. In these organisms, PHE exposure has been linked to a range of physiological, developmental, and behavioural effects such as immune suppression and oxidative stress, DNA damage and impaired reproductive success (Correia et al., 2007; Bhagat et al., 2016; Peng et al., 2019; Wang et al., 2024; Yifei et al., 2024).

Despite extensive research describing the toxicological effects of MPs and their associated chemicals on marine species, the potential role of intraspecific diversity in modulating species' vulnerability to these contaminants remains largely neglected. In recent years, intraspecific diversity (i.e., the genotypic and phenotypic variation within and among populations of a single species) has been increasingly recognised as a crucial factor shaping species' responses to environmental stressors, including plastic pollution (Nicastro et al., 2023). Genotypic variation has been shown to affect the behavioural and physiological responses of mussels to microplastic leachates (Cozzolino et al., 2024). Similarly, within-species phenotypic variation affects patterns of MP ingestion and retention across different marine species (Nanninga et al., 2020a; Cozzolino et al., 2022; Nicastro et al., 2022). Beyond plastic pollution, intraspecific diversity plays a fundamental role in regulating ecological dynamics (Becks et al., 2010; Bolnick et al., 2011; Des Roches et al., 2018), shaping species interactions and community structure (Crutsinger et al., 2006; Palkovacs and Post, 2009; Nicastro et al., 2020), while affecting ecosystem functioning and resilience (Hughes and Stachowicz, 2004; Whitham et al., 2006; Raffard et al., 2019; Govaert et al., 2024).

Given its critical ecological role, understanding how intraspecific diversity influences vulnerability to MPs and PAHs is essential for assessing species' resilience in polluted environments. The European green crab, *Carcinus maenas,* provides an ideal model for investigating these questions. It is a widespread keystone species in coastal ecosystems and is particularly susceptible to the combined effects of MPs and PAHs due to its benthic lifestyle and feeding behaviour. As an opportunistic predator and scavenger, *C. maenas* consumes a wide range of prey and organic matter, increasing the likelihood of MP ingestion and exposure to associated contaminants (Suckling, 2021). Microplastic ingestion in *C. maenas* has been well-documented (Watts et al., 2014, 2015; McGoran et al., 2020; Cunningham et al., 2021), with indirect exposure occurring via trophic transfer (Farrell and Nelson, 2013) and branchial respiration mechanisms further enhancing exposure to MPs suspended in the water column (Watts et al., 2016).

Despite these findings, no research to date has explored whether distinct genotypic lineages of *C. maenas* exhibit varying physiological and behavioural responses to MPs and PAHs. This knowledge gap is particularly significant given its extensive geographic range and notable genetic diversity (Roman and Palumbi, 2004; Darling et al., 2008; Domingues et al., 2010). Native to the northeast Atlantic, the geographic range of the European green crab extends from Norway to Mauritania, including populations in Iceland and the Faroe Islands. However, due to its adaptability, high reproductive success, and extensive larval dispersal, *C. maenas* has rapidly expanded, establishing invasive populations in the northwestern Atlantic and the northeastern Pacific, often outcompeting native species (Grosholz et al., 2000; McDonald et al., 2001; de Rivera et al., 2011).

As a global invader, the population structure of *C. maenas* has been well described. High-resolution analysis of population genetics using mitochondrial DNA (specifically the cytochrome c oxidase I gene), microsatellite loci, and restriction-site-associated DNA sequencing (RAD-seq) has identified three distinct genetic lineages in the native range of *Carcinus maenas*. Specifically, these are one in northern Europe (Norway, Sweden, Finland, Denmark, Germany and Poland), one in southwest Europe (the Netherlands, Belgium, France, Spain and Portugal) and a third in Iceland and the Faroe Islands (Roman and Palumbi, 2004; Darling et al., 2008; Domingues et al., 2010). The Icelandic and Faroese populations are particularly notable for their unique haplotypes and deep genetic divergence from continental populations (Roman and Palumbi, 2004), likely driven by long-term isolation due to deep-water barriers, as observed in other marine species (see Foss et al., 1998; Shaw et al., 1999). Genetic variation within *C. maenas* may influence physiological traits, including tolerance to abiotic stressors, as evidenced by differences in thermal tolerance between the northern and southern European populations (Tepolt and Somero, 2014; Tepolt and Palumbi, 2015, 2020). These physiological differences suggest that distinct genetic populations of *C. maenas* may exhibit varying sensitivity to environmental pollutants.

In this study, we aim to investigate whether different genetic populations of *C. maenas* respond differently to MPs and PAHs, offering insights into their adaptability and resilience to these pollutants. We expose *C. maenas* to factory-fresh virgin polyethylene microplastics (PE) and PE contaminated through phenanthrene (PHE) adsorption (PE + PHE). We selected PE and PHE because they are among the most common polymer and polycyclic aromatic hydrocarbons (PAHs) in the marine environment, and because high-density polyethylene (HDPE) MPs often exhibit high sorption capacity (Rochman et al., 2013). In addition, research assessing the sorption-desorption dynamics of MPs and PAHs using various polymer types and pollutants has shown that PE and PHE have the highest potential for accumulation in organisms (Teuten et al., 2007; 2009; Bakir et al., 2014). Importantly, higher PHE desorption rates were observed in the presence of gut fluids, compared to seawater alone, suggesting that increased leaching will occur once MP is ingested (Teuten et al., 2007). We focused on two genetic lineages of the green crab, the southwest European and Icelandic populations to evaluate intraspecific differences in their behavioural and physiological responses to these contaminants. Based on their distinct physiological tolerance to abiotic stressors (Tepolt and Somero, 2014; Tepolt and Palumbi, 2015, 2020), we specifically hypothesised that (i) distinct genetic lineages of the green crab would show different behavioural and physiological responses to the two treatments (i.e., PE and PE + PHE) and that (ii) the responses observed within each lineage would differ between treatments. In particular, we expected the combined effect of

PE + PHE to be more pronounced than that of PE alone.

2. Material and methods

2.1. Study organism

Juvenile male green intermoult crabs, *Carcinus maenas* (carapace width: 2.74 ± 0.26 cm; mean ± SD) belonging to the southwest European (SWE) and Icelandic (ICE) clades were collected in June and September 2024 in Faro (N 37°0'35.95", W 7°59'46.23"; Portugal) and Sandgerði (N 64°1'59.8", W 22°42'42.18"; Iceland), respectively. At each location (Fig. 1), specimens were manually collected from the intertidal zone during low tide and brought to the laboratory in dark, cool conditions. Before each experiment, individuals of *C. maenas* were acclimatised for 5 days in glass aquaria under continuous seawater flow directly pumped from the experimental location and thus representative of *in situ* conditions (i.e., Portugal: S = 35 PSU, T = 17 °C; Iceland: S = 34 PSU, T = 10 °C). Crabs were maintained under natural photoperiods that reflected the environmental conditions of each collection site during the experimental periods. Specifically, individuals held in southern Portugal were exposed to approximately 15 h of daylight and 9 h of darkness, consistent with early summer at that latitude. In contrast, crabs from southwestern Iceland experienced a shorter photoperiod of approximately 11 h of daylight and 13 h of darkness, corresponding to early autumn conditions in that region.

All crabs used in this study were green morphotype males at comparable stages of the intermoult cycle, selected to minimise variation related to moult status and sex-specific physiological differences. Previous studies have demonstrated significant physiological and behavioural differences between green and red morphotypes of *Carcinus maenas* (Reid and Aldrich, 1989; Reid et al., 1989; Kaiser et al., 1990; Reid et al., 1994, 1997), and intermoult duration has been shown to influence susceptibility to toxicants such as pyrene, as well as metabolic capacity (Dam et al., 2006). Females were excluded to avoid confounding effects of reproductive investment, such as egg carrying, which can substantially alter metabolic and stress responses.

2.2. Experimental design

Prior to the laboratory experiments, crab carapace width (including the spines; cm) and surface area of the major chela (cm^2) were measured using ImageJ software.

Crabs were placed in individual glass jars (~ 3 L in volume) and exposed to one of three distinct treatments, including control seawater, seawater with factory-fresh (virgin) polyethylene (PE) microplastic (MP), and seawater with PE MP contaminated via phenanthrene adsorption onto their surface (i.e. PE + PHE). Control seawater consisted of the seawater used for acclimatisation. The solution of virgin PE MP was prepared from commercially available virgin ultra-high molecular weight PE MP powder (150 µm; Goodfellow Cambridge Ltd) at a concentration of 50 mg L^{-1} seawater, following the lowest concentration in Mbedzi et al. (2020). Phenanthrene was sorbed onto virgin PE MP at an environmentally relevant concentration (Teuten et al., 2007; 2009). Briefly, 375 g of virgin PE MP powder was added to a solution of phenanthrene (7.524 mg crystalline powder; Thermo Scientific) dissolved in 400 mL of absolute ethanol (Fisher Scientific). Ethanol was evaporated in a flume hood, and the resulting contaminated PE MP was washed three times in Milli-Q-purified water to remove ethanol and unbound phenanthrene (protocol adapted from Browne et al., 2013).

Prior to the experiment, crabs were fed ad libitum to standardise hunger levels. Each crab was assigned to a single treatment group and exposed for 72 h to either control seawater, PE MP, or PE + PHE. Every 24 h, each jar was cleaned and refilled with newly prepared treatment seawater. Aeration was used to aid the distribution of microplastics in

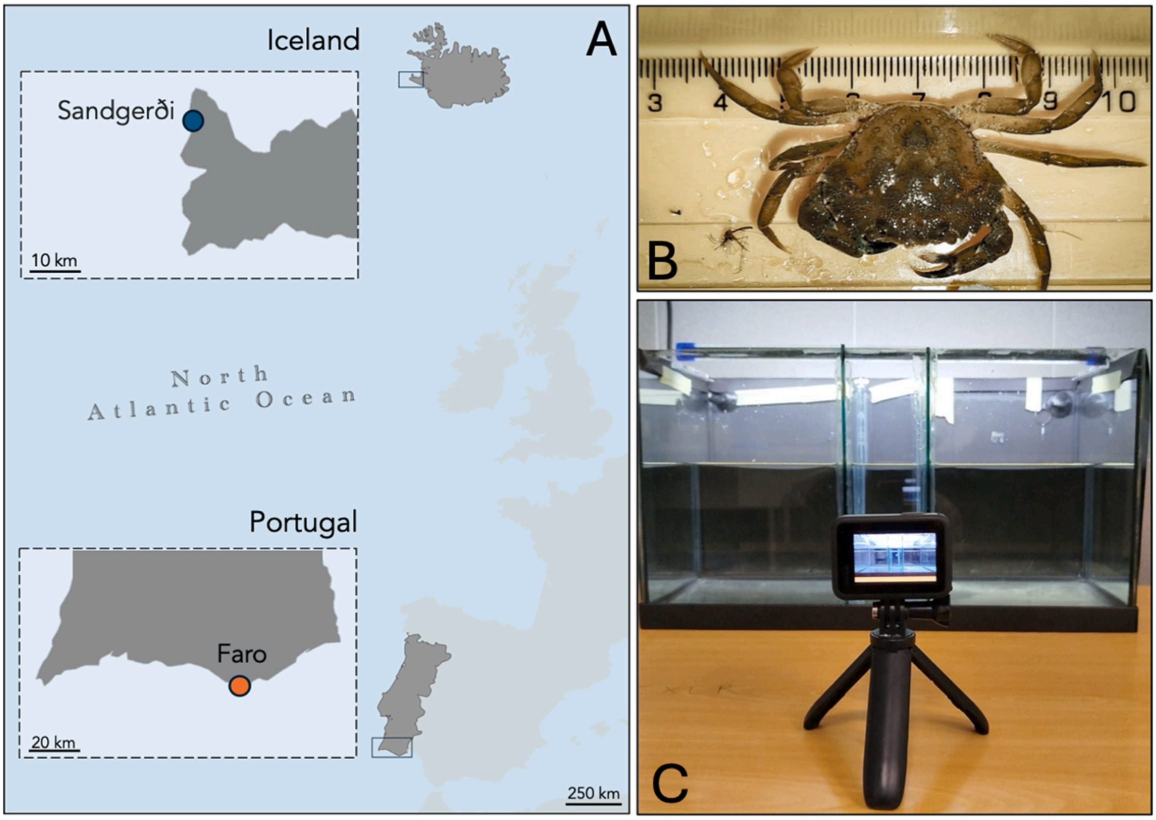

Fig. 1. (A) Sampling locations of Southwest European and Icelandic lineages of *Carcinus maenas*, collected in Faro (Portugal) and Sandgerði (Iceland), respectively. (B) Juvenile *C. maenas* individual used in the experiments. (C) Experimental tank and setup used for behavioural trait recording.

each jar. Crabs remained unfed throughout the 72-hour exposure experiment. At the end of the experiment, a total of 40 crabs were used in food contest trials to assess behavioural responses during competitive interactions. An additional 21 crabs were used for heart rate measurements to provide a proxy for physiological condition.

2.2.1. *Behavioural experiment (agonistic interaction for food)*

Individuals of *Carcinus maenas* (n = 40) were used in contest trials where a control crab was paired with a treated conspecific (i.e., PE or PE + PHE). Specimens were selected in each contest to ensure that there were no significant differences in carapace width (cm; ANOVA: $p = 0.263$ for SWE and $p = 0.197$ for ICE *C. maenas*) or major chela area (cm^2; ANOVA: $p = 0.312$ for SWE and $p = 0.396$ for ICE *C. maenas*) to avoid potential bias in contest success related to body size. Crabs (i.e., control vs treated) were positioned at opposite ends of a 50 × 25 × 25 cm (L × H × W) observation chamber, divided into three sections (21 × 8 × 21 cm) by vertically sliding glasses. The lateral and rear glasses were blacked out to minimise external visual stimuli that could potentially affect crab behaviour. The observation chamber was lit by a 2.5 W LED (AquaNice T4-L50; 18 × 500 mm). Specimens were given a 15-minute acclimation period before a whole mussel (*Mytilus edulis* in Iceland and *M. galloprovincialis* in Southern Europe), within its shell (size: 4.3 ± 0.6 cm; weight: 10.6 ± 2.3 g for SWE; size: 5.2 ± 0.4 cm; weight: 12.5 ± 1 g for ICE; mean ± SD), was placed in the central section of the chamber. The dividers were then raised to initiate the agonistic encounter. Contests were recorded for 15 min using GoPro Hero 10 cameras positioned at the front and overhead angles. *C maenas* exhibits typical agonistic behaviour during competition for limited resources (Sneddon et al., 1997). Agonistic encounters are characterised by crabs clutching, pushing, and pinching each other with their claws while attempting to get the food resource. These interactions typically involved low aggression and minimal injuries, although in some cases, they escalated into more intense encounters with rapid movements and frequent pinching of the peraeopods. Contest success and crab behaviours were evaluated using criteria adapted from Sneddon et al., (1997) and Dissanayake et al. (2009), as detailed in Table 1.

2.2.2. *Physiological experiment (heart rate measurement)*

Individuals of *Carcinus maenas* (n = 21) were used in physiological experiments to assess heart rates (heartbeat min^{-1}) using infrared (IR) sensors (Vishay CNY70) connected to a PULSE V2 heart rate logging system (Electricblue, Portugal). As in *C. maenas* heart rates vary with body size (smaller individuals show faster heart rate; Ahsanullah and Newell, 1971), specimens were selected to ensure no significant differences in carapace width (cm; ANOVA: $p = 0.89$ for SWE and $p = 0.78$ for ICE *C. maenas*). Crabs were individually transferred to glass arenas filled with 2 L of clean seawater as in control conditions. Crabs were left to acclimate for 10 min before heart rate was measured. Cardiac activity was continuously recorded at 10 Hz for 5 min (n = 7 individuals per genotype and treatment) using IR sensors placed on the carapace directly above the heart while each crab was submerged.

Table 1
Behavioural responses of agonistic encounter for food in juvenile male green *Carcinus maenas* (adapted from Sneddon et al., 1997 and Dissanayake et al., 2009). Time in each response is expressed in seconds (s).

Behavioural response	Abbreviation	Description
Time for first action	*T_firstaction*	Time each crab takes to initiate its first movement in a contest, either by engaging in wrestling or attempting to seize the resource
Resource possession	*T_resource*	Time in possession of the mussel. Crabs with higher resource possession were deemed the contest "winner".
Interbout interval duration	*T_interbout*	Time between an individual disengaging from agonistic interactions (i.e., wrestling or resource possession) and re-engaging in such behaviour. During this period, the crab neither possesses the resource nor approaches its opponent, maintaining its distance.
Number of interbout intervals	*N_interbout*	Total number of interbout intervals that each crab performs during a contest.

2.3. *Statistical analyses*

To evaluate the effects of treatment (i.e., C, PE, PE + PHE) on the behavioural and physiological responses of *Carcinus maenas,* all statistical analyses were conducted separately for SWE and ICE populations.

Behavioural responses were analysed using separate linear mixed-effects models (LMMs) with contest ID as a random factor accounting for contest-level variability and carapace width as a covariate. Dependent variables included (i) time to first action (*T_firstaction*), total interbout interval time (*T_interbout*), number of interbout intervals (*N_interbout*) and total time holding the resource (*T_resource*). Log transformation was applied to non-normally distributed variables (Shapiro-Wilk normality test). For dependent variables with negligible random effects, standard linear models were used (e.g., for *T_resource*). Chi-squared tests were performed to assess contest outcomes (i.e., contest winner) comparing observed winner frequencies (control vs. treatment groups) with expected 50:50 distributions. Separate tests were conducted for each paired contest (i.e., C vs. PE and C vs. PE+PHE) and within each lineage.

Physiological responses were analysed using one-way ANOVA with treatments as the factor and heart rate (expressed as heartbeat min^{-1}) as the dependent variable. Normality (Shapiro-Wilk normality test) and homogeneity of variances (Levene tests) were assessed before analyses. All statistical analyses and visualisations were performed in R software (R Studio). Significance was assessed at $p < 0.05$.

3. Results

3.1. *Behavioural experiment (agonistic interaction for food)*

3.1.1. *SW European population*

The morphological data for crab size measurements (i.e., carapace width and chelae size) of SWE *Carcinus maenas* followed a normal distribution (Shapiro-Wilk test, $p > 0.05$) and exhibited homoscedasticity (Levene's test, $p > 0.05$). Juvenile green crabs displayed no significant differences in morphological features (e.g., chelae size) across treatments (ANOVA, $p > 0.05$). Contests in which crabs failed to move (n = 3; i.e., n = 2 C vs PE and n = 1 C vs PE + PHE) were excluded from the analysis.

None of the variables analysed followed a normal distribution (Shapiro-Wilk test, $p < 0.05$) and were log-transformed.

"T_firstaction" was tested using a linear mixed-effects models fitted using restricted maximum likelihood (REML) and t-tests (Satterthwaite's method), revealing no significant effect of treatments ($p = 0.213$ for PE; $p = 0.572$ for PE+PHE) or carapace width ($p = 0.348$). These results indicate that exposure to polyethylene microplastics and phenanthrene does not affect the latency to initiate movement during food contests. On average, crabs in control seawater moved after 98 ± 73 s (mean ± SD) and those in PE and PE+PHE moved after 153 ± 122 and 110 ± 91 s, respectively (Fig. 2A).

"T_resource" was tested using a simpler linear model (LM), which included treatment as a factor and carapace width as a covariate. This model was preferred over linear mixed-effects models due to negligible random effect variance (= 0). The model revealed no significant effect of treatments ($p = 0.435$ for PE; $p = 0.735$ for PE+PHE) or carapace width ($p = 0.890$). On average, crabs from control seawater held the resource for 186 ± 236 s (mean ± SD) and those from PE and PE+PHE for 208 ± 257 and 124 ± 206 s, respectively (Fig. 2B).

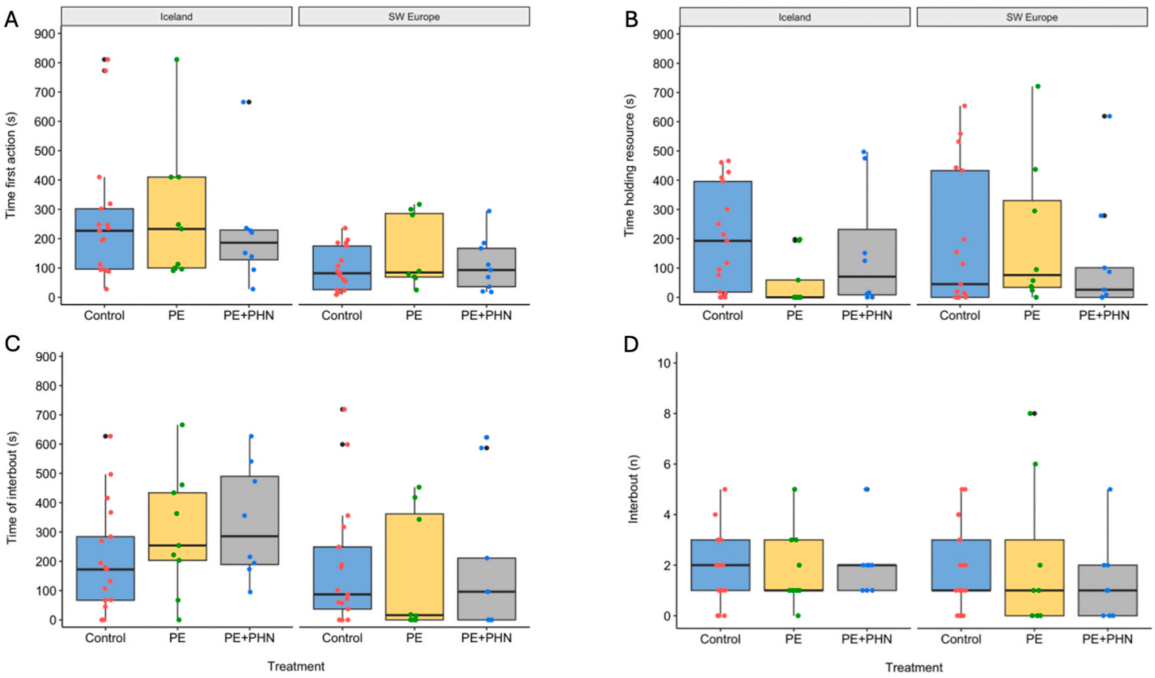

Fig. 2. Behavioural responses of treated (PE and PE+PHE) and untreated (Control) *Carcinus maenas* from the SW European and Icelandic lineages. Boxplots were used to visualise the behavioural responses during food contests, including (A) time to first action (*T_firstaction*), (B) time holding the resource (*T_resource*), (C) average interbout time (*T_interbout*), and (D) number of interbout intervals (*N_interbout*). Jittered data points overlaying the plots represent individual variability.

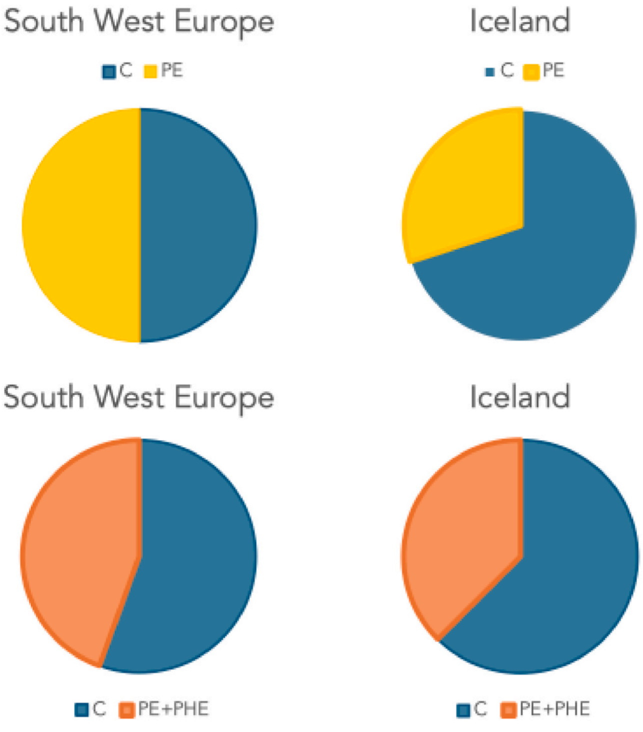

Fig. 3. The pie chart represents contest outcomes (C vs PE top two pies; C vs PE+PHE bottom two) for Icelandic and SW European populations of *C. maenas*.

"T_interbout" was tested using a linear mixed-effects models revealing no significant effect of treatment ($p = 0.423$ for PE; $p = 0.506$ for PE+PHE) or carapace width ($p = 0.831$). These findings suggest that exposure to polyethylene microplastics and phenanthrene does not influence the time between wrestles. On average, interbout duration was 177 ± 213 s (mean ± SD) for crabs in control seawater, and 155 ± 208 and 179 ± 251 s for crabs maintained in PE and PE+PHE seawater (Fig. 2C).

"N_interbout" was tested using a linear mixed-effects models revealing no significant effect of treatment ($p = 0.663$ for PE; $p = 0.444$ for PE+PHE) or carapace width ($p = 0.851$). On average, crabs kept in control seawater showed 1.9 ± 1.7 interbout (mean ± SD), compared to 1.8 ± 2.9 and 1.2 ± 1.6 of crabs from PE and PE+PHE-treated seawater, respectively (Fig. 2D).

The Chi-squared test revealed no significant deviation from the expected 50:50 distribution for contests between "C vs PE" ($\chi^2 = 0$, df = 1, $p = 1$) or "C vs PE+PHE" ($\chi^2 = 0.1$, df = 1, $p = 0.739$). These results suggest that exposure to polyethylene microplastics or polyethylene microplastics with sorbed phenanthrene did not influence the likelihood of winning an agonistic interaction for food in *C. maenas* (Fig. 3).

3.1.2. Icelandic population

The morphological data for crab size measurements (i.e., carapace width and chelae size) of ICE *Carcinus maenas* followed a normal distribution (Shapiro-Wilk test, $p > 0.05$) and exhibited homoscedasticity (Levene's test, $p > 0.05$). Juvenile green crabs displayed no significant differences in morphological features (e.g., chelae size) across treatments (ANOVA, $p > 0.05$). Contests in which crabs failed to move (n = 3; i.e., n = 1 C vs PE and n = 2 C vs PE+PHE) were excluded from the analysis.

As previously observed for the SWE population, none of the variables analysed showed normal distribution (Shapiro-Wilk test, $p < 0.05$) and were log-transformed. All tests were conducted using linear mixed-effects models.

For the *"T_firstaction"* the models revealed no significant effect of treatments ($p = 0.288$ for PE; $p = 0.119$ for PE+PHE) or carapace width ($p = 0.243$). On average, crabs in control seawater moved after 263 ± 222 s (mean ± SD) and those in PE and PE+PHE moved after 279 ± 237 and 220 ± 194 s, respectively (Fig. 2A).

For the *"T_resource"* the models revealed a significant effect of PE treatment ($p = 0.0131$; C > PE), yet no effect of PE+PHE ($p = 0.425$; C = PE+PHE) or carapace width ($p = 0.709$). On average, crabs from control seawater held the resource for 202 ± 178 s (mean ± SD) and those from PE and PE+PHE for 50 ± 85 and 159 ± 210 s, respectively (Fig. 2B).

For the *"T_interbout"* the linear mixed-effects models revealed no significant effect of treatments ($p = 0.582$ for PE; $p = 0.065$ for PE+PHE) or carapace width ($p = 0.148$). These findings suggest that exposure to polyethylene microplastics and phenanthrene does not influence the average time between wrestles. On average, interbout duration was 202 ± 186 s (mean ± SD) for crabs in control seawater, and 297 ± 207 and 334 ± 195 s for crabs maintained in PE and PE+PHE seawater respectively (Fig. 2C).

For the *"N_interbout"* models revealed no significant effect of treatments ($p = 0.639$ for PE; $p = 0.434$ for PE+PHE) or carapace width ($p = 0.163$). On average, crabs kept in control seawater showed 1.9 ± 1.4 interbout (mean ± SD), compared to 1.9 ± 1.5 and 2 ± 1.3 of crabs from PE and PE+PHE-treated seawater, respectively (Fig. 2D).

Chi-squared tests indicated no significant deviation from the expected 50:50 distribution for contests between "C vs PE" ($\chi^2 = 2.7$, df = 1, $p = 0.095$) and "C vs PE+PHE" ($\chi^2 = 0.5$, df = 1, $p = 0.479$). These findings suggest that exposure to polyethylene microplastics or polyethylene microplastics with sorbed phenanthrene does not significantly alter the likelihood of winning agonistic interaction for food in *C. maenas*.

3.2. Physiological experiment (heartbeat measurement)

3.2.1. SW European population

Juvenile green crabs displayed no significant differences in morphological features (e.g., carapace width) across treatments (ANOVA, $p > 0.05$). Treatments had no significant effect on the heart rate of SW European *C. maenas* (one-way ANOVA, $p = 0.428$), indicating that exposure to polyethylene microplastics or microplastics sorbed with phenanthrene did not influence heart rate function in the green crab (Fig. 4).

3.2.2. Icelandic population

Similarly to what observed for SW European *C. maenas*, treatments had no significant effect on the heart rate of Icelandic crabs (one-way ANOVA, $p = 0.759$; Fig. 4).

4. Discussion

This study provides the first comprehensive assessment of the responses of two genetically distinct lineages of *Carcinus maenas* to the combined effects of polyethylene microplastics (PE) and phenanthrene (PHE). Despite growing concern over the ecological implications of microplastics (MPs), our findings show limited behavioural and physiological effects after short-term exposure to environmentally relevant MP concentrations. This challenges prevailing assumptions and underscores the importance of using realistic exposure scenarios in ecotoxicological studies.

Our findings revealed that (i) short-term (72 h) exposure to PE, either virgin or PHE-contaminated, does not significantly affect the behavioural or physiological performance of *C. maenas*, and (ii) both Southwest European (SWE) and Icelandic (ICE) populations exhibited similar responses across treatments, except for reduced resource-holding time in ICE crabs exposed to virgin PE compared to control. The observed reduction in ICE crab resource-holding time suggest subtle physiological effects. Contaminants, including metals, have been shown to affect behavioural traits central to animal behavioural types (White and Briffa, 2017; Grunst et al., 2018). For instance, exposure to copper reduced aggressive behaviour in *Carcinus maenas* and *Pagurus bernhardus* (Dissanayake et al., 2009; White et al., 2013). However, the absence of a similar response in the PE+PHE treatment complicates this interpretation indicating that factors beyond toxicity, possibly behavioural variability, could be influencing outcomes.

While mean behavioural responses appeared generally consistent between SWE and ICE populations, high inter-individual variability was consistently observed. This aligns with previous studies on decapod crustaceans, demonstrating that behavioural responses to environmental stressors can vary considerably among individuals due to factors such as intrinsic physiological variation, competitive ability, prior acclimation history and behavioural plasticity (Koolhaas et al., 2007: Briffa et al., 2008; Gherardi et al., 2012; Sih et al., 2015). However, constraints on sensory capabilities, cognitive structure and physiological capacities may limit behavioural plasticity (Hazlett, 1995; Dall et al., 2004), leading to consistent behavioural differences both between and within populations (Wilson, 1998; Brown and Braithwaite, 2004). In crustaceans, consistent inter-individual variation in aggression,

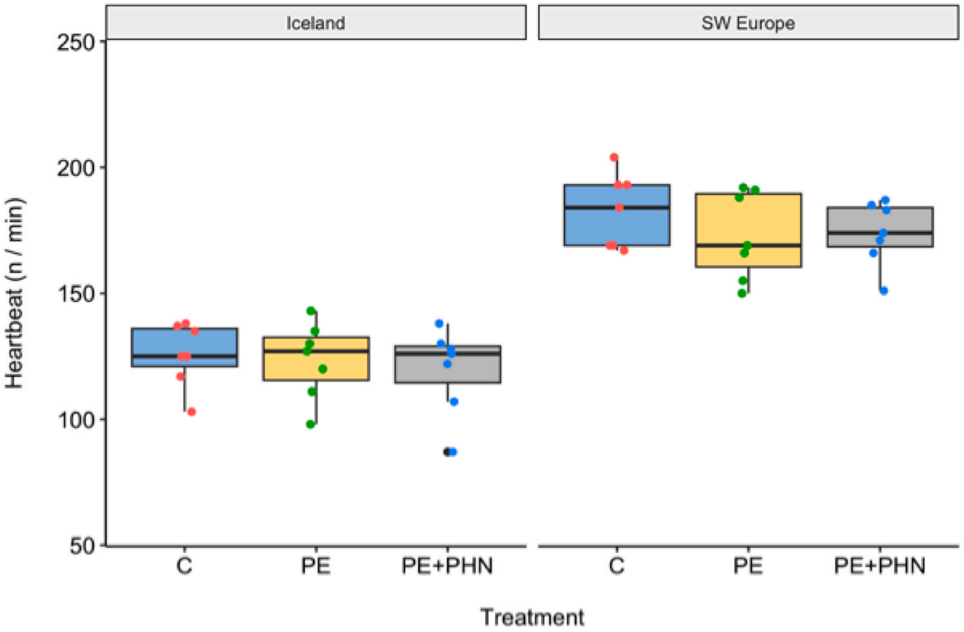

Fig. 4. Heart rate after 72 h of exposure to control seawater (C), virgin polyethylene microplastic (PE) and virgin polyethylene microplastic with sorbed phenanthrene (PE+PHE). Heart rate is expressed as mean heartbeat (HB) per minute. Jittered data points overlaying the plots represent individual variability.

boldness and exploration has been previously described (Mowles et al., 2012). When these differences in behavioural traits remain consistent across time and environmental or social contexts (e.g., presence or absence of predator), they are referred to as animal personalities (Dall et al., 2004; Réale et al., 2007). These personalities can form suites of correlated traits, known as behavioural syndromes (Sih et al., 2004a, 2004b), where, for instance, more aggressive individuals may also exhibit increased activity levels and lower risk aversion. Individual behavioural traits associated with personalities can also be affected by state-dependent behavioural dynamics (Dall et al., 2004; Sih et al., 2015), which may in turn be influenced by pollutant exposure (Montiglio and Royauté, 2014). Given these dynamics, we propose that the high inter-individual variability observed in our study may be at least partially explained by animal personalities and behavioural syndromes. Future studies should incorporate pre-exposure behavioural assays and environmental microplastic monitoring at collection sites to better characterise population-specific behavioural regimes and contextualise exposure responses.

As observed with behavioural responses, short-term exposure to PE microplastics, whether virgin or PHE-contaminated, did not significantly affect the heart rates of *C. maenas.* Heart rate (HR) was used as a proxy for physiological stress as it provides valuable insights into species responses to environmental changes, contaminants, and metabolic alterations (DeFur and Mangum, 1979; Bamber and Depledge, 1997; Handy and Depledge, 1999; Green, 2011). HR remained generally consistent across treatments within each population, yet ICE crabs exhibited generally lower HR than SWE ones. These differences are possibly linked to temperature-dependent metabolic variation, rather than genotypic intraspecific differences. Indeed, acclimation to colder temperatures in ICE crabs (10 °C) compared to SWE crabs (17 °C) might explain the intraspecific differences in HR (Tepolt and Somero, 2014).

C. maenas is a poikilotherm and its physiological and behavioural processes such as metabolism, growth, and activity, are strongly influenced by fluctuations in environmental temperature. Numerous studies on decapod crustaceans, particularly *C. maenas*, have shown a direct positive relationship between increased temperature and HR, likely driven by higher metabolic demand (Styrishave et al., 1999; Frederich and Pörtner, 2000; McGaw and Nancollas, 2018). For example, the HR of a 25 mm (CW) individual of *C. maenas* was reported to be ~ 80 HB min^{-1} at 10 °C, increasing to ~ 130 HB min^{-1} at 20 °C, yielding a Q_{10} of 1.7 in controlled-laboratory experiments (Ahsanullah and Newell, 1971). Similarly, Q_{10} for HR of *C. maenas* between 4 °C and 20 °C was estimated at 2.4 during *in situ* experiments (Styrishave et al., 1999). These findings align with our observed Q_{10} of 1.7 between ICE (11 °C) and SWE (17 °C) heart rate.

Contrary to our observations, previous research has consistently documented impaired behavioural and physiological responses in crustaceans exposed to MPs (reviewed in Yin et al., 2022; D'Costa, 2022; Zhang et al., 2023). For instance, swimming and feeding behaviours have been negatively affected in amphipods and copepods (Tosetto et al., 2016; Cole et al., 2015; Carrasco et al., 2019; Suwaki et al., 2020). In decapod crustaceans, MP exposure has been linked to increased susceptibility to predation (5 days; 100 μg L^{-1} (Nanninga et al., 2020b) and disruption of contest dynamics and shell selection in hermit crabs, with adverse effects on rapping behaviours and key cognitive pathways such as resource evaluation (5 days, 5 g L^{-1}; Cunningham et al., 2021a; Crump et al., 2023). Research has also shown that *C. maenas* can take up MPs through ingestion and inhalation across the gills (Farrell and Nelson, 2013; Watts et al., 2014; Yi et al., 2021), leading to retention within body tissues for up to 3 weeks and resulting in physiological impairment, including decreased gill function, reduced food consumption, and lower energy for growth (Watts et al., 2015, 2016).

Combined exposure to MPs and PAHs has also been observed to exacerbate the adverse effects of MPs (e.g., Sun et al., 2021; Su et al., 2022), primarily by altering their bioavailability and toxicity. For instance, the microbial degradation of PHE is significantly reduced when it is associated with PE, leading to its increased environmental persistence (Hatzinger and Alexander, 1997). Synergistic effects have also been observed with other pollutants; for example, co-exposure to PHE and copper (24 h) triggered behavioural changes in the amphipod *Hyalella azteca* (Gauthier et al., 2016), while dimethyl sulfide-contaminated nylon fibres reduced grazing rates of the copepod *Calanus helgolandicus* more significantly than virgin fibres (Procter et al., 2019). The effects of PHE have also been studied in relation to cardiac function, with research highlighting adverse impacts in various species (Brette et al., 2014, 2017; England et al., 2024). Based on these findings, we hypothesised that PHE-contaminated PE would have elicited more pronounced behavioural and physiological effects on *C. maenas* compared to virgin particles. However, our findings did not support this hypothesis.

The lack of significant effects in our study may be attributed to a combination of factors, including the relatively low (but realistic) concentrations of MPs and PHE, as well as the short exposure duration. Specifically, the concentration of PE employed in this study (50 mg L^{-1}) aligns with lower environmental exposure levels (Mbedzi et al., 2020), providing realistic scenarios for risk assessment. This approach aligns with recent calls from the scientific community to prioritise environmentally relevant experimental designs (Cunningham and Sigwart, 2019). Supporting this interpretation, research has shown that realistic concentrations of MPs did not significantly affect the emergence behaviour of the intertidal gastropod *Littorina littorea* (Doyle et al., 2020) or the feeding behaviour of *Carcinus maenas* (Cunningham et al., 2021b).

The low PHE concentration tested in this study (~1 μg L^{-1}), aligning with the maximum permissible level in seawater (Verbruggen and Van Herwijnen, 2012), likely limited its bioavailability and uptake. This concentration is two orders of magnitude lower than the safe level for the Chinese mitten crab *Eriocheir sinensis* (214 μg L^{-1}; Jiang et al., 2015) and the LC50 for *C. maenas* exposed to sulfoxaflor (288 μg L^{-1}; Damasceno et al., 2021). Given that bioaccumulation and elimination of polycyclic aromatic hydrocarbons like phenanthrene depend on exposure concentration and duration (Fillmann et al., 2002; Watson et al., 2004), the environmentally relevant PHE levels used here likely reduced the potential for toxic effects. These findings highlight a critical yet often overlooked aspect of ecotoxicological research: the necessity of testing pollutants at realistic environmental concentrations.

Research on the physiological and behavioural responses of decapods to higher PAHs concentrations supports this reasoning. For example, exposure to sublethal concentrations (200 ug L-1; 7–15 days) of pyrene significantly increased juvenile *Carcinus maenas* HR (from ~ 90 HB min^{-1} to ~ 120 HB min^{-1}) and enhanced competitive ability during agonistic interactions for food (Dissanayake et al., 2008, 2009). Similarly, higher concentrations of PHE were also linked to toxic effects (14 days; 50 μg L^{-1}; Yang et al., 2022) and acute mortality in *Eriocheir sinensis* (4 days; 7000 μg L^{-1}; Jiang et al., 2015). In contrast, low PHE concentrations (~ 1 μg L^{-1}) were sufficient to induce immunosuppression of hemocytes in mud crabs *Scylla paramamosain* (7 days; Yifei et al., 2024), trigger bioaccumulation in the lugworm *Arenicola marina* (Teuten et al., 2007; Browne et al., 2013), and reduce growth rates in two phytoplankton species (7 days; Jiang et al., 2022). These studies, however, were conducted over longer exposure periods.

These differences highlight the importance of exposure duration in ecotoxicological research. Studies on decapod crustaceans shows considerable variability in this regard, with exposures ranging from hours to weeks (D'Costa, 2022). For instance, prolonged exposure (15 days) to varying concentrations of PHE (0.5, 1.0 and 1.5 mg L^{-1}) reduced maximum HR in freshwater crayfish (*Pacifastacus leniusculus*) without affecting routine HR (Ainerua et al., 2021). Shorter exposures, such as those in our study, may fail to capture sublethal or chronic effects, which often require extended periods to emerge. Taken together, our findings suggest that the lack of significant effects observed after 72 h of exposure to PE and PHE possibly reflects the combined influence of low pollutant concentrations, short exposure duration and substantial inter-individual variability.

5. Conclusion

This study demonstrated that short-term exposure to environmentally relevant concentrations of virgin and phenanthrene-contaminated polyethylene microplastics had limited effects on the behavioural and physiological performance of *Carcinus maenas*. Genetically distinct populations of green crab responded similarly, except for a significant reduction in resource-holding behaviour in ICE crabs exposed to PE. High inter-individual variability in behavioural responses suggests that susceptibility to MPs and PAHs is not uniform within populations. While realistic MP concentrations enhance ecological relevance, short exposure duration and limited sample size for physiological endpoints may have constrained the detection of subtle or delayed effects. Future research should adopt long-term designs and individual-level approaches to better assess the interplay between plastic pollution, genetic diversity, and behavioural plasticity.

CRediT authorship contribution statement

Lima Fernando P: Writing – review & editing, Software. **Gerardo I Zardi:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Lorenzo Cozzolino:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Katy R Nicastro:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Christopher D McQuaid:** Writing – review & editing, Supervision. **Halldór P Halldórsson:** Writing – review & editing, Validation, Resources.

Declaration of Competing Interest

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

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Data availability

Data will be made available on request.

References

Ahsanullah, M., Newell, R.C., 1971. Factors affecting the heart rate of the shore crab *carcinus maenas* (L.). Comparative Biochemistry Physiology Part A Physiology 39 (2), 277–287.

Ainerua, M.O., Tinwell, J., Murphy, R., Galli, G.L., Van Dongen, B.E., White, K.N., Shiels, H.A., 2021. Prolonged phenanthrene exposure reduces cardiac function but fails to mount a significant oxidative stress response in the signal crayfish (*pacifastacus leniusculus*). Chemosphere 268, 129297.

Ali, M., Xu, D., Yang, X., Hu, J., 2024. Microplastics and PAHs mixed contamination: an in-depth review on the sources, co-occurrence, and fate in marine ecosystems. Water Res., 121622

Ali, N., Khan, M.H., Ali, M., Ahmad, S., Khan, A., Nabi, G., Kyzas, G.Z., 2023. Insight into microplastics in the aquatic ecosystem: properties, sources, threats and mitigation strategies. Sci. Total Environ., 169489

Anyahara, J.N., 2021. Effects of polycyclic aromatic hydrocarbons (PAHs) on the environment: a systematic review. Int. J. Adv. Acad. Res. 7 (3).

Bakir, A., Rowland, S.J., Thompson, R.C., 2014. Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. Environ. Pollut. 185, 16–23.

Bamber, S.D., Depledge, M.H., 1997. Responses of shore crabs to physiological challenges following exposure to selected environmental contaminants. Aquat. Toxicol. 40 (1), 79–92.

Becks, L., Ellner, S.P., Jones, L.E., Hairston Jr, N.G., 2010. Reduction of adaptive genetic diversity radically alters eco-evolutionary community dynamics. Ecol. Lett. 13 (8), 989–997.

Besseling, E., Wegner, A., Foekema, E.M., Van Den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *arenicola marina* (L.). Environ. Sci. Technol. 47 (1), 593–600.

Bhagat, J., Sarkar, A., Ingole, B.S., 2016. DNA damage and oxidative stress in marine gastropod *morula granulata* exposed to phenanthrene. Water Air Soil Pollut. 227, 1–12.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Vasseur, D.A., 2011. Why intraspecific trait variation matters in community ecology. Trends Ecol. Evol. 26 (4), 183–192.

Borrelle, S.B., Ringma, J., Law, K.L., Monnahan, C.C., Lebreton, L., McGivern, A., Rochman, C.M., 2020. Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. Science 369 (6510), 1515–1518.

Brette, F., Cros, C., Machado, B., Incardona, J.P., Scholz, N.L., Block, B.A., 2014. Crude oil impairs cardiac excitation-contraction coupling in fish. Biophys. J. 106 (2), 732a.

Brette, F., Shiels, H.A., Galli, G.L., Cros, C., Incardona, J.P., Scholz, N.L., Block, B.A., 2017. A novel cardiotoxic mechanism for a pervasive global pollutant. Sci. Rep. 7 (1), 41476.

Briffa, M., Rundle, S.D., Fryer, A., 2008. Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab *pagurus bernhardus*. Proc. R. Soc. B Biol. Sci. 275 (1640), 1305–1311.

Brown, C., Braithwaite, V.A., 2004. Size matters: a test of boldness in eight populations of the poeciliid *brachyraphis episcopi*. Anim. Behav. 68 (6), 1325–1329.

Browne, M.A., Niven, S.J., Galloway, T.S., Rowland, S.J., Thompson, R.C., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol. 23 (23), 2388–2392.

Carbery, M., O'Connor, W., Palanisami, T., 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environ. Int. 115, 400–409.

Carrasco, A., Pulgar, J., Quintanilla-Ahumada, D., Perez-Venegas, D., Quijón, P.A., Duarte, C., 2019. The influence of microplastics pollution on the feeding behavior of a prominent sandy beach amphipod, *orchestoidea tuberculata* (Nicolet, 1849). Mar. Pollut. Bull. 145, 23–27.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *calanus helgolandicus*. Environ. Sci. Technol. 49 (2), 1130–1137.

Correia, A.D., Gonçalves, R., Scholze, M., Ferreira, M., Henriques, M.A.R., 2007. Biochemical and behavioral responses in gilthead seabream (*sparus aurata*) to phenanthrene. J. Exp. Mar. Biol. Ecol. 347 (1-2), 109–122.

Cozzolino, L., Nicastro, K.R., Seuront, L., McQuaid, C.D., Zardi, G.I., 2022. The relative effects of interspecific and intraspecific diversity on microplastic trapping in coastal biogenic habitats. Sci. Total Environ. 848, 157771.

Cozzolino, L., Nicastro, K.R., Hubbard, P.C., Seuront, L., McQuaid, C.D., Zardi, G.I., 2024. Intraspecific genetic lineages of a marine mussel show behavioural divergence when exposed to microplastic leachates. Environ. Pollut. 340, 122779.

Crump, A., Aiken, C., Cunningham, E.M., Arnott, G., 2023. Short-term microplastic exposure impairs cognition in hermit crabs. Animals 13, 1055, 2023.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C., Sanders, N.J., 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. Science 313 (5789), 966–968.

Cunningham, E.M., Sigwart, J.D., 2019. Environmentally accurate microplastic levels and their absence from exposure studies. Integr. Comp. Biol. 59 (6), 1485–1496.

Cunningham, E.M., Mundye, A., Kregting, L., Dick, J.T., Crump, A., Riddell, G., Arnott, G., 2021b. Animal contests and microplastics: evidence of disrupted behaviour in hermit crabs *pagurus bernhardus*. R. Soc. Open Sci. 8 (10), 211089.

Cunningham, E.M., Cuthbert, R.N., Coughlan, N.E., Kregting, L., Cairnduff, V., Dick, J.T., 2021a. Microplastics do not affect the feeding rates of a marine predator. Sci. Total Environ. 779, 146487.

Dai, C., Han, Y., Duan, Y., Lai, X., Fu, R., Liu, S., Zhou, L., 2022. Review on the contamination and remediation of polycyclic aromatic hydrocarbons (PAHs) in coastal soil and sediments. Environ. Res. 205, 112423.

Dall, S.R., Houston, A.I., McNamara, J.M., 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecol. Lett. 7 (8), 734–739.

Dam, E., Styrishave, B., Rewitz, K.F., Andersen, O., 2006. Intermoult duration affects the susceptibility of shore crabs carcinus maenas (L.) to pyrene and their ability to metabolise it. Aquat. Toxicol. 80 (3), 290–297.

Damasceno, J.M., Rato, L.D., Simões, T., Morão, I.F., Meireles, G., Novais, S.C., Lemos, M.F., 2021. Exposure to the insecticide sulfoxaflor affects behaviour and biomarkers responses of *carcinus maenas* (Crustacea: Decapoda). Biology 10 (12), 1234.

Darling, J.A., Bagley, M.J., Roman, J.O.E., Tepolt, C.K., Geller, J.B., 2008. Genetic patterns across multiple introductions of the globally invasive crab genus carcinus. Mol. Ecol. 17 (23), 4992–5007.

D'Costa, A.H., 2022. Microplastics in decapod crustaceans: accumulation, toxicity and impacts, a review. Sci. Total Environ. 832, 154963.

DeFur, P.L., Mangum, C.P., 1979. The effects of environmental variables on the heart rates of invertebrates. Comparative Biochemistry Physiology Part A Physiology 62 (2), 283–294.

Des Roches, S., Post, D.M., Turley, N.E., Bailey, J.K., Hendry, A.P., Kinnison, M.T., Palkovacs, E.P., 2018. The ecological importance of intraspecific variation. Nat. Ecol. Evol. 2 (1), 57–64.

Dissanayake, A., Galloway, T.S., Jones, M.B., 2008. Physiological responses of juvenile and adult shore crabs *carcinus maenas* (Crustacea: Decapoda) to pyrene exposure. Mar. Environ. Res. 66 (4), 445–450.

Dissanayake, A., Galloway, T.S., Jones, M.B., 2009. Physiological condition and intraspecific agonistic behaviour in *carcinus maenas* (Crustacea: Decapoda). J. Exp. Mar. Biol. Ecol. 375 (12), 57–63.

Domingues, C.P., Creer, S., Taylor, M.I., Queiroga, H., Carvalho, G.R., 2010. Genetic structure of *carcinus maenas* within its native range: larval dispersal and oceanographic variability. Mar. Ecol. Prog. Ser. 410, 111–123.

Douben, P.E.T., 2003. PAHs: an ecotoxicological perspective. John Wiley & Sons. https://doi.org/10.1002/0470867132.

Doyle, D., Frias, J., Nash, R., Gammell, M., 2020. Current environmental microplastic levels do not alter emergence behaviour in the intertidal gastropod *littorina littorea*. Mar. Pollut. Bull. 151, 110859.

England, E., Morris, J.W., Bussy, C., Hancox, J.C., Shiels, H.A., 2024. The key characteristics of cardiotoxicity for the pervasive pollutant phenanthrene. J. Hazard. Mater. 469, 133853.

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *mytilus edulis* (L.) to *carcinus maenas* (L.). Environ. Pollut. 177, 1–3.

Fillmann, G., Watson, G.M., Francioni, E., Readman, J.W., Depledge, M.H., 2002. A non-destructive assessment of the exposure of crabs to PAH using ELISA analyses of their urine and haemolymph. Mar. Environ. Res. 54 (3-5), 823–828.

Foss, A., Imsland, A.K., Nævdal, G., 1998. Population genetic studies of the atlantic halibut in the north Atlantic Ocean. J. Fish. Biol. 53 (4), 901–905.

Frederich, M., Pörtner, H.O., 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *maja squinado*. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279 (5), R1531–R1538.

Fries, E., Zarfl, C., 2012. Sorption of polycyclic aromatic hydrocarbons (PAHs) to low and high density polyethylene (PE). Environ. Sci. Pollut. Res. 19, 1296–1304.

Gauthier, P.T., Norwood, W.P., Prepas, E.E., Pyle, G.G., 2016. Behavioural alterations from exposure to cu, phenanthrene, and Cu-phenanthrene mixtures: linking behaviour to acute toxic mechanisms in the aquatic amphipod, *hyalella azteca*. Aquat. Toxicol. 170, 377–383.

Gherardi, F., Aquiloni, L., Tricarico, E., 2012. Behavioral plasticity, behavioral syndromes and animal personality in crustacean decapods: an imperfect map is better than no map. Curr. Zool. 58 (4), 567–579.

Govaert, L., Hendry, A.P., Fattahi, F., Möst, M., 2024. Quantifying interspecific and intraspecific diversity effects on ecosystem functioning. Ecology 105 (1), e4199.

Green, J.A., 2011. The heart rate method for estimating metabolic rate: a review and recommendations. Comp. Biochem. Physiol. A. 158, 287–304.

Grosholz, E.D., Ruiz, G.M., Dean, C.A., Shirley, K.A., Maron, J.L., Connors, P.G., 2000. The impacts of a nonindigenous marine predator in a california bay. Ecology 81 (5), 1206–1224.

Grunst, A.S., Grunst, M.L., Thys, B., Raap, T., Daem, N., Pinxten, R., Eens, M., 2018. Variation in personality traits across a metal pollution gradient in a free-living songbird. Sci. Total Environ. 630, 668–678.

Gunaalan, K., Fabbri, E., Capolupo, M., 2020. The hidden threat of plastic leachates: a critical review on their impacts on aquatic organisms. Water Res. 184, 116170.

Guzzetti, E., Sureda, A., Tejada, S., Faggio, C., 2018. Microplastic in marine organism: environmental and toxicological effects. Environ. Toxicol. Pharmacol. 64, 164–171.

Handy, R.D., Depledge, M.H., 1999. Physiological responses: their measurement and use as environmental biomarkers in ecotoxicology. Ecotoxicology 8 (5), 329–349.

Hatzinger, P.B., Alexander, M., 1997. Biodegradation of organic compounds sequestered in organic solids or in nanopores within silica particles. Environ. Toxicol. Chem. 16 (11), 2215–2221.

Hazlett, B.A., 1995. Behavioral plasticity in crustacea: why not more? J. Exp. Mar. Biol. Ecol. 193 (1-2), 57–66.

Hong, X., Qin, J., Chen, R., Yuan, L., Zha, J., Huang, C., Wang, Z., 2017. Phenanthrene-induced apoptosis and its underlying mechanism. Environ. Sci. Technol. 51 (24), 14397–14405.

Hou, G., Zhao, X., Zhao, T., Wu, X., Pu, S., Tang, Z., Wu, F., 2023. The adsorption of PAHs on microplastics and desorption in the simulated human digestive system. Chem. Eng. J. 473, 145157.

Hughes, A.R., Stachowicz, J.J., 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proc. Natl. Acad. Sci. 101 (24), 8998–9002.

Isobe, A., Iwasaki, S., Uchida, K., Tokai, T., 2019. Abundance of non-conservative microplastics in the upper ocean from 1957 to 2066. Nat. Commun. 10 (1), 417.

Jeong, E., Lee, J.Y., Redwan, M., 2024. Animal exposure to microplastics and health effects: a review. Emerg. Contam., 100369

Jiang, L., Pan, Y., Zhu, S., Qiu, J., Shang, Y., Xu, J., Wang, H., 2022. Stimulatory and inhibitory effects of phenanthrene on physiological performance of *chlorella vulgaris* and *skeletonema costatum*. Sci. Rep. 12 (1), 5194.

Jiang, R.L., Xiao, B.C., Zhang, F., Feng, D.X., Yu, N., Du, Z.Y., Chen, L.Q., 2015. Acute toxic effects of phenanthrene on Chinese mitten crab eriocheir sinensis. Chin. J. Appl. Environ. Biol. 21 (1), 108–114.

Kaiser, M.J., Hughes, R.N., Reid, D.G., 1990. Chelal morphometry, prey-size selection and aggressive competition in Green and red forms of carcinus maenas (L.). J. Exp. Mar. Biol. Ecol. 140 (1-2), 121–134.

Koolhaas, J.M., De Boer, S.F., Buwalda, B., Van Reenen, K., 2007. Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. Brain Behav. Evol. 70 (4), 218–226.

Kumar, R., Verma, A., Shome, A., Sinha, R., Sinha, S., Jha, P.K., Vara Prasad, P.V., 2021. Impacts of plastic pollution on ecosystem services, sustainable development goals, and need to focus on circular economy and policy interventions. Sustainability 13 (17), 9963.

Li, Y., Yang, G., Wang, J., Lu, L., Li, X., Zheng, Y., Ru, S., 2022. Microplastics increase the accumulation of phenanthrene in the ovaries of marine medaka (*oryzias melastigma*) and its transgenerational toxicity. J. Hazard. Mater. 424, 127754.

Ma, Y., Huang, A., Cao, S., Sun, F., Wang, L., Guo, H., Ji, R., 2016. Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water. Environ. Pollut. 219, 166–173.

Maletić, S.P., Beljin, J.M., Rončević, S.D., Grgić, M.G., Dalmacija, B.D., 2019. State of the art and future challenges for polycyclic aromatic hydrocarbons is sediments: sources, fate, bioavailability and remediation techniques. J. Hazard. Mater. 365, 467–482.

Mbedzi, R., Dalu, T., Wasserman, R.J., Murungweni, F., Cuthbert, R.N., 2020. Functional response quantifies microplastic uptake by a widespread African fish species. Sci. Total Environ. 700, 134522.

McDonald, P.S., Jensen, G.C., Armstrong, D.A., 2001. The competitive and predatory impacts of the nonindigenous crab *carcinus maenas* (L.) on early benthic phase dungeness crab *cancer magister* dana. J. Exp. Mar. Biol. Ecol. 258 (1), 39–54.

McGaw, I.J., Nancollas, S.J., 2018. Experimental setup influences the cardiovascular responses of decapod crustaceans to environmental change. Can. J. Zool. 96 (9), 1043–1052.

McGoran, A.R., Clark, P.F., Smith, B.D., Morritt, D., 2020. High prevalence of plastic ingestion by eriocheir sinensis and *carcinus maenas* (Crustacea: Decapoda: Brachyura) in the thames estuary. Environ. Pollut. 265, 114972.

Means, J.C., Wood, S.G., Hassett, J.J., Banwart, W.L., 1980. Sorption of polynuclear aromatic hydrocarbons by sediments and soils. Environ. Sci. Technol. 14 (12), 1524–1528.

Miller, M.E., Hamann, M., Kroon, F.J., 2020. Bioaccumulation and biomagnification of microplastics in marine organisms: a review and meta-analysis of current data. PloS One 15 (10), e0240792.

Mojiri, A., Zhou, J.L., Ohashi, A., Ozaki, N., Kindaichi, T., 2019. Comprehensive review of polycyclic aromatic hydrocarbons in water sources, their effects and treatments. Sci. Total Environ. 696, 133971.

Montiglio, P.O., Royauté, R., 2014. Contaminants as a neglected source of behavioural variation. Anim. Behav. 88 (1), 29–35.

Mowles, S.L., Cotton, P.A., Briffa, M., 2012. Consistent crustaceans: the identification of stable behavioural syndromes in hermit crabs. Behav. Ecol. Sociobiol. 66, 1087–1094.

Nanninga, G.B., Scott, A., Manica, A., 2020a. Microplastic ingestion rates are phenotype-dependent in juvenile anemonefish. Environ. Pollut. 259, 113855.

Nanninga, G.B., Horswill, C., Lane, S.M., Manica, A., Briffa, M., 2020b. Microplastic exposure increases predictability of predator avoidance strategies in hermit crabs. J. Hazard. Mater. Lett. 1, 100005.

Nicastro, K.R., McQuaid, C.D., Dievart, A., Zardi, G.I., 2020. Intraspecific diversity in an ecological engineer functionally trumps interspecific diversity in shaping community structure. Sci. Total Environ. 743, 140723.

Nicastro, K.R., Seuront, L., McQuaid, C.D., Zardi, G.I., 2022. Symbiont-induced intraspecific phenotypic variation enhances plastic trapping and ingestion in biogenic habitats. Sci. Total Environ. 826, 153922.

Nicastro, K.R., Seuront, L., Cozzolino, L., Froneman, W., Zardi, G.I., 2023. The neglected role of intraspecific variation in plastic pollution research. Anthr. Sci. 2 (2), 141–147.

Palkovacs, E.P., Post, D.M., 2009. Experimental evidence that phenotypic divergence in predators drives community divergence in prey. Ecology 90 (2), 300–305.

Peng, X., Sun, X., Yu, M., Fu, W., Chen, H., Chen, J., 2019. Chronic exposure to environmental concentrations of phenanthrene impairs zebrafish reproduction. Ecotoxicol. Environ. Saf. 182, 109376.

Phuong, N.N., Fauvelle, V., Grenz, C., Ourgaud, M., Schmidt, N., Strady, E., Sempéré, R., 2021. Highlights from a review of microplastics in marine sediments. Sci. Total Environ. 777, 146225.

Procter, J., Hopkins, F.E., Fileman, E.S., Lindeque, P.K., 2019. Smells good enough to eat: dimethyl sulfide (DMS) enhances copepod ingestion of microplastics. Mar. Pollut. Bull. 138, 1–6.

Raffard, A., Santoul, F., Cucherousset, J., Blanchet, S., 2019. The community and ecosystem consequences of intraspecific diversity: a meta-analysis. Biol. Rev. 94 (2), 648–661.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. Biol. Rev. 82 (2), 291–318.

Reid, D.G., Aldrich, J.C., 1989. Variations in response to environmental hypoxia of different colour forms of the shore crab, carcinus maenas. Comparative Biochemistry Physiology Part A Physiology 92 (4), 535–539.

Reid, D.G., Abelló, P., McGaw, I., Naylor, E., 1989. Differential tolerances of desiccation and hypo-osmotic stress in sub- and inter-tidal carcinus maenas. In: Aldrich, J.C. (Ed.), Phenotypic Responses in Aquatic Ectotherms. Japaga, Dublin, pp. 89–96.

Reid, D.G., Abelló, P., Warman, C.G., Naylor, E., 1994. Size-related mating success in the shore crab carcinus maenas (Crustacea: Brachyura). J. Zool. 232 (3), 397–407.

Reid, D.G., Abelló, P., Kaiser, M.J., Warman, C.G., 1997. Carapace colour, inter-moult duration and the behavioural and physiological ecology of the shore crabcarcinus maenas. Estuar. Coast. Shelf Sci. 44 (2), 203–211.

de Rivera, C.E., Grosholz, E.D., Ruiz, G.M., 2011. Multiple and long-term effects of an introduced predatory crab. Mar. Ecol. Prog. Ser. 429, 145–155.

Rochman, C.M., Hoh, E., Hentschel, B.T., Kaye, S., 2013. Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: implications for plastic marine debris. Environ. Sci. Technol. 47 (3), 1646–1654.

Rodrigues, J.P., Duarte, A.C., Santos-Echeandía, J., Rocha-Santos, T., 2019. Significance of interactions between microplastics and POPs in the marine environment: a critical overview. TrAC Trends Anal. Chem. 111, 252–260.

Roman, J.O.E., Palumbi, S.R., 2004. A global invader at home: population structure of the Green crab, *carcinus maenas*, in Europe. Mol. Ecol. 13 (10), 2891–2898.

Shaw, P.W., Turan, C., Wright, J.M., O'connell, M., Carvalho, G.R., 1999. Microsatellite DNA analysis of population structure in atlantic herring (*clupea harengus*), with direct comparison to allozyme and mtDNA RFLP analyses. Heredity 83 (4), 490–499.

Sih, A., Bell, A.M., Johnson, J.C., Ziemba, R.E., 2004b. Behavioral syndromes: an integrative overview. Q. Rev. Biol. 79 (3), 241–277.

Sih, A., Bell, A., Johnson, J.C., 2004a. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol. Evol. 19 (7), 372–378.

Sih, A., Mathot, K.J., Moiron, M., Montiglio, P.O., Wolf, M., Dingemanse, N.J., 2015. Animal personality and state–behaviour feedbacks: a review and guide for empiricists. Trends Ecol. Evol. 30 (1), 50–60.

Sneddon, L.U., Huntingford, F.A., Taylor, A.C., 1997. The influence of resource value on the agonistic behaviour of the shore crab, *carcinus maenas* (L.). Mar. Freshw. Behav. Physiol. 30 (4), 225–237.

Sørensen, L., Rogers, E., Altin, D., Salaberria, I., Booth, A.M., 2020. Sorption of PAHs to microplastic and their bioavailability and toxicity to marine copepods under co-exposure conditions. Environ. Pollut. 258, 113844.

Styrishave, B., Aagaard, A., Andersen, O., 1999. In situ studies on physiology and behaviour in two colour forms of the shore crab *carcinus maenas* in relation to season. Mar. Ecol. Prog. Ser. 189, 221–231.

Su, Y., Qi, H., Hou, Y., Gao, M., Li, J., Cai, M., Peng, L., 2022. Combined effects of microplastics and benzo [a] pyrene on the marine diatom *chaetoceros muelleri*. Front. Mar. Sci. 8, 779321.

Suckling, C.C., 2021. Responses to environmentally relevant microplastics are species-specific with dietary habit as a potential sensitivity indicator. Sci. Total Environ. 751, 142341.

Sun, S., Shi, W., Tang, Y., Han, Y., Du, X., Zhou, W., Liu, G., 2021. The toxic impacts of microplastics (MPs) and polycyclic aromatic hydrocarbons (PAHs) on haematic parameters in a marine bivalve species and their potential mechanisms of action. Sci. Total Environ. 783, 147003.

Suwaki, C.H., De-La-Cruz, L.T., Lopes, R.M., 2020. Impacts of microplastics on the swimming behavior of the copepod *temora turbinata* (Dana, 1849). Fluids 5 (3), 103.

Tepolt, C.K., Palumbi, S.R., 2015. Transcriptome sequencing reveals both neutral and adaptive genome dynamics in a marine invader. Mol. Ecol. 24 (16), 4145–4158.

Tepolt, C.K., Palumbi, S.R., 2020. Rapid adaptation to temperature via a potential genomic island of divergence in the invasive Green crab, *carcinus maenas*. Front. Ecol. Evol. 8, 580701.

Tepolt, C.K., Somero, G.N., 2014. Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the european Green crab, *carcinus maenas*. J. Exp. Biol. 217 (7), 1129–1138.

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ. Sci. Technol. 41 (22), 7759–7764.

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., et al., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364, 2027–2045.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304 (5672), 838-838.

Thushari, G.G.N., Senevirathna, J.D.M., 2020. Plastic pollution in the marine environment. Heliyon 6 (8), e04709.

Tosetto, L., Brown, C., Williamson, J.E., 2016. Microplastics on beaches: ingestion and behavioural consequences for beachhoppers. Mar. Biol. 163, 1–13.

Ugwu, K., Herrera, A., Gómez, M., 2021. Microplastics in marine biota: a review. Mar. Pollut. Bull. 169, 112540.

Verbruggen, E.M.J., Van Herwijnen, R., 2012. RIVM letter report. Environ. risk Limits phenanthrene, 601357007.

Wang, S., Ma, L., Chen, L., Sokolova, I.M., Huang, W., Li, D., Wang, Y., 2024. The combined effects of phenanthrene and micro-/nanoplastics mixtures on the cellular stress responses of the thick-shell mussel *mytilus coruscus*. Environ. Pollut. 341, 122999.

Wang, W., Gao, H., Jin, S., Li, R., Na, G., 2019. The ecotoxicological effects of microplastics on aquatic food web, from primary producer to human: a review. Ecotoxicol. Environ. Saf. 173, 110–117.

Watson, G.M., Andersen, O.K., Galloway, T.S., Depledge, M.H., 2004. Rapid assessment of polycyclic aromatic hydrocarbon (PAH) exposure in decapod crustaceans by fluorimetric analysis of urine and haemolymph. Aquat. Toxicol. 67 (2), 127–142.

Watts, A.J., Lewis, C., Goodhead, R.M., Beckett, S.J., Moger, J., Tyler, C.R., Galloway, T. S., 2014. Uptake and retention of microplastics by the shore crab *carcinus maenas*. Environ. Sci. Ttechnology 48 (15), 8823–8830.

Watts, A.J., Urbina, M.A., Corr, S., Lewis, C., Galloway, T.S., 2015. Ingestion of plastic microfibers by the crab *carcinus maenas* and its effect on food consumption and energy balance. Environ. Sci. Technol. 49 (24), 14597–14604.

Watts, A.J., Urbina, M.A., Goodhead, R., Moger, J., Lewis, C., Galloway, T.S., 2016. Effect of microplastic on the gills of the shore crab *carcinus maenas*. Environ. Sci. Technol. 50 (10), 5364–5369.

White, S.J., Briffa, M., 2017. How do anthropogenic contaminants (ACs) affect behaviour? Multi-level analysis of the effects of copper on boldness in hermit crabs. Oecologia 183, 391–400.

White, S.J., Pipe, R.K., Fisher, A., Briffa, M., 2013. Asymmetric effects of contaminant exposure during asymmetric contests in the hermit crab *pagurus bernhardus*. Anim. Behav. 86 (4), 773–781.

Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster, S.M., Bangert, R.K., LeRoy, C.J., Wooley, S.C., 2006. A framework for community and ecosystem genetics: from genes to ecosystems. Nat. Rev. Genet. 7 (7), 510–523.

Wilson, D.S., 1998. Adaptive individual differences within single populations. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 353 (1366), 199–205.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.

Yang, Z., Fang, Y., Liu, J., Chen, A., Cheng, Y., Wang, Y., 2022. Moderate acidification mitigates the toxic effects of phenanthrene on the mitten crab *eriocheir sinensis*. Chemosphere 294, 133783.

Yi, Y.Z., Azman, S., Primus, A., Said, M.I.M., Abideen, M.Z., 2021. Microplastic ingestion by crabs. The 6th proceeding of civil engineering. Sch. Civ. Eng. 363–375.

Yifei, Y., Zhixiong, Z., Luna, C., Qihui, C., Zuoyuan, W., Xinqi, L., Xiujuan, Z., 2024. Marine pollutant phenanthrene (PHE) exposure causes immunosuppression of hemocytes in crustacean species, *scylla paramamosain*. Comparative Biochemistry Physiology Part C Toxicology Pharmacology 275, 109761.

Yin, J., Li, J.Y., Craig, N.J., Su, L., 2022. Microplastic pollution in wild populations of decapod crustaceans: a review. Chemosphere 291, 132985.

Zhang, S., Wu, H., Hou, J., 2023. Progress on the effects of microplastics on aquatic crustaceans: a review. Int. J. Mol. Sci. 24 (6), 5523.