Journal of Hazardous Materials 487 (2025) 137069

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

First evidence of molecular response of the shrimp *Hippolyte inermis* to biodegradable microplastics

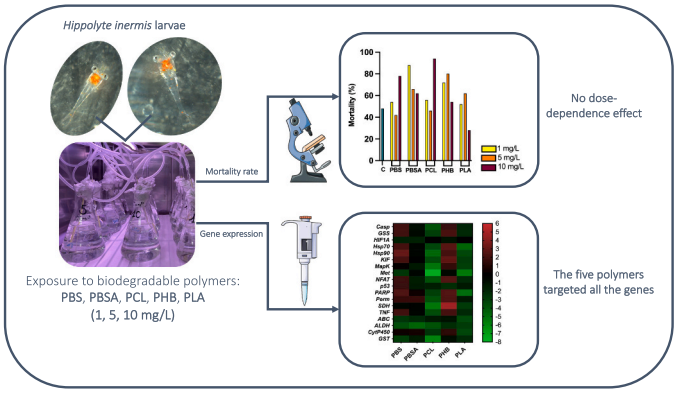
Amalia Amato [a,b,c], Roberta Esposito [a], Bruno Pinto [a,c], Thomas Viel [a], Francesca Glaviano [c], Mariacristina Cocca [d], Loredana Manfra [a,e], Giovanni Libralato [a,b], Eliahu D. Aflalo [f,g], Amir Sagi [f], Maria Costantini [a,*,1], Valerio Zupo [c,1]

[a] *Stazione Zoologica Anton Dohrn, Department of Ecosustainable Marine Biotechnology, Via Ammiraglio Ferdinando Acton 55, Naples 80133, Italy*
[b] *Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, Naples 80126, Italy*
[c] *Stazione Zoologica Anton Dohrn, Department of Ecosustainable Marine Biotechnology, Ischia Marine Centre, Via Francesco Buonocore, 42, Ischia 80077, Italy*
[d] *Institute of Polymers, Composites and Biomaterials, National Research Council of Italy, Via Campi Flegrei, 34, Pozzuoli, Napoli 80078, Italy*
[e] *Institute for Environmental Protection and Research (ISPRA), Via Vitaliano Brancati 48, Rome 00144, Italy*
[f] *Department of Life Sciences, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 8410501, Israel*
[g] *Department of Life Sciences, Achva Academic College, Arugot 7980400, Israel*

HIGHLIGHTS

- After ten days of exposure of *H. inermis* larvae to BPs mortality was triggered by all plastics.
- PCL triggered higher mortality rates compared to other BPs.
- PCL and PLA were the most harmful biodegradable polymers.
- As an excellent model organism *H. inermis* was proposed as a sentinel for the assessment of the effects of BMPs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Crustaceans
Genes
Plastics
Biodegradable polymers
Gene pathaways

ABSTRACT

The increasing demand for sustainable alternatives to conventional plastics has propelled the interest in bioplastics. A few papers reported on the effects of plastics on crustaceans, but no indication about biodegradable polymers is available. *Hippolyte inermis* Leach, 1816 is a protandric shrimp commonly living on leaves of the seagrass *Posidonia oceanica,* in the Mediterranean Sea. This crustacean is typically chosen as a model to study sex differentiation processes. Here, we demonstrated its convenience as a model organism to study the effects of biodegradable polymers (BPs). Five BPs were studied: polybutylene succinate (PBS), polybutylene succinate-co-butylene adipate (PBSA), polycaprolactone (PCL), poly-3-hydroxybutyrates (PHB) and polylactic acid (PLA).

* Corresponding author.
E-mail address: maria.costantini@szn.it (M. Costantini).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jhazmat.2024.137069
Received 12 September 2024; Received in revised form 20 December 2024; Accepted 30 December 2024
Available online 31 December 2024

Larvae of *H. inermis* were exposed to three concentrations of each BP (1, 5 and 10 mg/L, respectively) for ten days. After exposure, the expression levels of eighteen genes involved in stress response and detoxification processes, retrieved from a *H. inermis* transcriptomic library, were validated by Real Time qPCR. This study is the first using a molecular approach to detect *H. inermis* responses to contaminants and in particular to biodegradable polymers, through the evaluation of functional gene's pathways.

1. Introduction

Our interest in biodegradable polymers (BPs), among contaminants of aquatic environments, is progressively increasing due to the notion that they serve as alternatives to "traditional" plastics [1]. However, BPs could still represent dangerous elements for aquatic environments [2], due to variable degrees of degradation and the fact that many are not readily biodegraded at all [3]. As reviewed by Nik Mut et al. [4], BPs can cause negative effects on a range of invertebrates, including crustaceans. Such effects include increased mortality, decreased mobility and influences on reproductive processes. Large amounts of BPs debris can be gradually broken-down into small particles and float in the oceans to form biodegradable microplastics (BMPs) [5,6]. Since crustaceans have an open circulatory system, with internal organs immersed into an haemocoel, the risks of translocation of microplastics (MPs) among organs via the blood circulation is increased [7].

Decapod crustaceans are characterized by wide diversity in terms of physiology, size, morphology and behavior [8]. This diversity facilitates their ubiquitous life in a range of aquatic environments [9]. In addition, decapods are important keystone organisms, playing key roles in benthic food webs and detritus food chains, greatly contributing to the structure of marine ecosystems [10]. In parallel, they are important prey for many organisms, such as pelagic and demersal fish, rays, sharks, reptiles, aquatic mammals and birds [11–13]. From a functional point of view, they own a complex digestive system, as compared to that of other invertebrates [14], comprising three functional segments, besides the mouth and the muscular gut: the anterior, the middle and the posterior intestine. The midgut, which contains numerous tubules and is connected to the hepatopancreas, represents a fundamental region for the main digestive processes [15]. In addition, the hepatopancreas, i.e., the midgut gland (or "perigastric organ" or "perigaster") [16] of decapod crustaceans represents 2–6 % of the total body weight and allows for the synthesis and secretion of digestive enzymes. Consequently, the absorption of food is mainly concentrated in this segment of the digestive tract, while the posterior intestine is mainly devoted to further absorption and concentration of fecal pellets towards the anus, located closer to the tail [14]. Decapods are commonly used in ecotoxicological studies as model organisms, aiming at detecting how contaminants and micropollutants dissolved in aquatic environments may negatively affect the biodiversity of animal communities and impact the physiology of crustaceans and other marine organisms [17]. The evaluation of xenobiotic effects in aquatic environments may use crustaceans at various stages of their life cycle, from larvae to the adults [18].

Remarkably, decapod crustaceans are characterized by complex life cycles, comprising stages with variable levels of tolerance towards contaminants and allelochemicals [19]. Consequently, their ability to cope with stressors changes across ontogenetic processes [20]. *Hippolyte inermis* Leach 1816 is a protandric shrimp often present in the leaf stratum of *Posidonia oceanica* [21] meadows. This shrimp shows selective feeding habits, being an opportunistic herbivore. This shrimp has been extensively used in studies aimed at elucidating the mechanisms of sexual differentiation in crustaceans [22,23]. It has been also studied to clarify the mechanisms of proterandric sex reversal [24–26]. In fact, it has been demonstrated that the feeding on the benthic diatoms *Cocconeis* sp. induces the destruction of its androgenic gland (AG) due to programmed cell death (PCD). In particular, ferroptosis followed by apoptosis are the main PCD processes leading to an early sex reversal of these shrimps upon the ingestion of selected diatoms [6,27].

We conducted our bioassays on the first zoeal stages to evaluate the acute effects of MPs, since these stages are considered the most sensitive to pollutants [28]. In the preset study, we analyzed the responses of *H. inermis* to the exposure to five BMPs obtained from the degradation of PHB (poly-3-hydroxybutyrate); PBS (poly-butylene succinate); PBSA (poly-butylene succinate-co-butylene adipate); PCL (poly-ε-caprolactone) and PLA (poly-lactic acid). The alteration of gene expression of eighteen genes retrieved from a *H. inermis* transcriptomic library was evaluated [29]. These genes were chosen as orthologous of the sea urchin *Paracentrotus lividus* genes, analyzed in previous researches in response to various natural toxins and xenobiotics [30–33]. These genes exhibit key roles in various functional processes, such as stress response and detoxification; consequently, they have been adopted in the present study to evaluate the effects of BP pollutants.

2. Materials and methods

2.1. BMP preparation

BMPs were prepared as described by Viel et al. (2023). PBSA (grade FD92PM) and PBS (grade FZ91PM) were kindly supplied by PTT MCC Biochem Company Limited, Bangkok, Thailand; PCL (CAPA 6506, Mw = 50,000 g/mol), PHB (Biomer T19) was supplied by Biomer (Germany), PLA (LX 175Corbion PLA) was supplied by Corbion, from Solvay). PLA, PBS and PBSA pellets were milled with liquid nitrogen using a Retsch centrifugal grinding machine ZM1 equipped with a 500 μm grid to obtain BMPs. PHB and PCL powders were not submitted to any mechanical treatment.

The size of all BMPs was determined by analyzing optical (OM) and Scanning Electron Microscope (SEM) micrographs and resulted to be (average diameter): for PBS microplastics 193.10 ± 148.40 μm; for PBSA 207.70 ± 131.40 μm; for PCL 164.90 ± 99.20 μm; for PHB 0.64 ± 0.30 μm; for PLA 335.00 ± 182.01 μm. The measure of their specific surface area (SSA) revealed that PBS and PBSA had a similar SSA (0.025 and 0.023 cm^2/g respectively); for PCL the SSA was 0.032 cm^2/g; for PHB it was 7.56 cm^2/g; for PLA it was 0.014 cm^2/g.

2.2. Collection, larval culture and exposure to BPs

Ovigerous females of *H. inermis* were collected in September over the leaves of a *Posidonia oceanica* meadow located off Lacco Ameno d'Ischia (Bay of Naples, Italy; 40° 45' 00" N, 13° 54' 00" E), dragging a plankton net (0.1 mm mesh) from a boat. Ovigerous females were transferred to Erlenmayer flasks containing 1.5 L of filtered seawater (0.22 μm) in pools of three individuals. The flasks were kept in a thermostatic chamber at a constant temperature of 18°C, with 12/12 light/dark cycles. The water was daily changed and sieved through a 0.60 μm plastic mesh to detect the presence of just hatched larvae. The larvae produced in the next weeks were collected, counted and transferred to 400 mL Erlenmeyer flasks at a density of one larva per 10 mL of water. Consequently, 25 larvae were transferred into 250 mL of filtered (0.22 μm) seawater. Two flasks were set for each polymer treatment and for each of three concentrations tested, i.e., 1 mg/L, 5 mg/L and 10 mg/L according to Trifuoggi et al. [34] and our previous investigations on sea urchin embryos. All treatment and control larvae were daily fed on newly hatched *Artemia salina* nauplii (premium Artemia cysts, Super High Group, Ovada, Italy) at a density of 5 nauplia. mL^{-1}. In addition, treated larvae received daily administrations of BMPs, as indicated above. The

experiment lasted ten days. The seawater was changed daily in each flask, and larvae were monitored and counted under a light microscope (Leica MZ16), prior to being sticked in the experimental system with newly hatched nauplii (all control and treatment replicates) and BPs (only in the treatment replicates).

2.3. Mortality rates

After ten days of exposure, the mortality rates (M) were compared between treatments and controls adopting the following formula:

$$M = M_T - M_C \quad (1)$$

Table 1
Eighteen genes herein analyzed, pooled according to the pathways to which they belong, with acronyms, accession numbers, primer sequences and lengths of PCR amplified fragments is reported. A reference gene is also reported.

	Gene name	Acronym	Accession number	Primer	Sequences (5′ → 3′)	Amplicon length (bp)
Stress	*Caspase activity and apoptosis inhibitor 1*	*CASP*	HIPPOLYTE_123_TRINITY_DN205178_c7_g1_i4	CASP_Hi_F1 CASP_Hi_R1	GTGAGAAGCGATCTTCTGAC CATCCTTATTTTCACTGCTG	210
	Glutamine synthetase	*GSS*	HIPPOLYTE_3_TRINITY_DN37082_c0_g3_i1	GSS_Hi_F1 GSS_Hi_R1	CGGAATACGGATAGACGCAG CTGAGCAGGCGCTGTACTAC	167
	Heat shock protein 70	*Hsp70*	Hippolyte_Eye_TRINITY_DN32603_c0_g1_i1 ()	Hsp70_Hi_F1 Hsp70_Hi_R1	GCTGGAGGTGTTATGACTGC CTCTTGCTGCTGGAGGTATTC	193
	Heat shock protein 90	*Hsp90*	Hippolyte_Eye_TRINITY_DN255111_c0_g1_i1	Hsp90_Hi_F1 Hsp90_Hi_R1	GCATTGCTATTTATTCCTCG CTGCTGGAGCATTTCTCGGG	203
	Hypoxia induced protein conserved region (HIG1 domain family member 2 A)	*HIF1A*	HIPPOLYTE_3_TRINITY_DN48930_c1_g2_i4	HIF1A_Hi_F3 HIF1A_Hi_R3	GAGTAAATGAGACCCTCTCAG CTTATATGTAGATGTATATTTTC	204
	Kinesin-related protein 1	*KIF*	HIPPOLYTE_3_TRINITY_DN76048_c3_g3_i1	KIF_Hi_F1 KIF_Hi_R1	CCTCATCACGTGCCCATACC CTGCTAAAGCATGGATACAG	219
	MAP kinase-interacting serine/threonine-protein kinase 1	*MAPK*	HIPPOLYTE_3_TRINITY_DN68909_c1_g1_i3	MAPK_Hi_F1 MAPK_Hi_R1	GATTGATACGCGCACCAAAG CAGATTCTTGGTTAGCACTTGC	206
	Methyltransferase (Methyltransferase-like protein 24)	*MET*	HIPPOLYTE_3_TRINITY_DN62772_c1_g1_i1	Met_Hi_F1 Met_Hi_R1	GGCGAAATACTCGTGTCGAG CACTGGATTTCATCACAGCTG	206
	Nuclear Factor of Activated T Cells 2-interacting protein	*NFAT*	HIPPOLYTE_3_TRINITY_DN61342_c1_g3_i1	NFAT_Hi_F4 NFAT_Hi_R4	GCCTCGTAAAGTGTATGTGC GGGGGGAGAGTTTTGACAGG	216
	Tumor protein p53-inducible protein 11	*p53*	HIPPOLYTE_3_TRINITY_DN68758_c0_g1_i5	p53_Hi_F1 p53_Hi_R1	GGTGGAGCTGTTGGAGGTAG GAGACGTGAGTGTAGGTCGC	207
	Poly [ADP-ribose] polymerase	*PARP*	HIPPOLYTE_3_TRINITY_DN75580_c0_g2_i1	PARP_Hi_F3 PARP_Hi_R3	AGATAACTCTCTCTGATTCTA CTGAAAGATAAAAGGTGCATC	213
	Permease	*PERM*	HIPPOLYTE_3_TRINITY_DN46023_c2_g4_i1	PERM_Hi_F2 PERM_Hi_R2	CGATTTATCTGGTCTGTTTCTG CGACGCAGTAGAGAAAGCGG	190
	Succinate dehydrogenase	*SDH*	HIPPOLYTE_3_TRINITY_DN46023_c2_g9_i1	SDH_Hi_F1 SDH_Hi_R1	GAATACCGCCCATCATGTAG GAAGGTCGCGGCTGTGATG	187
	Tumor necrosis factor alpha-induced protein 8-like protein	*TNF*	HIPPOLYTE_3_TRINITY_DN58245_c2_g1_i2	TNF_Hi_F1 TNF_Hi_R1	CATTCAGATTCTGCCTTCATCG GAAACCATCTCACCGATAAG	170
Detoxification	*Aldehyde dehydrogenase*	*ALDH*	Hippolyte_Eye_TRINITY_DN2021_c2_g1_i1	ALDH_Hi_F1 ALDH_Hi_R1	GCTGAAAGACATGTCGATCG CTTCTACATGTGATTCCACG	193
	ATP-binding cassette sub-family F member 3	*ABC*	HIPPOLYTE_3_TRINITY_DN69699_c3_g1_i2	ABC_Hi_F1 ABC_Hi_R1	GACCTATTGCATACTCTATGC CACTCCTATTCTACAGTTAGACG	196
	Cytochrome P450	*CytP450*	HIPPOLYTE_3_TRINITY_DN76392_c4_g1_i1	CytP450_Hi_F1 CytP450_Hi_R1	GGCAAAGGAAGGTATGGCTG CATCAGAGATAAGAAGGTAAGG	183
	Glutathione S-transferase	*GST*	HIPPOLYTE_3_TRINITY_DN53717_c0_g2_i5	GST_H1_F1 GST_H1_F1	CCAGCCTATTCGATTGATGC CTCTACTTTGAGATATCTTTAC	182
Housekeeping	*Cytochrome oxidase subunit*	*COI*		Coi_Hi_F1 Coi_Hi_R1	CTGAAGAGGTATAGTAGGAAC CTCGGTGCCCCTGACATAGC	204

where M_T = mortality (reported as percentage) of duplicates for each treated sample with mortality values > 0 and M_C = mortality (reported as percentage) of replicates for each control.

Statistical analyses included two-way ANOVA on the matrix of mortalities, to compare the significance of effects among the treatments against controls using Prism 4.0 for Windows (GraphPad, San Diego California USA).

2.4. RNA extraction and cDNA synthesis

While mortality rates were evaluated at three concentrations of BPs, the analyses of their effects on individual genes were performed only at the concentration of 5 mg/L, to facilitate comparisons with previous studies on other marine invertebrates, conducted at the same concentration [6,35]. Since the minimum amount of RNA required for cDNA synthesis, according to the *iScript cDNA synthesis kit* (Bio-Rad), is ~30 ng/μL, we set an RNA extraction method starting from a pool of three larvae. The RNA extraction protocol was applied to individuals fed on BMPs as well as controls. The *RNeasy Mini Kit* extraction kit (Qiagen) was used, according to the manufacturer instructions. The amount of total RNA extracted was estimated by absorbance at 260 nm and its purity was determined by measuring 260/280 and 260/230 nm ratios using a *NanoDrop* spectrophotometer (ND-1000 UV–VIS spectrophotometer; NanoDrop Technologies, Wilmington, DE, USA). For each sample, 600 ng of total RNA was retrotranscribed with an *iScript cDNA synthesis kit* (Bio-Rad).

2.5. Gene isolation

Starting from the information available in a transcriptomic library of *H. inermis* [29], the sequences of eighteen genes were retrieved. The genes of interest belong to various molecular pathways (Table 1), including stress response (*Casp*, *GSS*, *HIF1A*, *Hsp70*, *Hsp90*, *KIF*, *MapK*, *Met*, *NFAT*, *PARP*, *Perm*, *p53*, *SDH* and *TNF*) and detoxification processes (*ALDH*, *ABC*, *CytP450* and *GST*). For each gene, a specific pair of primers was designed based on the nucleotide sequence. The fragments were amplified by PCR and the products were subsequently purified from agarose gel using the *QIAquick Gel Extraction kit* (Qiagen, Milan, Italy). The specificity of the products was evaluated by DNA sequencing. PCR products were then aligned with gene sequences of the complete gene by *MultAlin* Software (http://multalin.toulouse.inra.fr/multalin/).

2.6. Real-time qPCR

The variations in the expression levels of eighteen genes in treated samples with BMPs with respect to controls (in seawater without plastics) under analysis was followed by Real-Time qPCR. cDNA (1 μL) was used as a template in a reaction containing a final concentration of 0.7 μM for each primer and 2 × Quantitative Master Mix with SYBR Green low ROX (total volume of 10 μL). PCR amplifications were performed in a thermal cycler *CFX384 Real Time System* using the following thermal profile: 95°C for 20 seconds, one cycle for cDNA denaturation; 95°C for 1 second and 60°C for 20 seconds, 40 cycles for amplification; 95°C for 15 seconds and 60°C for 15 seconds for final elongation. The fluorescence was measured using a *Bio-Rad CFX Maestro software* (Bio-Rad Laboratories, Inc., Milano, Italy). The expression level of each gene was analyzed and normalized with the housekeeping and reference gene *Cytochrome oxidase subunit (COI)*, using the *REST* software (Relative Expression Software Tool; Weihenstephan, Germany), and the expression values of the gene of interest in treated animals relative to the control were reported [36,37]. Values above or below 1.5 were considered significant (REST-MCS© - version 2).

3. Results

3.1. Mortality rates

After ten days of exposure of *H. inermis* larvae to BPs at three concentrations, differences were detected both among the mortality rates triggered by all plastics and among the three concentrations tested (Fig. 1). PBS triggered the highest mortality rates (31 %) at the maximum concentration tested (10 mg/L). Similarly, PCL triggered the highest mortality rates (48 %) at the maximum concentration. PBSA, in turn, induced the highest mortality rates (40 %) at the lowest concentration (1 mg/L), and the mortality rates were inversely related to the three concentrations. PHB prompted the highest mortality rates (37 %) at the intermediate concentration (5 mg/L), but the effects were comparable with those observed at the lowest concentration (28 % mortality), while the highest concentration (10 mg/L) promoted the lowest (6 %) mortality. PLA was the least harmful plastic, inducing low mortality rates, not significantly different from controls, only at 1 mg/L (4 %) and 5 mg/L (13 %); however, mortality rates were null at the highest (10 mg/L) dose.

The differences detected among the mortality rates triggered by all plastics were significant (at $p < 0.0001$), as well as the differences among the three concentrations tested (Table 1). Consequently, ANOVA indicated that individual BPs produce selective effects on the mortality of *H. inermis* larvae. A dose-dependence of effects, however, cannot be sustained according to the results of statistical analyses, for all the tested BPs. While PBS, PCL and PLA exhibited an increasing mortality proportional to the doses, PHB and PBSA exhibited mortality levels inversely related to the doses, although they were all significantly different (Table 2).

3.2. Gene expression

Molecular analyses focused on *H. inermis* larvae exposed to the concentration of 5 mg/L since this concentration permits comparisons with previous studies on other marine invertebrates [6]. All analyzed genes were targeted by five BMPs of interest (Fig. 2 and Table S1). We evaluated the variations in the expression levels of fourteen genes involved in the stress response and four genes involved in detoxification processes, orthologous to genes isolated from the crustacean isopod *Idotea balthica basteri* Audouin, 1826 used in a previous ecotoxicology work [35]. In particular, PBS induced an increased expression of 70 % of genes involved in stress response (*Casp*, *GSS*, *Hsp70*, *Hsp90*, *KIF*, *NFAT*, *PARP*, *Perm*, *p53* and *TNF*), while *HIF1A* and *Met* genes were

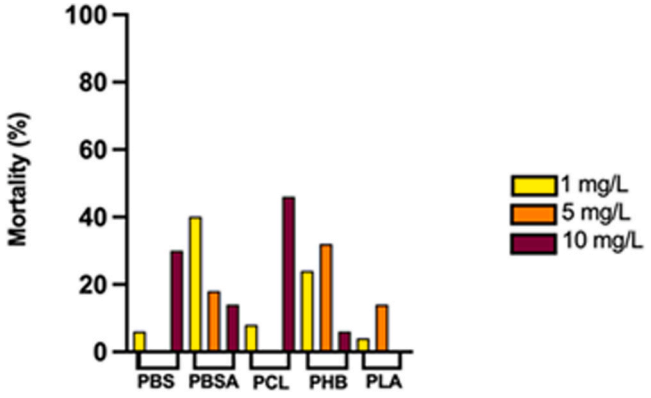


Fig. 1. The histograms show the mortality rate reported as percentage (%), calculated according to the formula reported in the Materials and Methods section of *H. inermis* exposed to three different concentrations of BPs (1, 5, 10 mg/L) obtained from PHB, PBS, PBSA, PLA and PCL. For PBS (42 %) and PCL (46 %) at 5 mg/L and PLA (28 %) at 10 mg/L values of mortality rate lower than the control was detected. Statistical differences are reported in Table 1.

Table 2
Results of two-way ANOVA applied to the data on mortality rates triggered by five different BPs tested at three concentrations (reported in Fig. 1).

Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	77,29	< 0,0001	* ** *	Yes	
Row Factor	19,39	< 0,0001	* ** *	Yes	
Column Factor	3234	< 0,0001	* ** *	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	4919	8	614,9	F (8, 15) = 1653	P < 0,0001
Row Factor	1234	4	308,5	F (4, 15) = 829,4	P < 0,0001
Column Factor	205,9	2	102,9	F (2, 15) = 276,7	P < 0,0001
Residual	5,58	15	0372		

down-regulated. The expression levels of the stress genes *MapK* and *SDH* were not affected by this polymer. Concerning detoxification genes, PBS induced down-regulation of *ABC*, *ALDH* and *GST*, whereas only *CytP450* was not targeted. PBSA induced changes in the expression levels of the following genes: *HIF1A*, *ABC*, *ALDH* (down-regulated) and *Perm* (up-regulated). *H. inermis* larvae treated with PCL revealed a strong decrease of the expression levels of all targeted genes. In particular, PCL determined an alteration of all the analyzed genes, with the exception of the *ABC* gene, involved in detoxification processes. Furthermore, the exposure to PCL induced a strong down-regulation of *HIF1A* gene such as its Ct values resulted undetermined, with Ct values major than 38. PHB triggered an up-regulation of the majority of the analyzed genes involved in stress response, i.e., *Casp*, *GSS*, *Hsp70*, *Hsp90, KIF*, *MapK*, *NFAT, Perm*, *PARP*, *SDH* and *TNF*. Only the *p53* gene was down-regulated, while *HIF1A* and *Met* were not affected by this polymer. As for the genes involved in detoxification processes, only *ALDH* showed variation in its expression levels. Finally, PLA affected approximately 80 % of the genes, resulting in downregulation of all involved genes, with the only exception of the stress genes *p53* and *Perm*, whose expression did not change after the exposure. Similar to PCL, *HIF1A* was found to be strongly downregulated, being Ct values undetermined.

A graphical summary of these molecular results was reported in the heatmap (see Fig. 3).

4. Discussion

A variety of chemical and physical parameters, in several environments and marine ecosystems influence the degradation rates of BPs [38]. Physical and chemical properties of polymers, along with the environmental conditions they are exposed to, affect the time they will last till degradation [39]. Such environmental factors (including the intensity of UV radiations) enhance the toxicity of BPs even before biodegradation [40]. However, the production of micro- and nano-plastics (MPs and NPs) caused by incomplete degradation are to be considered [41]. In this view, BPs may not be the ultimate solution to mitigate pollution by anthropogenic activities and require proper management and disposal [1,42]. They are considered to be promising materials, but investigations will be needed to fully understand their interactions with environmental factors, besides their degradation kinetics [43]. Several studies demonstrated the influence of BMPs on the aquatic biota [44], showing the complexity of the degradation processes.

The polymers used in this work were previously tested, at the same concentrations, on embryos of the sea urchin, *Paracentrotus lividus* [6]. Exposure to PBS and PBSA microplastics showed no detectable effects on the survival rates of sea urchin embryos. However, PCL, PHB and PLA induced delays and malformations of embryonic development, targeting the expression levels of eighty-seven genes involved in various cellular processes, such as stress response, differentiation and development, skeletogenic and detoxification. The results of the present study are insufficient to demonstrate a clear dose-dependence of the mortality of *H. inermis* larvae in response to the BPs. In fact, in some incidents, lower concentrations induced even higher mortality than the highest concentrations. These findings suggested the triggering of subtle mechanisms that organisms can activate following an insult due to an environmental xenobiotic [45,46]. Several mechanisms can be responsible for such effect, e.g, hormesis in which a low dose of a chemical agent may trigger in an organism the opposite physiologic response as compared to higher doses [47]. Consequently, a substance might produce a stimulatory effect at low doses and toxic effects at high doses. In the case of microplastics, small amounts stimulate defense or adaptation mechanisms in marine invertebrates, while large doses could overwhelm these mechanisms and cause damage [48]. In addition, microplastics can interact with other pollutants, by absorbing them [49]. At low doses, the amount of toxins adsorbed could be sufficient to cause a biological effect, while at high doses, microplastics are likely to become saturated with toxins, leading to complex interactions that may follow a non-linear response [50,51]. As an alternative hypothesis, a low-dose exposure to microplastics could trigger an immune response or moderate physiological stress that stimulates repair or defense mechanisms, while at high doses, the stress may be too high, leading to cell damage and a reduction in the ability to adequately respond [52]. These hypotheses may explain the concurring results of mortality rates and the molecular analyses. However, it is important to highlight that several features of BMPs, including their molecular weight, the shape and size of particles (as described above in the method section), the degree of crystallinity, besides their chemical composition, may largely influence the results, because the biological effects observed are due to chemical, mechanical and physical interactions. Given the multi-factorial nature of their biological activities, further studies on BMPs will be needed to fully explain specific effects and the influence of individual characters.

Molecular findings reveal that PCL and PLA were the most harmful biodegradable polymers, causing a strong decrease in the expression level of seventeen genes. This downregulation can be explained as a sort of surrender mechanism to the stress induced by these two plastics, to which *H. inermis* reacts by decreasing the expression of genes. Furthermore, these two BPs were also harmful for *P. lividus* embryos, along with PHB, which was the strongest BP, inducing the variation of expression levels in 78 % of the genes under analysis [6]. *H. inermis* differently reacted to the insults induced by PBS and PHB, since the majority of genes were upregulated, suggesting that the larvae try to survive by increasing the expression of the analyzed genes by enhancing the action of the gene pathways to which they belong. The PBSA was the least harmful polymer, since only five genes, among those analyzed, were targeted. This could be explained considering that *H. inermis* might activate other genes to react to this plastic, belonging to different molecular pathways, not analyzed in this work. Alternatively, it could activate the so called “first response genes”; this mechanism consists in a set of immediate-early genes able to respond to both extrinsic and intrinsic cell signaling activated and transcribed within minutes after stimulation, not requiring *de novo* protein synthesis for their expression (Fowler et al., 2011; Bahrami and Drabløs 2016). These genes have essential biological roles, being mainly involved in stress response, the immune system, and the differentiation.

These data demonstrate the broad spectrum of toxicity for which BPs can be responsible in organisms living in several aquatic environments and how marine organisms try to use the defensome to counteract an environmental insult (Varrella et al., 2014). Differently from *H. inermis,*

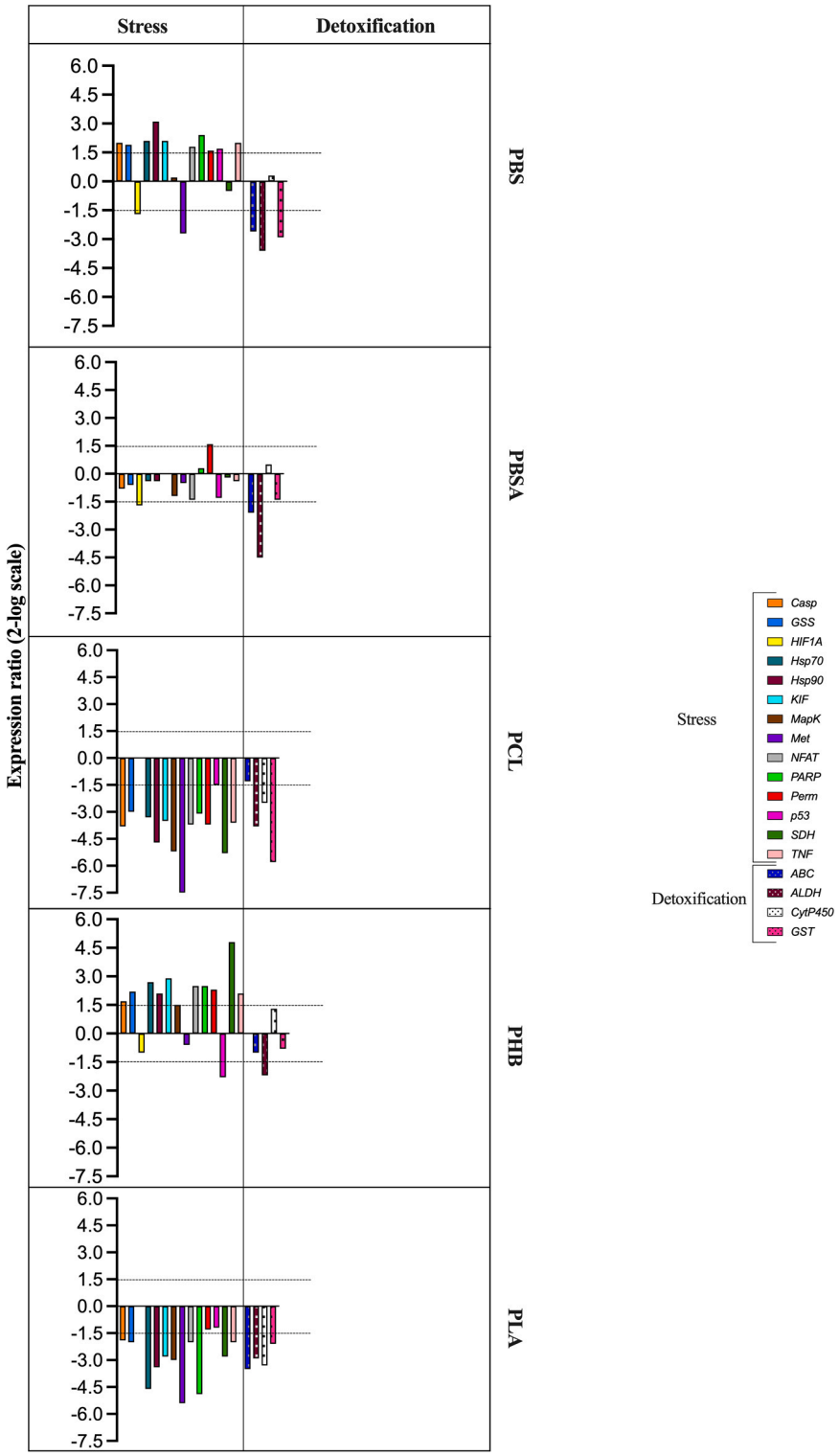


Fig. 2. Gene expression variations (reported as fold changes) of eighteen genes, involved in stress responses and detoxification processes, in *H. inermis* larvae exposed for ten days to five BPs under analysis at the concentration of 5 mg/L. Fold differences larger than ± 1.5 (see dashed horizontal guidelines at values of +1.5 and −1.5) were considered significant.

in which the analyzed genes were mainly down-regulated, sea urchin embryos showed up-regulated genes. The effects of BMPs have been evaluated on the survival rates of several aquatic organisms. In particular, the mortality rates were investigated on a freshwater cladoceran crustacean, *Daphnia magna,* [53], and on the marine mollusk *Perna viridis* [54]. Other crustaceans, as *Idotea balthica basteri,* were adopted as models for the study of the effects of BPs by Amato et al. [35]. Studying molecular responses to contaminants showed a decrease in the expression of the *GST* gene and an up-regulation for *Hsp70* in *Artemia franciscana* after exposure to synthetic PS for 14 days at a concentration of 1 μg/mL [55]. Yoo et al. [56] studied the molecular response of *Diaphanosoma celebensis* at 48 hours of exposure to PS with a diameter of 0.05,

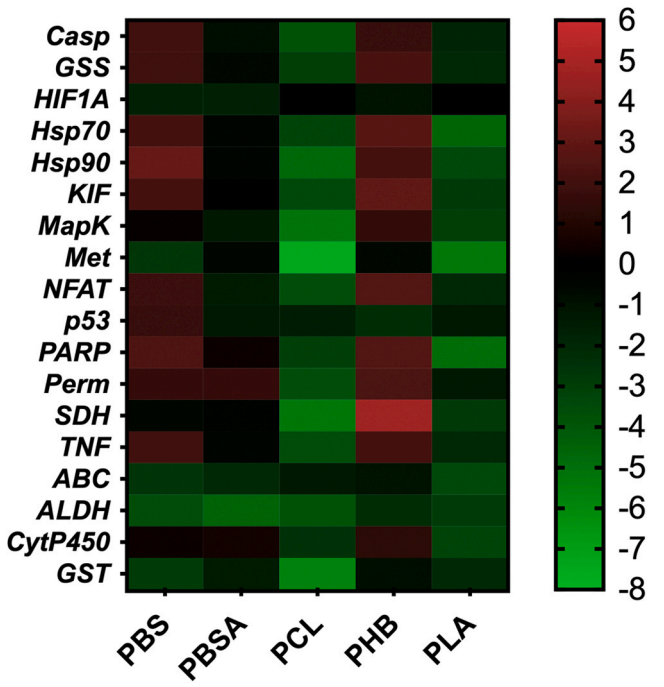


Fig. 3. Heatmap (GraphPad prism software version 9.5.1) showing the expression profile of stress genes and detoxification genes for a total of eighteen genes analysed by Real-Time PCR in *H. inermis* larvae treated with BMPs. Color code: green for down-regulated genes and red for up-regulated genes.

0.5 and 6 μm. In that case, the transcriptional levels and enzyme activities of antioxidants, such as the *GST* gene, were modulated in a dose-dependent manner according to the size of the PS beads. Furthermore, PS induced an alteration in the expression level of *GST* in the prawn *Macrobrachium nipponense* when exposed to a range of concentrations (0.04, 0.4, 4 and 40 mg/L) for 21 days [57].

The present study is the first attempt to analyze the molecular effects of BPs on *H. inermis*. This research was facilitated by a previously published *de novo* transcriptome sequencing and assembly of this shrimp [29] and here we isolated eighteen genes from the shrimp transcriptome, which were orthologous of the crustacean isopod *I. balthica basteri.* Remarkably, these genes are functionally intercorrelated, demonstrating that five different BPs impact specific molecular pathways [35]. Differently from *H. inermis*, investigations on *I. balthica basteri* showed that the polymers inducing the strongest effect were PBSA and PLA, mainly up-regulating most genes. Previous studies mainly focused on the gene expression induction by synthetic plastics [58] referring to the same genes used in the present study (*Hsp70*, *Hsp90* and *GST)* in *D. magna* after the administration of mixtures of MPs along with feeds, for 48 hours. The mixture consisted of four polymers commonly found in freshwater ecosystems (polyamide, polycarbonate, polyethylene terephthalate and polyvinylchloride) and some polymers with a higher market share, (acrylonitrile-burtdiene-styrene terpolymer, plasticized polyvinyl chloride, polyoxymethylene homopolymer and styrene- acrylonitrile copolymer). Similar effects observed in other invertebrates raised environmental concerns and call for further investigations.

5. Conclusions

We demonstrated that changes in gene expression levels represent a useful tool in the evaluation of the stress caused by BMPs and to understand the cellular mechanisms underlying the response of benthic organisms to MPs [31]. Marine invertebrates employ specific gene networks (called “defensome”) to counteract the effects of plastics, and other environmental pollution [30]. Since decapod crustaceans are critical trophic links in several environments between primary producers (often polluted with microplastics) and further consumers [59], the harmful and toxic effects of BPs may be magnified and impact even human health. The effect of microplastics on marine invertebrates can be largely variable, as a function of concentration, and this increases the importance of studying biological and molecular responses over a range of concentrations and organisms, to fully understand their ecological impacts. Concluding, *H. inermis* is an excellent model organism proposed as a sentinel for the assessment of BMPs effects, being able to provide an early detection of the effects of environmental pollution.

Environmental implication

This is the first study to use a molecular approach to detect *Hipployte inermis* responses to contaminants and in particular to biodegradable polymers. Since decapod crustaceans are critical trophic links in several environments between primary producers (often polluted with microplastics) and further consumers, the harmful and toxic effects of BPs may be magnified and impact even human health. The effect of microplastics on marine invertebrates can be largely variable as a function of concentration, and this increases the importance of studying biological and molecular responses over a wide range of concentrations and organisms, to fully understand their ecological impacts.

CRediT authorship contribution statement

Valerio Zupo: Writing – review & editing, Writing – original draft, Supervision, Resources, Formal analysis, Data curation, Conceptualization. **Maria Costantini:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Roberta Esposito:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Bruno Pinto:** Formal analysis, Methodology, Writing – review & editing. **Amalia Amato:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Amir Sagi:** Writing – review & editing, Investigation. **Eliahu Aflalo:** Writing – review & editing, Investigation, Formal analysis. **Giovanni Libralato:** Writing – review & editing, Investigation, Formal analysis. **Francesca Glaviano:** Writing – review & editing, Methodology. **Thomas Viel:** Writing – review & editing, Methodology, Formal analysis. **Loredana Manfra:** Writing – review & editing, Investigation, Formal analysis. **Mariacristina Cocca:** Writing – review & editing, Investigation, Formal analysis.

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.

Acknowledgments

We thank Vincenzo Rando for his assistance in the collection of shrimps on board the vessel Phoenicia owned by the Stazione Zoologica Anton Dohrn. Amalia Amato was fully supported by a PhD fellowship (PhD in Biology, University of Naples Federico II) funded by the project “Biomonitoraggio di micro e nanoplastiche biodegradabili: dall’ambiente all’uomo in una prospettiva one health” (BioPlast4Safe), Piano Nazionale per gli Investimenti Complementari (PNC) E.1 Salute, Ambiente, Biodiversità e Clima. Shrimp feeds were kindly provided by SHG group (Ovada, Italy). Bruno Pinto was supported by a PhD fellowship cofounded by the Stazione Zoologica Anton Dohrn of Naples and King Abdullah University of Science and Technology (KAUST – Saudi Arabia). We thank the Marine Protected Area “Regno di Nettuno” off the Ischia Island (Italy) for allowing the collections of shrimps at Lacco Ameno. We thank the Bioinformatic Core Facility at BGU for enabling the analysis of the transcriptomic libraries. Antonia Chiarore

and Alice Mirasole (Medas Center, Stazione Zoologica Anton Dohrn) aided the experimental routines.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.137069.

Data availability

The data will be available upon request by the corresponding author.

References

[1] Manfra, L., Marengo, V., Libralato, G., Costantini, M., De Falco, F., Cocca, M., 2021. Biodegradable polymers: A real opportunity to solve marine plastic pollution? J Hazard Mater 416, 125763. https://doi.org/10.1016/j.jhazmat.2021.125763.

[2] Malafeev, K.V., Apicella, A., Incarnato, L., Scarfato, P., 2023. Understanding the Impact of Biodegradable Microplastics on Living Organisms Entering the Food Chain: A Review. Polymers 15, 3680. https://doi.org/10.3390/polym15183680.

[3] Singh, R., Kumar, N., Mehrotra, T., Bisaria, K., Sinha, S., 2020. Bioremediation for Environmental Sustainability Toxicity, Mechanisms of Contaminants Degradation, Detoxification, and Challenges, in: Saxena, G., Kumar, V., Shah, M. (Eds.), Bioremediation for Environmental Sustainability, 1st ed., Elsevier, Amsterdam, The Netherlands, pp. 193–207.193207.

[4] Nik Mut, N.N., Na, J., Jung, J., 2024. A review on fate and ecotoxicity of biodegradable microplastics in aquatic system: are biodegradable plastics truly safe for the environment? Environ Pollut 344, 123399. https://doi.org/10.1016/j.envpol.2024.123399.

[5] Wang, C., Yu, J., Lu, Y., Hua, D., Wang, X., Zou, X., 2021. Biodegradable microplastics (BMPs): a new cause for concern? Environ Sci Pollut Res 28, 66511–66518. https://doi.org/10.1007/s11356-021-16435-4/Published.

[6] Viel, T., Cocca, M., Manfra, L., Caramiello, D., Libralato, G., Zupo, V., Costantini, M., 2023. Effects of biodegradable-based microplastics in *Paracentrotus lividus* Lmk embryos: Morphological and gene expression analysis. Environ Pollut 334, 122129. https://doi.org/10.1016/j.envpol.2023.122129.

[7] Yin, J., Li, J.Y., Craig, N.J., Su, L., 2022. Microplastic pollution in wild populations of decapod crustaceans: a review. Chemosphere 291, 132985. https://doi.org/10.1016/j.chemosphere.2021.132985.

[8] Costa, F.O., DeWaard, J.R., Boutillier, J., Ratnasingham, S., Dooh, R.T., Hajibabaei, M., Hebert, P.D.N., 2007. Biological identifications through DNA barcodes: the case of the Crustacea. Can J Fish Aquat Sci 64, 272–295. https://doi.org/10.1139/F07-008.

[9] Hobbs, H.H., Lodge, D.M., 2010. Decapoda, in: Thorp, J.H., Covich, A.P. (Eds.), Ecology and Classification of North American Freshwater Invertebrates, 3rd ed., Academic Press, San Diego, pp. 901–968. https://doi.org/10.1016/10.1016/B978-0-12-374855-3.00022-4..

[10] Boudreau, S.A., Worm, B., 2012. Ecological role of large benthic decapods in marine ecosystems: a review. Mar Ecol Prog Ser 469, 195–213. https://doi.org/10.3354/meps09862.

[11] Wallace, K.M., Leslie, A.J., 2008. Diet of the Nile Crocodile (*Crocodylus niloticus*) in the Okavango Delta, Botswana. J Herpetol 42, 361–368. https://doi.org/10.1670/07-1071.1.

[12] Fanelli, E., Rey, J., Torres, P., Gil De Sola, L., 2009. Feeding habits of blackmouth catshark *Galeus melastomus* Rafinesque, 1810 and velvet belly lantern shark *Etmopterus spinax* (Linnaeus, 1758) in the western Mediterranean. J Appl Ichthyol 25, 83–93. https://doi.org/10.1111/j.1439-0426.2008.01112.x.

[13] Šantić, M., Rada, B., Pallaoro, A., 2012. Diet and feeding strategy of thornback ray *Raja clavata*. J Fish Biol 81, 1070–1084. https://doi.org/10.1111/j.1095-8649.2012.03382.x.

[14] Ceccaldi, H., 1989. Anatomy and physiology of digestive tract of Crustaceans Decapods reared in aquaculture. : Adv Trop Aquac, Workshop Tahiti, Fr Polyn 243–259.

[15] D'Costa, A.H., 2022. Microplastics in decapod crustaceans: Accumulation, toxicity and impacts, a review. Sci Total Environ 832, 154963. https://doi.org/10.1016/j.scitotenv.2022.154963.

[16] Cervellione, F., McGurk, C., Van Den Broeck, W., 2017. Perigastric organ": a replacement name for the "hepatopancreas" of Decapoda. J Crustace Biol 37, 353–355. https://doi.org/10.1093/jcbiol/rux020.

[17] Patrick, R., Egbuatu, M., 2024. A Review and Meta-Analysis of the Studies Conducted on the Impact of Micropollutants on the Aggressive Behaviors of Some Crustaceans. Uttar Pradesh J Zool 45, 161–174. https://doi.org/10.56557/upjoz/2024/v45i53942.

[18] Anderson, B., Phillips, B., 2016. Saltwater Toxicity Tests. in: Marine Ecotoxicology. Elsevier, Amsterdam, The Netherlands, pp. 167–197. https://doi.org/10.1016/B978-0-12-803371-5.00006-0.

[19] Anger, K., 2016. Adaptation to Life in Fresh Water by Decapod Crustaceans: Evolutionary Challenges in the Early Life-History Stages, in: T. Kaway, N. Cumberlidge (Eds.), A Global Overview of the Conservation of Freshwater Decapod Crustaceans, Springer, pp. 127–168..

[20] Ocampo, E.H., Nuñez, J.D., Ribeiro, P.D., Pérez García, M., Bas, C.C., Luppi, T.A., 2024. Disparate response of decapods to low pH: A meta-analysis of life history, physiology and behavior traits across life stages and environments. Mar Pollut Bull 202, 116293. https://doi.org/10.1016/j.marpolbul.2024.116293.

[21] Zupo, V., Messina, P., 2007. How do dietary diatoms cause the sex reversal of the shrimp *Hippolyte inermis* Leach (Crustacea, Decapoda). Mar Biol 151, 907–917. https://doi.org/10.1007/s00227-006-0524-9.

[22] Chandler, J.C., Elizur, A., Ventura, T., 2018. The decapod researcher's guide to the galaxy of sex determination. Hydrobiologia 825, 61–80. https://doi.org/10.1007/s10750-017-3452-4.

[23] Levy, T., Zupo, V., Mutalipassi, M., Somma, E., Ruocco, N., Costantini, M., Abehsera, S., Manor, R., Chalifa-Caspi, V., Sagi, A., Aflalo, E.D., 2021. Protandric Transcriptomes to Uncover Parts of the Crustacean Sex-Differentiation Puzzle. Front Mar Sci 8, 745540. https://doi.org/10.3389/fmars.2021.745540.

[24] Reverberi, G., 1950. La situazione sessuale di *Hyppolyte viridis* e le condizioni che la reggono. Bolletino Di Zool 17, 91–94. https://doi.org/10.1080/11250005009436805.

[25] Zupo, V., Maibam, C., 2010. Effects of natural compounds in the sex determination of decapod crustaceans and possible aquaculture applications: a review. : Convegno-La Risorsa Dei Crostacei Nel Mediterr: Ric, Prod e Mercato 113–120.

[26] Cobos, V., Díaz, V., Raso, J.E.G., Manjón-Cabeza, M.E., 2011. The male reproductive system of *Hippolyte inermis* Leach 1815 (Decapoda, Caridea). Helgol Mar Res 65, 17–24. https://doi.org/10.1007/s10152-010-0196-1.

[27] Zupo, V., 1994. Strategies of sexual inversion in *Hippolyte inermis* Leach (Crustacea, Decapoda) from a Mediterranean seagrass meadow. J Exp Mar Biol Ecol 178, 131–145. https://doi.org/10.1016/0022-0981(94)90229-1.

[28] Di Muzio, G., Mussat Sartor, R., Nurra, N., Battuello, M., Pessani, D., Cervella, P., Cuesta, J.A., 2019. Morphology of planktonic zoeal stages of *Palicus caronii* (Decapoda, Brachyura), identified by DNA barcoding, provides novelties to Palicoidea larval systematics. Sci Rep 9, 19132. https://doi.org/10.1038/s41598-019-55412-3.

[29] Zupo, V., Costantini, M., Aflalo, E.D., Levy, T., Chalifa-Caspi, V., Obayomi, O., Mutalipassi, M., Ruocco, N., Glaviano, F., Somma, E., Nieri, P., Sagi, A., 2023. Ferroptosis precedes apoptosis to facilitate specific death signalling by fatty acids. P Roy SocB-Biol Sci 290, 20231327. https://doi.org/10.1098/rspb.2023.1327.

[30] Varrella, S., Romano, G., Ianora, A., Bentley, M.G., Ruocco, N., Costantini, M., 2014. Molecular response to toxic diatom-derived aldehydes in the sea urchin *Paracentrotus lividus*. Mar Drugs 12, 2089–2113. https://doi.org/10.3390/md12042089.

[31] Varrella, S., Romano, G., Ruocco, N., Ianora, A., Bentley, M.G., Costantini, M., 2016. First morphological and molecular evidence of the negative impact of diatom-derived hydroxyacids on the sea urchin *Paracentrotus lividus*. Toxicol Sci 151, 419–433. https://doi.org/10.1093/toxsci/kfw053.

[32] Ruocco, N., Varrella, S., Romano, G., Ianora, A., Bentley, M.G., Somma, D., Leonardi, A., Mellone, S., Zuppa, A., Costantini, M., 2016. Diatom-derived oxylipins induce cell death in sea urchin embryos activating caspase-8 and caspase 3/7. Aquat Toxicol 176, 128–140. https://doi.org/10.1016/j.aquatox.2016.04.012.

[33] Ruocco, N., Maria Fedele, A., Costantini, S., Romano, G., Ianora, A., Costantini, M., 2017. New inter-correlated genes targeted by diatom-derived polyunsaturated aldehydes in the sea urchin *Paracentrotus lividus*. Ecotoxicol Environ Saf 142, 355–362. https://doi.org/10.1016/j.ecoenv.2017.04.022.

[34] Trifuoggi, M., Pagano, G., Oral, R., Pavičić-Hamer, D., Burić, P., Kovačić, I., Siciliano, A., Toscanesi, M., Thomas, P.J., Paduano, L., Guida, M., Lyons, D.M., 2019. Microplastic-induced damage in early embryonal development of sea urchin *Sphaerechinus granularis*. Environ Res 179, 108815. https://doi.org/10.1016/j.envres.2019.108815.

[35] Amato, A., Esposito, R., Viel, T., Glaviano, F., Cocca, M., Manfra, L., Libralato, G., Somma, E., Lorenti, M., Costantini, M., Zupo, V., 2024. Effects of biodegradable microplastics on the crustacean isopod *Idotea balthica basteri* Audouin, 1826. Environ Pollut, 124897. https://doi.org/10.1016/j.envpol.2024.124897 (In Press)).

[36] Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29, 045–e45. https://doi.org/10.1093/nar/29.9.e45.

[37] Pfaffl, M.W., Horgan, G.W., Dempfle, L., 2002. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 30, e36–e39. https://doi.org/10.1093/nar/30.9.e36.

[38] Miksch, L., Köck, M., Gutow, L., Saborowski, R., 2022. Bioplastics in the sea: rapid *In-Vitro* evaluation of degradability and persistence at natural temperatures. Front Mar Sci 9, 920293. https://doi.org/10.3389/fmars.2022.920293.

[39] Emadian, S.M., Onay, T.T., Demirel, B., 2017. Biodegradation of bioplastics in natural environments. Waste Manag 59, 526–536. https://doi.org/10.1016/j.wasman.2016.10.006.

[40] Quade, J., López-Ibáñez, S., Beiras, R., 2022. Mesocosm trials reveal the potential toxic risk of degrading bioplastics to marine life. Mar Pollut Bull 179, 113673. https://doi.org/10.1016/j.marpolbul.2022.113673.

[41] Islam, M., Xayachak, T., Haque, N., Lau, D., Bhuiyan, M., Pramanik, B.K., 2024. Impact of bioplastics on environment from its production to end-of-life. Process Saf Environ 188, 151–166. https://doi.org/10.1016/j.psep.2024.05.113.

[42] Mastrolia, C., Giaquinto, D., Gatz, C., Pervez, M.N., Hasan, S.W., Zarra, T., Li, C.W., Belgiorno, V., Naddeo, V., 2022. Plastic Pollution: Are Bioplastics the Right Solution?, 14, 3596. https://doi.org/10.3390/w14223596.

[43] Green, D.S., 2020. Biological and ecological impacts of plastic debris in aquatic ecosystems, in: Barcelò, D., Kostianoy, A.G. (Eds.), Plastics in the Aquatic Environment-Part I: Current Status and Challenges, Springer International Publishing, pp. 111–133. http://link.springer.com/bookseries/698..

[44] Wang, D., Xiong, F., Wu, L., Liu, Z., Xu, K., Huang, J., Liu, J., Ding, Q., Zhang, J., Pu, Y., Sun, R., 2024. A progress update on the biological effects of biodegradable microplastics on soil and ocean environment: A perfect substitute or new threat? Environ Res 252, 118960. https://doi.org/10.1016/j.envres.2024.118960.
[45] Carrier-Belleau, C., Drolet, D., McKindsey, C.W., Archambault, P., 2021. Environmental stressors, complex interactions and marine benthic communities' responses. Sci Rep 11, 4194. https://doi.org/10.1038/s41598-021-83533-1.
[46] Marcharla, E., Vinayagam, S., Gnanasekaran, L., Soto-Moscoso, M., Chen, W.H., Thanigaivel, S., Ganesan, S., 2024. Microplastics in marine ecosystems: A comprehensive review of biological and ecological implications and its mitigation approach using nanotechnology for the sustainable environment. Environ Res 256, 119181. https://doi.org/10.1016/j.envres.2024.119181.
[47] Calabrese, E.J., Mattson, M.P., 2017. How does hormesis impact biology, toxicology, and medicine? NPJ Aging Mech Dis 3, 1–8. https://doi.org/10.1038/s41514-017-0013-z.
[48] Moffett, D.B., Mumtaz, M.M., Sullivan, D.W., Fowler, B.A., 2015. General Considerations of Dose-Effect and Dose-Response Relationships, in: Nordberg, G.F., Fowler, B.A., Nordberg, M. (Eds. Handbook on the Toxicology of Metals. Fourth, Academic Press, pp. 197–212.
[49] Xiang, Y., Jiang, L., Zhou, Y., Luo, Z., Zhi, D., Yang, J., Lam, S.S., 2022. Microplastics and environmental pollutants: key interaction and toxicology in aquatic and soil environments. J Hazard Mater 422, 126843. https://doi.org/10.1016/j.jhazmat.2021.126843.
[50] Joo, S.H., Liang, Y., Kim, M., Byun, J., Choi, H., 2021. Microplastics with adsorbed contaminants: mechanisms and treatment. Environ Chall 3, 100042. https://doi.org/10.1016/j.envc.2021.100042.
[51] Rafa, N., Ahmed, B., Zohora, F., Bakya, J., Ahmed, S., Ahmed, S.F., Mofijur, M., Chowdhury, A.A., Almomani, F., 2023. Microplastics as carriers of toxic pollutants: source, transport, and toxicological effects. Environ Pollut 343, 123190. https://doi.org/10.1016/j.envpol.2023.123190.
[52] Yang, W., Jannatun, N., Zeng, Y., Liu, T., Zhang, G., Chen, C., Li, Y., 2022. Impacts of microplastics on immunity. Front Toxicol 4, 956885. https://doi.org/10.3389/ftox.2022.956885.
[53] Zimmermann, L., Göttlich, S., Oehlmann, J., Wagner, M., Völker, C., 2020. What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*. Environ Pollut 267, 115392. https://doi.org/10.1016/j.envpol.2020.115392.
[54] Phothakwanpracha, J., Lirdwitayaprasit, T., Pairohakul, S., 2021. Effects of sizes and concentrations of different types of microplastics on bioaccumulation and lethality rate in the green mussel, *Perna viridis*. Mar Pollut Bull 173, 112954. https://doi.org/10.1016/j.marpolbul.2021.112954.
[55] Varó, I., Perini, A., Torreblanca, A., Garcia, Y., Bergami, E., Vannuccini, M.L., Corsi, I., 2019. Time-dependent effects of polystyrene nanoparticles in brine shrimp *Artemia franciscana* at physiological, biochemical and molecular levels. Sci Total Environ 675, 570–580. https://doi.org/10.1016/j.scitotenv.2019.04.157.
[56] Yoo, J.W., Cho, H., Jeon, M.J., Jeong, C.B., Jung, J.H., Lee, Y.M., 2021. Effects of polystyrene in the brackish water flea *Diaphanosoma celebensis*: size-dependent acute toxicity, ingestion, egestion, and antioxidant response. Aquat Toxicol 235, 105821. https://doi.org/10.1016/j.aquatox.2021.105821.
[57] Fan, W., Yang, P., Qiao, Y., Su, M., Zhang, G., 2022. Polystyrene nanoplastics decrease molting and induce oxidative stress in adult *Macrobrachium nipponense*. Fish Shellfish Immunol 122, 419–425. https://doi.org/10.1016/j.fsi.2022.02.028.
[58] Imhof, H.K., Rusek, J., Thiel, M., Wolinska, J., Laforsch, C., 2017. Do microplastic particles affect *Daphnia magna* at the morphological, life history and molecular level? PLoS One 12, e0187590. https://doi.org/10.1371/journal.pone.0187590.
[59] Briones-Fourzán, P., Hendrickx, M.E., 2022. Ecology and Diversity of Marine Decapod Crustaceans. MDPI-Multidisciplinary Digital Publishing Institute.