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Bioindicator species of plastic toxicity in tropical environments

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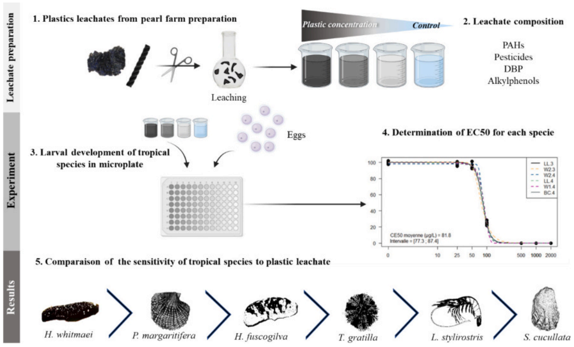
HIGHLIGHTS

- The composition of plastic leachates used in pearl farming has been evaluated.
- We describe the first toxicity tests using a tropical sea cucumber.
- The plastic sensitivity of 5 tropical species was measured for the first time.
- Sensitivity: *H. whitmaei* > *P. margaritifera* > *T. gratilla* > *L. stylirostris* > *S. cucullata*

GRAPHICAL ABSTRACT



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ABSTRACT

In French Polynesia, pearl farming represents the second economic resource of the country. The distinctive black pearls produced there are globally recognized and appreciated. However, pearl farms extensively use submerged plastic materials. Through gas chromatography coupled with tandem mass spectrometry detection (GC/MSMS) analysis, we were able to identify various POPs (Persistent Organic Pollutants) and additives released after 24 h of leaching into seawater from these "pearl plastics" composed of PE (Polyethylene) and PP (Polypropylene). Subsequently, we tested different concentrations of this plastic leachate on five tropical species commonly raised in the pearl and aquaculture sector in Polynesia: *Pinctada margaritifera*, *Saccostrea cucullata*, *Holothuria whitmaei*, *Litopenaeus stylirostris*, and *Tripneustes gratilla*. Monitoring the embryo-larval development of these organisms allowed us to establish a correlation between the decrease in the percentage of normal larvae and the plastic concentration. Through the use of regression models, the EC50 (Effective Concentration) of the plastic leachate for each species was determined, and demonstrated to range from 6.6 to 71.5 g/L, depending on the species. The most sensitive species was the black teatfish *Holothuria whitmaei*, a tropical sea cucumber used for the first time for ecotoxicological tests. The sensitivity of this species, its large distribution in tropical areas, and the various advantages presented by its cultivation make it an interesting bio-indicator species for monitoring plastic pollution in tropical lagoons.

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1. Introduction

Plastics are ubiquitous in modern life due to their versatility and low cost, with global production reaching a record 390 million tonnes in 2021, a 4 % increase from the previous year (Plastics Europe, 2022). Unfortunately, a portion of the produced plastic will end up in the oceans due to mismanaged plastic waste. In 2015, they estimated that between 4.8 and 12.7 million tonnes were being introduced into the oceans annually (Jambeck et al., 2015), this quantity was revised to be between 0.8 and 2.7 million tonnes (Meijer et al., 2021) and to 0.5 million tonnes with 49,000 to 53,000 t consisting of microplastics (plastic particles <5 mm) (Kaandorp et al., 2023).

Once in the marine environment, plastics are subject to mechanical, photolytic, and biological processes that fragment them into smaller particles (Ali et al., 2021). These plastics are now present in both shallow and deep-sea sediments (Zalasiewicz et al., 2016, review in Galgani et al., 2022), marking a significant geological signature of the Anthropocene, often termed the "Plastic Age" (Rangel-Buitrago et al., 2023). The presence of plastics in marine systems induces physical, biological, and chemical pollution. Physical pollution includes macro- and microplastics that can cause harm to marine life, such as suffocation, intestinal damage or disruption of plankton motility (Laist, 1997; Gall and Thompson, 2015; Garnier et al., 2022). Biological pollution arises from plastics providing habitats for invasive and/or pathogenic species through the "raft effect" (Dussud et al., 2018; Haram et al., 2023). Finally, the third type of impact resulting from the presence of plastic in the marine environment is the release of chemicals from polymers. Indeed, upon immersion in the marine compartment, plastics will release various chemicals, including additives with kinetics that depend on numerous parameters such as temperature, salinity, pH, UV exposure, and the physical characteristics of the plastic itself (Hahladakis et al., 2018; Luo et al., 2019; Paluselli et al., 2019). Certain additives present in plastics, such as phthalates, or chemical compounds accumulated during the storage or transport of plastics (Wang et al., 2016), such as pesticides (acetochlor, metolachlor, chlorpyrifos), are often incriminated as endocrine disruptors. They may cause delays in growth, feminization of male individuals in certain species, as well as reproductive toxicity and carcinogenic effects (Mai et al., 2013; Guo and Wang, 2019; Stara et al., 2019; Zhang et al., 2023). More generally, among the 16,325 currently known plastic chemicals, 2760 (16.9 %) are considered to be toxic for the aquatic environment following the EU's REACH regulation and 10,726 (65.7 %) other chemicals do not present any hazard data (Wagner et al., 2024).

At present, while the majority of studies into the toxicity of plastics have traditionally focused on microplastics or nanoplastics to highlight the physical and mechanical risks induced by the presence of these microparticles in the environment (Leistenschneider et al., 2023), there is a growing body of research investigating the chemical pollution effects generated by the leaching of plastic materials. Indeed, recent studies are increasingly exploring the toxicological impacts of chemical additives and leachates from various plastic types, including micro and nanoplastics (Li et al., 2024; Luo et al., 2022; Meng et al., 2024). Furthermore, most tested polymers are PS (Polystyrene), PE, or PVC (Polyvinyl chloride). The toxicity of PP materials is still underexplored in ecotoxicological tests, despite being the second most prevalent polymer on the ocean's surface (Leistenschneider et al., 2023).

In 2022, French Polynesia exported 9 t of pearls, generating 5 billion USD. This ranks the pearl sector in the second place on the economic resources (DRM, 2022). Pearl farms are largely using plastic materials, due to their low cost, resistance, lightweight, and effectiveness in larval capture. A pearl farm is organized as follows: long lines of 200 m ropes are aligned in parallel within the hectares of the concessions. These lines are anchored by mooring blocks and supported at the surface at an average depth of 5 m by buoys. Along these lines, oysters are suspended in strings or baskets. These ropes also contain spat collectors, a kind of plastic lace used to catch oyster larvae. If there is a significant presence of predators, pearl farmers generally choose to protect their stock with mesh protection devices. Photographs of these different plastic materials are available in the article by Crusot et al. (2023). In tropical environments, plastics are exposed to higher temperatures, intense sunlight, and intense marine biological activity, having then shorter lifespans (Crusot et al., 2023). The extensive use of plastic, coupled with poor waste management practices, has made pearl farming one of the identified sources of macro and microplastic litter (Andréfouët et al., 2014). Estimates of the amount of waste in pearl lagoons by the local management bodies (DRM, 2015) revealed high concentrations of plastics in some pearl atolls, reaching up to 3800 t of marine litter in the single atoll of Takaroa (Tuamotu archipelago). Previous studies have also revealed the omnipresence of microplastics not only floating at the surface water, and in the water column of the lagoons, but also accumulated in the tissues of oysters in pearl atolls (Gardon et al., 2021). Recently, an estimate of the annual waste generated by pearl archipelagos, based on prevalent practices, reported >369 t of waste per year for Mangareva island in the Gambier Archipelago (Crusot et al., 2023). This raises the question of toxicity of leachates from polymers used in pearl oyster aquaculture, and how they may affect the biodiversity of coral reef ecosystems. A preliminary study has demonstrated the lethality and possible teratogenic effects of plastic leachates from pearl materials on larvae from the black-lipped pearl oyster *Pinctada margaritifera* (Gardon et al., 2020). The percentage of abnormalities observed in these oyster larvae increases with the concentration of the tested plastic leachates. The same authors showed that leachates obtained from newly submerged pearl plastics for 24 h had higher toxicity than leachates from aged plastics submerged for the same period. Chemical analyses of these first leachates revealed the presence of 19 PAHs in new rope and spat collectors as well as four additives (dimethyl phthalate, dibutyl phtalate, bis(2-ethylhexyl) phthalate and Irgafos 168®). As a consequence, there is a need for toxicological tests adapted to the monitoring of plastic impact in tropical environments, further providing the scientific and technical basis for mitigation measures. Typically, good bioindicator species should possess several characteristics, such as a well-known biological cycle, homogeneous responsiveness to a pollutant, and the existence of identifiable toxic effects associated with the degree of pollution (Li et al., 2019). In recent years, numerous ecotoxicological tests have been conducted, generally using echinoderms or bivalves (His et al., 1999; Bonaventura et al., 2021), well adapted to temperate waters. Tropical species are largely underrepresented in the literature, or focusing on issues related to the toxicity of metals from mining (Gissi et al., 2018; Markich, 2021), particularly on coral species (Binet et al., 2023; Hédouin et al., 2016). For now, and while these species are of greatest interest for tropical aquaculture, no ecotoxicological tests had been conducted on tropical sea cucumber black teatfish *Holothuria whitmaei*.

Here, our study was aimed to (i) assess the toxicity of ropes and collectors, made of PE and PP co-polymer, that are largely use in pearl aquaculture industry, (ii) to test various tropical species for the development of ecotoxicological tests adapted to the local context, and (iii) identify the best bioindicator species for chemical pollution induced by plastic leaching. Overall, this will support a better understanding of the impact of leachates from PE and PP plastic materials on the embryo-larval development of tropical species.

2. Materials et methods

2.1. Plastics selection and leachate preparation

In this study, we specifically investigated the impact of chemical pollution induced by the immersion of new plastic materials used in pearl farms on marine organisms. We selected new ropes and collectors, which are among the most frequently used materials in pearl farming (Crusot et al., 2023). Macroplastics from new ropes and collectors made of PE and PP co-polymer were cut with scissors, sterilised with 70 % ethanol, into smaller plastic particles of approximately 5 mm. For leachate preparation, all glassware (flasks, filter funnels, Erlenmeyer flasks, borosilicate bottles) was autoclaved. The seawater used for all experiments was directly pumped in front of the Pacific Ifremer Center (CIP) in the lagoon of Vairao (Tahiti, French Polynesia). It went through several filtration steps: strainer, sand filter, 25 μm, 10 μm, and 1 μm pocket filters, followed by a double 1 μm cartridge filter, UV sterilization, and finally autoclaving (sterile seawater - SW). Water parameters were measured using a multiparameter probe, and salinity was readjusted with MilliQ water if necessary (salinity: 36 psu; pH: 8.2). The leaching step was adapted from the protocol used by Gardon (Gardon et al., 2020). Four litres of pearl plastic leachate (PPL) and four litres of control leachate (CL) were prepared. For PPL, 200 g of collector microplastics and 200 g of rope microplastics were added to 4 L of sterile seawater in a 10 L flask. For CL, 4 L of sterile seawater was placed in a 10 L flask. Leaching was conducted at room temperature (26 °C), under natural light, with a magnetic stirrer set at 600 rpm. After 24 h of leaching the materials in water, both solutions were filtered with GF/C filters (1.2 mm of porosity, Ø 90 mm, Whatman™). PPL and CL were aliquoted into borosilicate bottles wrapped in aluminium foil, each containing 250 mL, and stored in the freezer at −20 °C.

2.2. *Polymer and chemical analysis*

The identification of polymers in pearl plastic materials, specifically ropes and collectors used throughout our study, was performed using Raman spectroscopy (AlphaR 300 Raman micro-spectrometer, Oxford Instrument/WITec, LDCM/ Brest, IFREMER) equipped with two lasers (532 nm and 785 nm).

Chemical characterization of leachates was conducted in duplicate by CEDRE (Brest, France) using gas chromatography (HP 7890 N, multifunction injection Combipal MPS2, Gerstel) coupled with tandem mass spectrometry (GC/MSMS). Firstly, the extraction of organic compounds from the leachates was carried out with 10 mL of an internal standard solution added to the leachate samples. A magnetic stir bar (Stir Bar Sorptive Extraction, SBSE) coated with a PDMS (Polydimethylsiloxane) phase was then immersed in the vial containing the leachate solution to proceed with the extraction of organic compounds. The extraction is performed by magnetically stirring the bar at 750 rpm for 16 h in the dark. After this step, the bar is retrieved, dried, and then transferred to a desorption tube. Then, the interface temperature was set at 300 °C, with a preprogramed injection of 50 °C (0.5 min) to 280 °C (6 min) at 15 °C/min, coupled with a temperature-programmed injector (Cooling Injection Device, Gerstel: −10 °C (0.05 min) to 300 °C (10 min) at 12 °C/s). The temperature program of the oven was: from 70 °C (0.5 min) to 150 °C at 20 °C/min, then 320 °C (5 min) at 7 °C/min. Helium was used as the carrier gas. The capillary column used was an RXi 5-ms (Restek, Bellefonte, USA). The chromatograph was coupled with a tandem mass spectrometry detector (Agilent 7000 Triple Quad). Quantitative analysis of PAHs, PCBs, PBDEs, pesticides, and additives was performed by internal standard calibration in MRM mode with two transitions for each compound (Quantifier/Qualifier). The acquisition frequency of each fragment was 2 cycles/s. For the analysis of organic compounds, chromatograms were reprocessed using MassHunter Workstation V10.0 software (Agilent Technologies). The list of chemical compounds studied by gas chromatography coupled to tandem mass spectrometry (GC/MSMS) carried out by CEDRE is available in the appendix Table A.1.

2.3. *Larval development and tests design*

Five species were selected to evaluate the potential toxicity of plastic leachates from pearl farms: *Pinctada margaritifera* (pearl oyster), *Saccostrea cucullata* (rock oyster), *Holothuria whitmaei* (sea cucumber black teatfish), *Litopenaeus stylirostris* (blue shrimp) and *Tripneustes gratilla* (collector urchin). The method of induction through thermal shocks on oysters and sea urchins was adapted according to the protocol by Ky et al. (2015). The animals were kept at 24 °C for 3 days and then rapidly immersed in a tank at 32 °C (Ky et al., 2015). For osmotic shock performed on *Saccostrea cucullata*, oysters were air-dried and rinsed with fresh water (Coerolie et al., 1984). After thermal or osmotic shocks, individuals emitting gametes were isolated in individual beakers and sexed. Once the quality of the gametes was verified, fertilization was performed. Holothurians were obtained from the commercial hatchery Tahiti Marine Products (TMP), where gametes were naturally spawned. Males and females were mixed in holding tanks, and the water was then filtered, after fecundation, to concentrate the emitted eggs and adjust their concentrations. For oysters, sea urchins and sea cucumbers, the eggs were exposed to leachates immediately after fertilization. *Litopenaeus stylirostris* larvae were directly supplied to us at the nauplii 1–2, *i.e.* approximately 8 h after egg hatching, stage by the Centre Technique Aquacole of VAIA (Tahiti). The number of nauplii was adjusted through successive filtration, before immediate use. For all species, the fertilization percentage was estimated 1 h after combining male and female gametes (Table A.2), through observations under a microscope and counting with a Malassez cell.

2.4. *Microplate tests*

The contact between the larvae and the different solutions was carried out in untreated cell culture plates (TPP, Techno Plastic Products, Switzerland). The volume and concentration of larvae per well were optimized during the various pre-tests and adapted for each species (Table 1). Once the larvae are added to the wells, the plates are kept in the dark in a Memmert climatic chamber to maintain a constant humidity level (64 % rh) and a temperature chosen for each species.

2.5. *Positive control*

Copper is often used as a reference toxicant because of its widespread use in various industries, its natural presence in the environment and its potential to cause adverse effects on aquatic organisms. Copper in the form of copper sulphate ($CuSO_4$) was diluted in sterile water and used as a positive control to monitor the quality of batches of larvae. The concentration ranges tested for each species will be expressed as equivalent Cu^{2+} concentrations in the remainder of this study (Table A.3). The tests were performed in five replicates.

2.6. *Negative controls*

In our tests, we used two negative controls. Firstly, the control leachate (CL) allowed us to verify that there has been no contamination of our solutions during the preparation and conditioning phases of the leachates. Secondly, we also prepared a sterile seawater control to test the quality of seawater used as a diluent for PPL. The tests were performed in five replicates.

2.7. *Ecotoxicological tests*

To test the potential toxicity of plastic leachates from pearl farms, we conducted a range of concentrations, expressed in grams of plastic per litre of seawater (g/L), ranging from 0.1 to 100 g/L (Table A.4). Dilutions were made with sterile seawater and the toxicity test was conducted in five replicates. During the testing phases, the development of larvae was regularly monitored using a Leica DMI3000B inverted microscope. Considering the different developmental stages, we predefined a target stage for each species that was easily identifiable, and before the larvae started to feed to avoid interferences (Fig. 1). When at least 80 % of the larvae under control conditions had reached this stage, the test was stopped for all conditions by adding 10 μL/mL of 8 % formaldehyde per well. After fixation, all the larvae in each well were observed and divided into two categories. Larvae that reached the predefined target stage were counted in the “normal larvae” category. Any

Table 1
Summary of larval rearing conditions in cell culture microplates.

Species	Number of wells per plate	Volume of well (mL)	Number of larvae/mL	Incubation temperature (C°)	Targeted stage	Post-fertilization fixation time (hours)
Pinctada margaritifera	12	4	60	29	Larva D	24
Saccostrea cucculata	12	4	50	27	Larva D	24
Holothuria whitmaei	12	4	25	28	Mid-Auricularia	93
Litopenaeus stylirostris	24	2	15	29	Zoe	40
Tripneustes gratilla	24	3	33	27	Pluteus	70

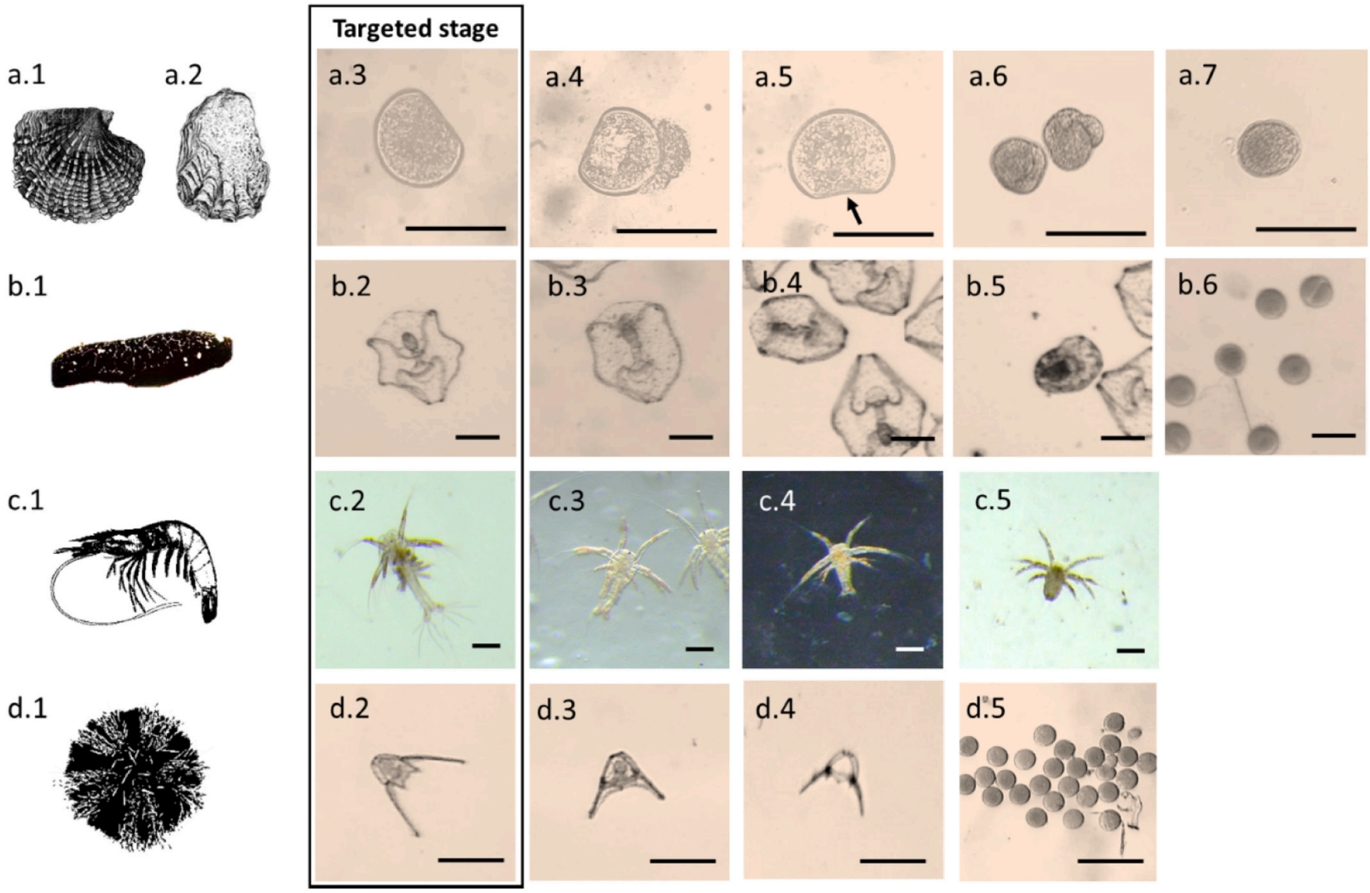

Fig. 1. Illustration depicting a *Pinctada margaritifera* (a.1), *Saccostrea cucullata* (a.2), *Holothuria whitmaei* (b.1), *Litopenaeus stylirostris* (c.1) and *Tripneustes gratilla* (d.1). Photographs of different encountered stages: normal D-larvae (a.3), D-larvae with mantle anomaly (a.4), shell anomaly (a.5), embryonic stage (a.6), oocytes (a.7), mid-auricularia (b.2), early-auricularia larvae (b.3, b.4), malformed larva (b.5), oocytes (b.6), zoe larvae (c.2), nauplii 6 larvae (c.3), nauplii 5/4 larvae (c.4), nauplii 3/2 larvae (c.5), pluteus larvae (d.2), early pluteus larvae (d.3), dead larva (d.4), oocytes (d.5).
Scale bar = 100 μm.

showing growth delay or malformation was counted as "abnormal larvae."

2.8. Determination of EC50

In order to compare the sensitivity of the five model species to plastic leachate in a consistent manner, we compared their effective concentration at which 50 % of the larvae exhibit a developmental anomaly compared to the control condition (EC50). For each species studied, the EC50 values for PPL and $CuSO_4$ were estimated using predictive models. Dose-response curves were generated using the "drc: Analysis of Dose-Response Curve" package. Among the regression models available in "drc", the best-fitted model for each dataset was selected. Model selection was based on various criteria such as "Log-Likelihood," "IC" (Information Criterion), "Lack of fit", "Residual standard error," and "Variance of the residuals," which assess the quality of the fit for each model. The analyses were conducted using R 4.0.5 (Fig. 2).

3. Results

3.1. Nature of pearl farm plastics and leachates analysis

After Raman spectroscopic analyses, we identified the polymers constituting the ropes and collectors as polyethylene (PE) and polypropylene (PP). Chemical composition analyses of control leachate (CL) and pearl plastic leachate (PPL) revealed the presence of numerous compounds (Table 2). The concentrations mentioned below result from the average of the concentrations from the two replicates. Firstly, in the

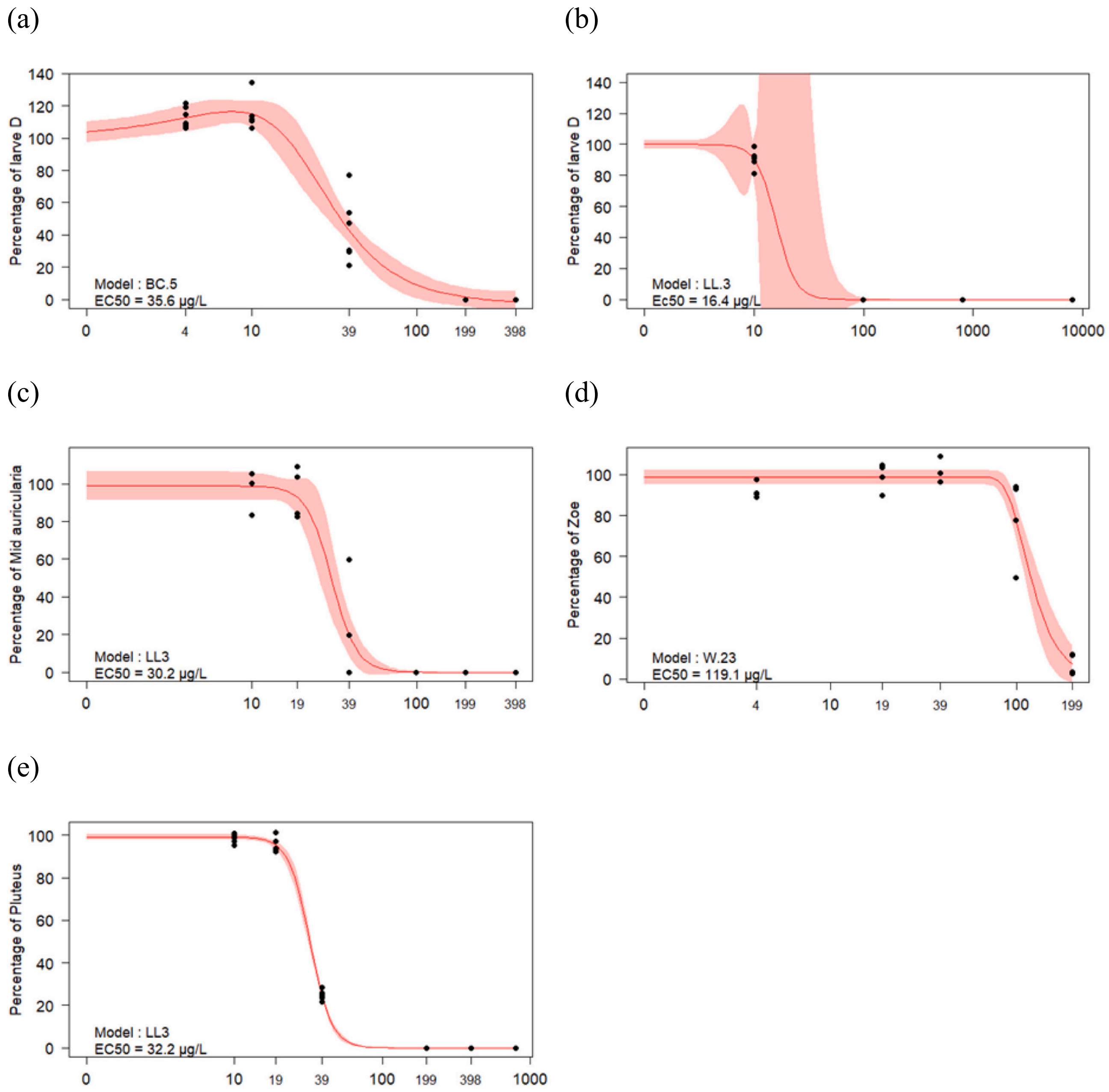

Fig. 2. Dose-response curves after exposure to Cu^{2+}, concentration in µg/L, and estimation of the EC50 and 95 % confidence interval for larvae *Pinctada margaritifera* (a), *Saccostrea cucullata* (b), *Holothuria whitmaei* (c), *Litopenaeus stylirostris* (d), *Tripneuste gratilla* (e).

CL, the only organic compound in a concentration high enough to be detectable was nonylphenols with an average concentration of 13.53 ng/L (±0.29).

For the PPL, four polycyclic aromatic hydrocarbons (PAHs) were quantified: phenanthrene at 21.37 ng/L (±0.54), pyrene at 6.93 ng/L (±0.23), fluoranthene at 4.65 ng/L (±0.06) and anthracene at 2.66 ng/L. The sum of the concentrations of PAHs is 35.6 ng/L (±0.82). Numerous pesticides were also identified. In descending order of concentrations, they were: acetochlor 425 ng/L (±25.67), metolachlor 78.37 ng/L (±4.4), chlorpyrifos 27.03 ng/L (±0.31), endosulfan alpha 3.92 ng/L (±0.13), endosulfan beta 1.77 ng/L (±0.07), beta-BHC (β-Hexachlorocyclohexane) 1.35 ng/L (±0.15), and gamma-BHC (gamma-hexachlorocyclohexane) 0.84 ng/L (±0.02). Summing the concentrations of the 7 pesticides listed yields 538.7 ng/L (±30.47). In the phthalate category, only dibutyl phthalate (DBP) was found, with a concentration of 169.46 ng/L (±54.05). Finally, the last category of identified organic pollutants was made up of alkylphenols. Three were identified: nonylphenols 88.36 ng/L (±1.57), 4-tOP (4-tert-Octylphenol) 6.21 ng/L (±0.69), and 4-OP (4-Octylphenol) 1.18 ng/L (±0.01). The analyzed PPL thus contains 95.75 ng/L (±2.25) of alkylphenols of three different types. No detectable level of PCBs or PBDEs was detected in any of the replicates.

3.2. Cu^{2+} as referent toxic

Five dose-response curves were obtained after exposure of *Pinctada margaritifera*, *Saccostrea cucullata*, *Holothuria whitmaei*, *Litopenaeus stylirostris* or *Tripneustes gratilla* larva to $CuSO_4$ pollutant (Fig. 3). Whatever the species considered, the rate of normally developed larvae in negative controls was above 80 % which allowed to calculate EC50. The five species were ranked from the most to the less sensitive to cupper cation, as follows: *Saccostrea cucullata*, *Holothuria whitmaei*, *Tripneustes gratilla*,

Table 2
Chemical compositions of new pearl plastic leachates (rope and collector), obtained through gas chromatography coupled with tandem mass spectrometry (GC/MSMS) conducted by CEDRE in Brest (Center for Documentation, Research, and Experimentation on Accidental Water Pollution). The limits of detection (LD in blue color) and quantification (LQ) are given for each chemical compound.

			Control		**Pearl plastic leachate 100g/L**	
LD (ng/L)	LQ (ng/L)	chemical compounds	1	2	1	2
1.5	5	Phenanthrene	<LD	<LQ	21.9	20.83
0.15	0.5	anthracene	<LD	<LD	2.66	2.67
0.3	1	fluoranthene	<LD	<LD	4.7	4.59
1.5	5	pyrene	<LD	<LD	7.16	6.7
		∑ HAPs	**0**	**0**	**36.42**	**34.79**
0.3	1	Beta-BHC	<LD	<LD	1.2	1.5
0.15	0.5	gama bhc	<LD	<LD	0.86	0.82
0.3	1	acetochlore	<LD	<LD	451.08	399.75
0.3	1	metolachlore	<LD	<LD	82.77	73.96
0.15	0.5	chlorpyrifos	<LQ	<LQ	27.34	26.72
0.15	0.5	endosulfan alpha	<LD	<LD	4.07	3.79
0.15	0.5	endosulfan beta	<LD	<LD	1.84	1.7
		∑ Pesticides	**0**	**0**	**569.16**	**508.24**
30	100	DBP	<LD	<LD	223.5	115.41
		∑ Phtalates	**0**	**0**	**223.5**	**115.41**
0.3	1	4 tOP	<LQ	<LQ	6.9	5.52
0.3	1	4-OP	<LD	<LD	1.18	1.19
3	10	NPs	13.82	13.25	89.91	86.79
		∑ Alkylphenols	**13.82**	**13.25**	**97.99**	**93.5**

Pinctada margaritifera, *Litopenaeus stylirostris*, with EC50 values ranging from 16.4 to 119.1 μg/L. The linear regression model best suited to each data set, the EC50 obtained with this model and a range of EC50 obtained with estimates from the other models are specified in Table 3.

3.3. PPL tests

The results of exposure tests on larvae of the five tropical model species to pearl plastic leachate are presented in Fig. 3. The tested PPL resulted in malformations and growth delays for all considered species. However, the species appear to exhibit varying sensitivities to the PPL. Calculations of EC50 values allowed for ranking these species based on their sensitivity. *Holothuria whitmaei* emerged as the most sensitive species, followed by *Pinctada margaritifera*, *Tripneustes gratilla*, *Litopenaeus stylirostris*, and finally, the least sensitive to PPL was *Saccostrea cucullata*. The EC50 values for PPL ranged from 6.6 to 71.5 g of plastic per litre of seawater. The summary of all obtained EC50 values for PPL is presented in Table 4 for each species, the linear regression model best suited to each data set, the EC50 obtained with this model and a range of EC50 obtained with estimates from the other models are specified in Table 4.

4. Discussion

In recent years, the number of studies on plastic pollution has exponentially increased. In order to compare the results highlighted by various research teams worldwide addressing this issue, it seems essential to establish a unique and harmonized protocol, such as ISO standards for other oyster species (ISO 17244, 2015). The identification of reference model species for each biotope is crucial for the overall comparison of the toxicity induced by plastic pollution as well as the use of referent toxic products as positive controls in order to be able to compare geographical distant ecotoxicology studies using the same model species. In this study, we have endeavoured to test several tropical species with the aim of defining criteria resulting from practical aspects (ease of obtaining breeders, ease of triggering spawning, larval capacity to develop in small volumes without water renewal, ease of identifying development stages with easily recognizable morphological characteristics) as well as toxicological relevance (sensitivity of species to tested toxins). The ultimate aim is to identify the best bioindicator species for chemical pollution caused by plastic leaching. The routine application of these simple tests could facilitate the establishment of a monitoring network to alert about the exceeding of pollution thresholds in high-risk areas such as pearl farming zones.

4.1. Contamination of the control leachate

To validate the leaching protocol and ensure there was no contamination during various preparation phases, control leachates were sent to CEDRE (France). Only the presence of nonylphenols was identified in the control leachates (13.2–13.9 ng/L). This contamination can have several origins. One possibility is that the measured nonylphenols were present on the glassware used. Another possibility, the presence of nonylphenols is attributed to the contamination of the Vairao lagoon, from which the seawater was pumped. Nonylphenols are widely used as surfactants, antioxidants, detergents, emulsifiers, among other applications and can be found in paints, pesticides, cleaning products, and are also used as plasticizers (Noorimotlagh et al., 2020). The concentrations measured in our control leachates remain far below the concentrations considered risky for the environment (1.7 μg/L) (Brooke and Thursby, 2005) and do not seem capable of affecting the larval development of the tested species (Hong et al., 2022). Other chemical compounds in the

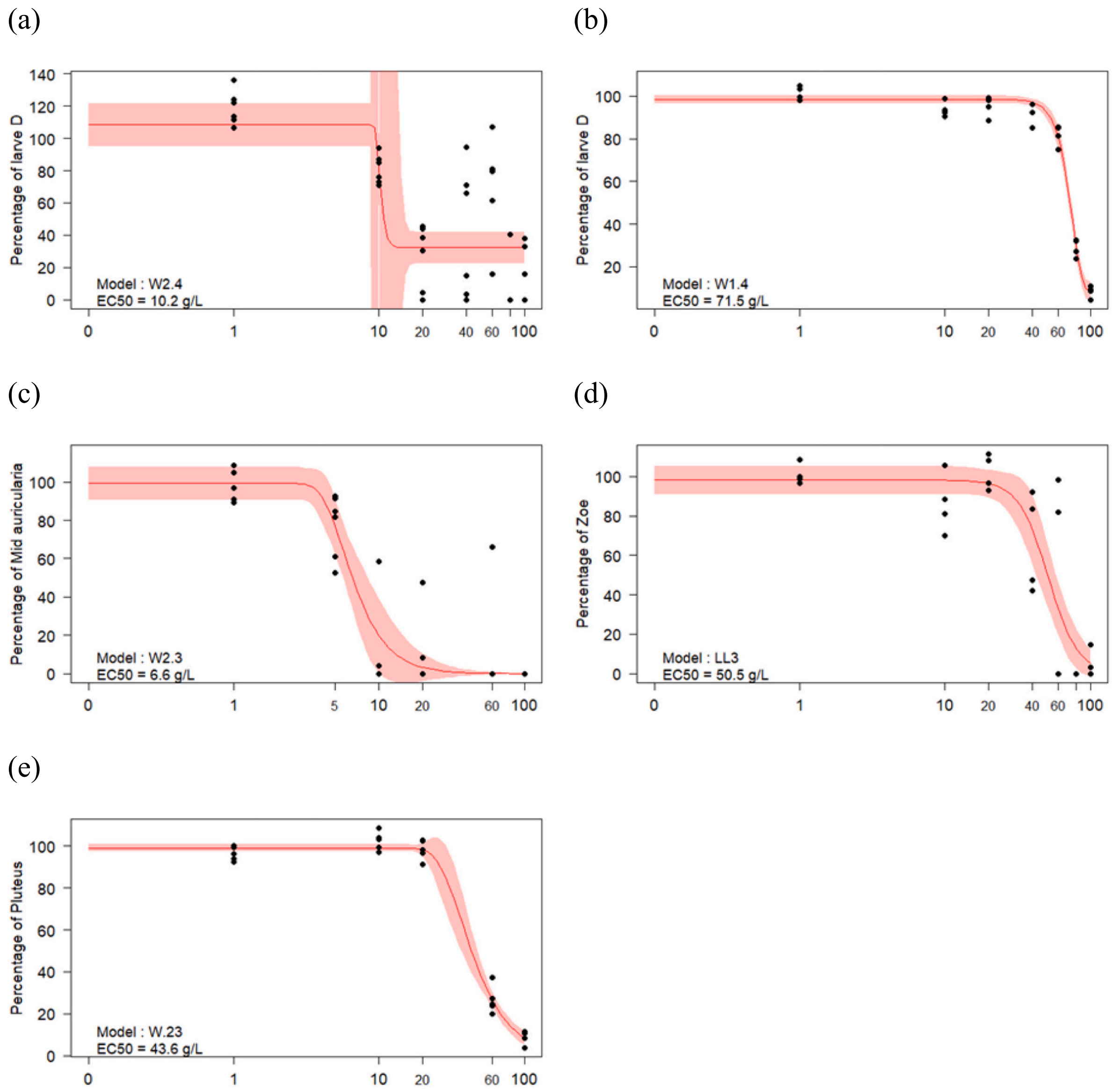

Fig. 3. Dose-response curves after exposure to pearl plastic leachate, PPL concentration in g/L, and estimation of the EC50 and 95 % confidence interval for larvae. *Pinctada margaritifera* (a), *Saccostrea cucullata* (b), *Holothuria whitmaei* (c), *Litopenaeus stylirostris* (d), *Tripneuste gratilla* (e).

Table 3
EC50 Cu^{2+} for each species and the evaluation of EC50 with other models.

Specie	Model	EC50 of Cu^{2+} (µg/L)	Range of assessment of EC50 by other models (µg/L)
Pinctada margaritifera	BC.5	35.6	[35.4; 38.6]
Saccostrea cucullata	LL.3	16.4	[10.8; 35.3]
Holothuria whitmaei	LL.3	30.2	[29.1; 33.6]
Litopenaeus stylirostris	W2.3	119.1	[112.6; 135.1]
Tripneustes gratilla	LL.3	32.2	[33.0; 37.7]

Table 4
Summary of the EC50 values obtained for the pearl plastic leachate for all studied species.

Specie	Model	EC50 (g/L)	Range of assessment of EC50 by other models (g/L)
Pinctada margaritifera	W2.4	10.2	[10.2; 26.3]
Saccostrea cucullata	W1.4	71.5	[70.7; 71.8]
Holothuria whitmaei	W2.3	6.6	[6.0; 6.8]
Litopenaeus stylirostris	LL.3	50.5	[48.9; 61.9]
Tripneustes gratilla	W2.3	43.6	[43.3; 50.8]

control leachate were below detection or quantification limits.

4.2. Variability of leachates products

Compared to a study performed in 2020 first highlighting the toxicity of leachates from pearl plastic materials (Gardon et al., 2020), and using the same protocol, in the same laboratory, leachate composition was found different, with the concentration of phthalates decreased from 6680.92 ng/L in 2020 to 169.46 ng/L in our work, while pesticides were increased from 21.69 ng/L in 2020 to 538.71 ng/L in our experiment.

These facts highlight the difficulty of reproducing plastic leaching products and strongly encourage the chemical characterization of the leachates generated. The differences observed between the composition of these leachates may be due to a number of external factors. The main reason being the plastics themselves. Although the plastics used in these two studies were standard PE and PP ropes and collectors used in pearl farming, we cannot rule out a difference in the manufacturing processes during which additives have been intentionally added during the manufacturing of plastics to provide them with desirable physical or chemical properties, such as plasticizers, stabilizers, or flame retardants (Hermabessiere et al., 2017; Lithner et al., 2011), or they may have been absorbed during their transportation or storage (Wang et al., 2016). These factors could explain the differences in composition between these two plastic leachates produced with different batches of plastics several years apart.

4.3. Chemical analysis of pearl plastic leachates: presence of POPs and additives

Analytical techniques using gas chromatography coupled with tandem mass spectrometry (GC/MSMS) conducted on our PPL have revealed numerous persistent organic pollutants (POPs) and additives related to plastics. Among the identified POPs after 24 h of leaching 100 g of new pearl plastics in one litre of seawater, some have already been recognized as posing risks to marine ecosystems. The most represented class of POPs was pesticides and acetochlor was the main one. Among the noteworthy studies, Mahmood et al. (2021, 2022) demonstrated the numerous effects of acetochlor on the carp *Aristichthys nobilis*. Metolachlor, the second most prevalent pesticide in our leachates, has been linked to a significant increase in DNA and behavioural damage observed in oysters and crustaceans (Mai et al., 2012; Stara et al., 2019). Some research has highlighted the combined effect of pesticides and microplastics on copepods (Bellas and Gil, 2020) or the synergistic effect of chlorpyrifos, acetochlor and dicofol on marine diatoms (Zhang et al., 2023).

The second largest group in our PPL was phthalates. Phthalates, like other plastic additives, are not bound to the polymer matrix of plastics and are therefore easily and rapidly leached into water during plastic immersion (Clara et al., 2010; Paluselli et al., 2019). After studying the effects of 18 different phthalates on microorganisms, algae, invertebrates, and fish, a toxicity ranking was established (Staple et al., 1997). Low molecular weight phthalates such as dibutyl phthalate (DBP) and butyl benzyl phtalate (BBP) have higher acute and chronic toxicity than other tested molecules (Staple et al., 1997). A wide range of effects of DBP on marine organisms has been described, ranging from oxidative stress to enzymatic and metabolic alterations in marine animals at concentrations on the order of μg/L (Zhou et al., 2015). Teratogenic effects and increased mortality in *Danio rerio* (Ortiz-Zarragoitia et al., 2006) or feminizing the sexual characteristics of male fish and its impact on the gonadal development of juvenile fish (Bhatia et al., 2014) have occurred following exposure to DBP.

The predominantly found alkylphenol in our plastic leachates is nonylphenol. This chemical compound is identified as an endocrine disruptor, mimicking natural hormones such as 17β-œstradiol (Lee and Lee, 1996), which can impact the development, fertility and the production of female hormones in some fish (Kinnberg et al., 2000; Lee et al., 2003; Tabata et al., 2018).

Phenanthrene is the most toxic aromatic hydrocarbon tested by Black et al., its high toxicity is attributed to the large number of aromatic cycles it contains (Black et al., 1983). Since then, the toxicity of PAHs on the development of freshwater and marine fish larvae and juveniles has been demonstrated (Mu et al., 2014; Wessel et al., 2010).

When comparing the cited studies to the concentrations of additives measured in our leachates, certain distinctions become evident. Firstly, it is noteworthy that the orders of concentrations examined in previous studies were expressed in micrograms or milligrams per litre, whereas in our plastic pearl leachate (PPL), all concentrations were reported in nanograms per litre. If we relate the concentration of the compounds identified in our leachates to the estimated effective concentration for each species, the toxic order of magnitude of each compound is even lower. Thus, the effective concentration causing abnormal development in 50 % of the larvae of *H. whitmaei*, *P. margaritifera*, *T. gratilla*, *L. stylirostris* and *S. cucculata* would be due to a complex mixture of respectively 55 ng/L, 86 ng/L, 366 ng/L, 424 ng/L and 600 ng/L of the sum of PAHs, pesticides, phthalates and alkylphenols (Table A.5).

However, with regard toxenoestrogens, a body of evidence from various studies has demonstrated that the effects of two or more compounds capable of disrupting the endocrine system may be additive or synergistic, even at low concentrations (Kwak et al., 2001; Rajapakse et al., 2002; Sol Dourdin et al., 2023). A mixture of eight xenoestrogens, such as Benzophenones or Bisphenol A which can be found in the composition of plastic, at concentrations below their no observed effect concentration (NOEC), had significant estrogenic effects on cells *in vitro* (Silva et al., 2002). These results demonstrate that the combination of these chemicals can induce notable biological effects, challenging risk assessments based solely on individual agents. This observation is particularly significant given the intricate mixture present in our PPL, necessitating consideration of potential synergistic and/or cumulative effects.

4.4. Embryo-larval tests with copper

The ISO standard suggests Cu^{2+} as a reference toxicant to ensure the standardization of ecotoxicological results. In our experiments, all examined species responded to the presence of this molecule, albeit in varying degrees. Previous studies indicate that the EC50 for copper ion in different bivalve species falls between 6.0 and 12.8 μg/L (Markich, 2021; Nadella et al., 2009; Rosen et al., 2008). We found an EC50 for *Saccostrea cucullata* closely aligning with reference values, while the calculated EC50 for *Pinctada margaritifera* was found 3-fold higher than the EC50 range established by the same authors. Limited ecotoxicological studies, especially on tropical species, exist for sea cucumbers. Rakaj (Rakaj et al., 2021) and Morroni (Morroni et al., 2019) assessed the sensitivity of two species, *Holothuria tubulosa* and *Holothuria polii*, estimating their EC50 between 100 and 110 μg/L for the first, and 260 μg/L for the second. *Holothuria whitmaei* exhibited an EC50 of 30.2 μg/L for Cu^{2+}, showing a greater sensitivity compared to other species within the same genus. The sensitivity of the shrimp to copper varies based on multiple factors including the life stage and exposure time. Tests on a freshwater species, *Macrobrachium rosenbergii*, estimated the LC50 to copper at 0.46 mg/L after 48 h of exposure (Osunde et al., 2004). For *Litopenaeus stylirostris*, the copper EC50 was 119.1 μg/L, and a higher concentration would be expected if “lethal concentration” had been chosen over “effective concentration”. In *Paracentrotus lividus,* extensively studied in toxicology, exhibit similar EC50 estimates for copper ion, ranging from 60 to 70 μg/L (Pétinay et al., 2009) and 45.8 to 72.8 μg/L, depending on exposure time (Morroni et al., 2018). Other sea urchin species, such as *Echinometra mathaei* (29.85 μg/L ± 1.86) and *Strongylocentrotus purpuratus* (14.3–20.6 μg/L), display varying sensitivities. In our study, the tropical species *Tripneustes gratilla* had an EC50 of 33.1 μg/L, consistent with estimates from published studies.

Comparing the EC50 for Cu^{2+} among model species in this study with those from prior research reveals valuable insights. Methodology variations significantly influence sensitivity measurements, and even species within the same phylogenetic “order” can exhibit different sensitivities. This is evident in *Pinctada margaritifera* and *Saccostrea cucullata*, both belonging to the *Ostreida* order, with respective EC50 values of 35.6 and 16.4 μg/L for Cu^{2+}. The sources of variation in the observed differences in sensitivity to copper between species can nevertheless be attributed to a sensitivity specific to the species or to external parameters (spawning quality, heterogeneity of test conditions, sensitivity index chosen EC or LC). The EC50 values for copper obtained for the five model species in our study could be used as reference points for future toxicological tests on these tropical species, which may have different sensitivities compared with their temperate counterparts.

4.5. Embryo-larval tests with PPL

The overall results indicate a significant effect of leachate from pearl plastic on all studied species. The dose-response curves obtained show a correlation between an increase in plastic concentration and a decrease in normal larvae. The presence of plastic in the environment therefore favours an increase in growth anomalies, such as malformations and developmental delays. The EC50 values obtained from projections of different models adapted to each species show varying effective concentrations of leachate from pearl plastics. The most sensitive species to PPL is *H. whitmaei*, followed by *P. margaritifera*, with an T. gratilla, *L. stylirostris* and *S. cucullata* as the least sensitive species. The effective concentration values ranged from 6.6 g/L for the most sensitive species to 71.5 g/L for the most resistant species to plastic leachates.

The effective concentrations of plastics obtained for all species, on the order of grams per litre, appear to be distant from concentrations previously measured in the natural environment. However, it is important to remember that the toxicity results of this study are based on both limited exposure duration and short time leachate production. For the two oyster species, we chose to stop the test after 24 h of larval exposure to the leachates. A previous toxicity study using the same pearl plastics demonstrated significant effects of leachates at 0.1 g/L for oyster larvae exposed for 48 h, whereas no visible effect was reported for the same concentration after 24 h of exposure and even using a dose 100 times more concentrated of 10 g/L (Gardon et al., 2020). Furthermore, the same authors show that increasing leachates production time from 24 h to 120 h induced significant larval mortality rates of 85.9 % ± 3.0 % at the lowest tested concentration of 0.1 g/L in a 48 h exposure treatment, to be compared to 10.0 % ± 13.8 % for control animals.

Prolonged exposure to plastic leachates coupled with a prolonged leaching time of new pearl plastics, as encountered in natural environments, may thus lead to increased larval sensitivity. In French Polynesia, several decades of extensive use of plastic materials coupled with poor waste management have gradually led to significant plastic pollution of the Polynesian pearl atolls. Campaigns to estimate the amount of historical pearl farm litter accumulated since the 1980s have been conducted in Polynesia by the Direction of Marine Resources. One such campaign was carried out in Takapoto in 2018. For over forty years, this atoll, due to its unique environmental and ecological characteristics, a nearly enclosed lagoon and a large wild oyster population, has been the main supplier of oyster spat for the Tuamotu (Intes, 1984). Using a multibeam echo sounder, part of this lagoon was analyzed, revealing over 432 t of plastic waste (DRM, 2018). Far from being inert, aged plastic will continue to be toxic throughout its marine life by releasing chemical compounds and decomposing into microplastics that can be assimilated by organisms (Andréfouët et al., 2014; Gardon et al., 2020, 2021, 2024).

This historical amount of abandoned plastics is compounded by the annual renewal of pearl farming plastics. A study to estimate the annual amount of waste produced by this sector was conducted by (Crusot et al., 2023). For the Takapoto atoll, they estimated that the 141 ha of marine concessions generate nearly 38.9 ± 3.4 t of plastic waste per year. Assuming that the quantity of plastic waste in the water has not increased since the 2018 estimate, due to widespread environmental awareness and the introduction of legislation in 2017 condemning this type of practice, and that the annual waste production corresponds to newly introduced plastics in the lagoon, we can approximate the concentration of plastic in the Takapoto lagoon. This lagoon, covering 8100 ha, with an average depth of 25 m, semi-closed with a water renewal time of 268 days (Pagès and Andréfouët, 2001), contains approximately 2025 million cubic meters of water. The amount of historical waste is 432.2 t, and the newly introduced plastic per year is 38.9 t. This results in a concentration of 0.23 mg/L. This quantity is likely underestimated as our calculations do not account for undeclared pearl farming installations, which can be significant (Crusot et al., 2023). Additionally, this concentration of 0.23 mg/L assumes a homogeneous distribution of plastic throughout the water column and across the lagoon's surface, which is inaccurate. Thus, local plastic concentrations can be much higher than the global estimates generally made at the lagoon scale (Gardon et al., 2021). Conversely, it can be more accurately considered that currently, in the Takapoto lagoon, nearly 470 t of old and new plastics continue to release potentially toxic chemicals to numerous lagoon species, and at high concentrations locally, to the extent that many species live in close contact with plastics such as ropes and collectors. This concerns in particular i) the first stages of life of molluscs such as pearl oysters during the fixation phase of the larvae on these types of inert supports or ii) benthic animals such as holothurians found near pearl farming waste such as tangled rope mats or collection lines stranded on the seabed (Andréfouët et al., 2014). To prevent and act in case of suspected contamination threshold exceedance in the lagoons, and in addition to ecotoxicological tests on larvae, it would be pertinent to chemically characterise the water using passive samplers (Bartelt-Hunt et al., 2011; Net et al., 2015; Van Metre et al., 2017).

With regard to the technical and logistical aspects of reproduction mentioned in this study, we can state that species such as *P. margaritifera* and *T. gratilla* represent a challenge with regard to the conditioning of broodstock, with the aim of inducing spawning, as well as the success of fertilization and the hatching rate of eggs. Ongoing research programs at CIP focus on optimizing breeding and conditioning for inducing spawning in these species. *L. stylirostris* presents a different set of challenges. In shrimp farming, spawning induction requires a complex and technical procedure. Although fertilization rates were good in various tests with this species, the hatching rate was low. To avoid compromising water quality in the reduced volumes of microplates, we chose to collect viable larvae after hatching instead of fertilized eggs, some of which might be non-viable and lead to an increase organic matter and potential contamination. However, this choice limited the contact time between the larvae and the PPL, beginning only 8 h post-hatching.

Based on our experience, we can conclude that the tested species that were the easiest to raise and reproduce were *S. cucullata* and *H. whitmaei*. Due to its developed adaptive capabilities, *S. cucullata* has spread worldwide and thrives in both temperate and tropical environments (do Amaral et al., 2020; Pagenkopp Lohan et al., 2015; Ramadhaniaty et al., 2018; Ulman et al., 2017). Unlike representatives of this species in temperate environments, specimens of *S. cucullata* in tropical environments are capable of reproducing throughout the year (Legat et al., 2021). This characteristic allows for the production of *S. cucullata* larvae at any season, which is a major advantage for implementing routine environmental monitoring. The minimum size for first maturation of this species has been determined to be 32.8 mm and 28.3 mm for females and males, respectively (Mafambissa et al., 2023). To select mature breeders, especially if collecting wild individuals is considered, it is advisable to choose individuals whose shell length exceeds these sizes. In our spawning attempts, a brief emersion phase of the breeders followed by a rinse with fresh water before immersion in seawater has always been sufficient to trigger spawning in several breeders with fertilization and hatching rates exceeding 90 %. However, other reproduction inductions exist and have proven effective for the *Saccostrea* genus, such as the combination of decreased salinity with the addition of sperm to the breeders' water (Nowland et al., 2021).

In these tests, we also measured the potential of sea cucumbers *H. whitmaei* and the numerous logistical advantages that this species represents. This species, widely distributed in the Indo-Pacific, is highly present in French Polynesia. Research currently conducted by the company TMP contributes to increasing general knowledge about this species to enhance its breeding for pharmacological purposes. At present, limited information is available for this species. However, data on the breeding methods of sandfish sea cucumbers *H. scabra*, a tropical sea cucumber species with high market value belonging to the same genus, are available. The ideal weight for a mature breeder is 500 g (Agudo, 2006). According to the same author, several spawning induction protocols have already been tested. Among the most effective and non-lethal combinations, it is recommended to air-dry the animals, followed by a cold and then a hot thermal shock. Alternatively, natural spawns can be obtained by collecting the animals just before the full and new moons, as was our case, with the peak fertility of this species identified during the austral summer. Eggs obtained from natural spawns are generally more numerous, but the quantity of oocytes emitted by induction, even outside the natural spawning periods, is on the order of 1 to 2 million per female (Agudo, 2006), which is more than sufficient for conducting tests in microplates.

In the perspective of larval production for the implementation of ecotoxicological tests in tropical regions, we recommend using *S. cucullata* and/or *H. whitmaei*. To limit infrastructure, maintenance, and food production, we advise collecting a few breeders from the wild and employing non-lethal spawning induction methods so that these adult individuals can be returned to the natural environment once spawning is completed.

If we now compare the response of these two selected species in terms of their sensitivity to plastic leachate, it is observed that the 50 % development anomalies are reached at concentrations of 6.6 and 71.5 g/L of plastic for *H. whitmaei* and *S. cucullata* respectively. *H. whitmaei* is therefore almost 11 times more sensitive than *S. cucullata*. Considering these biological characteristics and the sensitivity demonstrated by *H. whitmaei* during exposure tests to plastic leachate, this species appears to be a good bioindicator of plastic pollution in tropical environments. The control of larval development in *H. whitmaei* opens the possibility of establishing a monitoring network for assessing pollution levels in pearl farming sites or monitoring the toxicity of aquaculture effluents in Polynesia. More broadly, the significance of this approach lies in the applicability of the results to the specific context of tropical island environments, particularly in lagoons and aquaculture areas.

For the future, it would be interesting to continue the development of microplate tests by confronting the larvae with multistress and thus better assessing their behaviour in the face of current global changes. Indeed, synergistic effects have been demonstrated between the effects of climate change and marine pollution (reviewied by Cabral et al., 2019). Synergistic effects are defined as stressors interplay resulting in a combined effect greater than the sum of their individual effects (Folt et al., 1999). The effects of increasing temperature or ocean acidification (Cao et al., 2018), two effects of global climate change, combined with metals or POPs (Su et al., 2017) or with plastic pollution (Bertucci and Bellas, 2021) have shown more significant deleterious effects on marine organisms than the effect of each stress evaluated separately.

5. Conclusion

In to this study, we identified the composition of leachate from ropes and spat collector oysters made of polyethylene and polypropylene plastic. The results suggest that pearl plastics leachates are complex mixtures of POPs and additives such as hydrocarbons, pesticides, phthalates and alkylphenols. These chemical compounds prove toxic even at low concentrations (ng/L). Indeed, thanks to acute exposure toxicity tests developed in microplates, the harmful effects of plastic pollution on the early stages of development of tropical organisms have been demonstrated. Among the studied species, *Holothuria whitmaei* had never been used for ecotoxicological testing. In addition to its effective sensitivity to plastic pollution, this species has logistical and biological advantages that make it an interesting bio-indicator species to develop for monitoring chemical contamination of tropical lagoons. The range of sensitivity to the reference toxicant Cu^{2+} has been established for *H. whitmaei* and could serve as a reference for future ecotoxicological tests. As the use of plastics in aquaculture and pearl farming is chronic and massive, further studies are now needed to provide information on the impact on exposed organisms over generations and to characterise possible synergies between mixtures of POPs and additives and environmental factors set to change with current climate change.

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CRediT authorship contribution statement

M. Goulais: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **D. Saulnier:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **J. Rouxel:** Writing – review & editing, Methodology, Investigation. **F. Galgani:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

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.

Data availability

Data will be made available on request.

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References

Agudo, N., 2006. Sandfish hatchery. In: Geographical (Vol. 19, Issue 3). http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12880789.

Ali, S.S., Elsamahy, T., Koutra, E., Kornaros, M., El-Sheekh, M., Abdelkarim, E.A., Zhu, D., Sun, J., 2021. Degradation of conventional plastic wastes in the environment: a review on current status of knowledge and future perspectives of disposal. Sci. Total Environ. 771, 144719 https://doi.org/10.1016/j.scitotenv.2020.144719.

Andréfouët, S., Thomas, Y., Lo, C., 2014. Amount and type of derelict gear from the declining black pearl oyster aquaculture in Ahe atoll lagoon, French Polynesia. Mar. Pollut. Bull. 83 (1), 224–230. https://doi.org/10.1016/j.marpolbul.2014.03.048.

Bartelt-Hunt, S.L., Snow, D.D., Damon-Powell, T., Brown, D.L., Prasai, G., Schwarz, M., Kolok, A.S., 2011. Quantitative evaluation of laboratory uptake rates for pesticides, pharmaceuticals, and steroid hormones using POCIS. Environ. Toxicol. Chem. 30 (6), 1412–1420. https://doi.org/10.1002/etc.514.

Bellas, J., Gil, I., 2020. Polyethylene microplastics increase the toxicity of chlorpyrifos to the marine copepod *Acartia tonsa*. Environ. Pollut. 260, 114059 https://doi.org/10.1016/j.envpol.2020.114059.

Bertucci, J.I., Bellas, J., 2021. Combined effect of microplastics and global warming factors on early growth and development of the sea urchin (*Paracentrotus lividus*). Sci. Total Environ. 782, 146888 https://doi.org/10.1016/j.scitotenv.2021.146888.

Bhatia, H., Kumar, A., Chapman, J.C., Mclaughlin, M.J., 2014. Long-term exposures to di-n-butyl phthalate inhibit body growth and impair gonad development in juvenile Murray rainbowfish (*Melanotaenia fluviatilis*). J. Appl. Toxicol. 35 (7), 806–816. https://doi.org/10.1002/jat.3076.

Binet, M.T., Reichelt-Brushett, A., McKnight, K., Golding, L.A., Humphrey, C., Stauber, J. L., 2023. Adult corals are uniquely more sensitive to manganese than coral early-life stages. Environ. Toxicol. Chem. 42 (6), 1359–1370. https://doi.org/10.1002/etc.5618.

Black, J.A., Birge, W.J., Westerman, A.G., Francis, P.C., 1983. Comparative aquatic toxicology of aromatic hydrocarbons. Toxicol. Sci. 3 (5), 353–358. https://doi.org/10.1093/toxsci/3.5.353.

Bonaventura, R., Zito, F., Morroni, L., Pellegrini, D., Regoli, F., Pinsino, A., 2021. Development and validation of new analytical methods using sea urchin embryo bioassay to evaluate dredged marine sediments. J. Environ. Manag. 281 (November 2020), 111862 https://doi.org/10.1016/j.jenvman.2020.111862.

Brooke, L.T., Thursby, G., 2005. Aquatic Life Ambient Water Quality Criteria Nonylphenol. US Environmental Protection Agency (EPA), Office of Water Office of Science and Technology, Washington DC, p. 88.

Cabral, H., Fonseca, V., Sousa, T., Leal, M.C., 2019. Synergistic effects of climate change and marine pollution: an overlooked interaction in coastal and estuarine areas. Int. J. Environ. Res. Public Health 16 (15), 1–17. https://doi.org/10.3390/ijerph16152737.

Cao, R., Liu, Y., Wang, Q., Zhang, Q., Yang, D., Liu, H., Qu, Y., Zhao, J., 2018. The impact of ocean acidification and cadmium on the immune responses of Pacific oyster, *Crassostrea gigas*. Fish Shellfish Immunol. 81 (April), 456–462. https://doi.org/10.1016/j.fsi.2018.07.055.

Clara, M., Windhofer, G., Hartl, W., Braun, K., Simon, M., Gans, O., Scheffknecht, C., Chovanec, A., 2010. Occurrence of phthalates in surface runoff, untreated and treated wastewater and fate during wastewater treatment. Chemosphere 78 (9), 1078–1084. https://doi.org/10.1016/j.chemosphere.2009.12.052.

Coerolie, M., De Gajllande, D., Landret, J.P., 1984. Recent innovations in cultivation of molluscs in frecnch Polynesia. Aquaculture 39, 45–67.

Crusot, M., Gaertner, J.-C., Rodriguez, T., Lo, C., Gaertner-Mazouni, N., 2023. Assessment of plastic waste generated by the aquaculture industry: the case study of pearl farming in French Polynesia. J. Clean. Prod. 425 (September), 138902 https://doi.org/10.1016/j.jclepro.2023.138902.

do Amaral, V.S., Simone, L.R.L., de Souza Tâmega, F.T., Barbieri, E., Calazans, S.H., Coutinho, R., Spotorno-Oliveira, P., 2020. New records of the non-indigenous oyster *Saccostrea cucullata* (Bivalvia: Ostreidae) from the southeast and south Brazilian coast. Reg. Stud. Mar. Sci. 33, 100924 https://doi.org/10.1016/j.rsma.2019.100924.

DRM (Direction des Ressources Marines), 2015. Rapport de repérage et d'évaluation des déchets immergés identification des hoa à curer, lagon de Takaroa, BC n°28469, décembre 2015, 22.

DRM (Direction des Ressources Marines), 2018. Levé bathymétrique et cartographie des déchets perlicoles du lagon de Takapoto (archipel des Tuamotu).

DRM (Direction des Ressources Marines), 2022. Statistiques 2022: La perliculture. https://www.ressources-marines.gov.pf/2023/06/12/statistiques-la-perliculture/.

Dussud, C., Hudec, C., George, M., Fabre, P., Higgs, P., Bruzaud, S., Delort, A.M., Eyheraguibel, B., Meistertzheim, A.L., Jacquin, J., Cheng, J., Callac, N., Odobel, C., Rabouille, S., Ghiglione, J.F., 2018. Colonization of non-biodegradable and biodegradable plastics by marine microorganisms. Front. Microbiol. 9 (JUL), 1–13. https://doi.org/10.3389/fmicb.2018.01571.

Folt, C.L., Chen, C.Y., Moore, M.V., Burnaford, J., 1999. Synergism and antagonism among multiple stressors. Limnol. Oceanogr. 44 (3 II), 864–877. https://doi.org/10.4319/lo.1999.44.3_part_2.0864.

Galgani, F., Michela, A., Gérigny, O., Maes, T., Tambutté, E., Harris, T., P., 2022. Marine Litter, Plastic, and Microplastics on the Seafloor. Plastics and the Ocean: Origin, Characterization, Fate, and Impacts. https://doi.org/10.1002/9781119768432.ch6.

Gall, S.C., Thompson, R.C., 2015. The impact of debris on marine life. Mar. Pollut. Bull. 92 (1–2), 170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041.

Gardon, T., Huvet, A., Paul-Pont, I., Cassone, A.L., Sham Koua, M., Soyez, C., Jezequel, R., Receveur, J., Le Moullac, G., 2020. Toxic effects of leachates from plastic pearl-farming gear on embryo-larval development in the pearl oyster *Pinctada margaritifera*. Water Res. 179, 115890 https://doi.org/10.1016/j.watres.2020.115890.

Gardon, T., El Rakwe, M., Paul-Pont, I., Le Luyer, J., Thomas, L., Prado, E., Boukerma, K., Cassone, A.L., Quillien, V., Soyez, C., Costes, L., Crusot, M., Dreanno, C., Le Moullac, G., Huvet, A., 2021. Microplastics contamination in pearl-farming lagoons of French Polynesia. J. Hazard. Mater. 419 https://doi.org/10.1016/j.jhazmat.2021.126396.

Gardon, T., Le Luyer, J., Le Moullac, G., Soyez, C., Lagarde, F., Dehaut, A., Paul-Pont, I., Huvet, A., 2024. Pearl farming micro-nanoplastics affect oyster physiology and pearl quality. Environ. Sci. Technol. 58 (1), 207–218. https://doi.org/10.1021/acs.est.3c06684.

Garnier, Y., Galgani, F., Claro, F., 2022. Physical impacts of microplastics on marine species. In: Rocha-Santos, T., Costa, M.F., Mouneyrac, C. (Eds.), Handbook of Microplastics in the Environment. Springer, Cham. https://doi.org/10.1007/978-3-030-39041-9_49.

Gissi, F., Stauber, J.L., Binet, M.T., Trenfield, M.A., Van Dam, J.W., Jolley, D.F., 2018. Assessing the chronic toxicity of nickel to a tropical marine gastropod and two crustaceans. Ecotoxicol. Environ. Saf. 159 (April), 284–292. https://doi.org/10.1016/j.ecoenv.2018.05.010.

Guo, X., Wang, J., 2019. The chemical behaviors of microplastics in marine environment: a review. Mar. Pollut. Bull. 142 (February), 1–14. https://doi.org/10.1016/j.marpolbul.2019.03.019.

Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard. Mater. 344, 179–199. https://doi.org/10.1016/j.jhazmat.2017.10.014.

Haram, L.E., Carlton, J.T., Centurioni, L., Choong, H., Cornwell, B., Crowley, M., Egger, M., Hafner, J., Hormann, V., Lebreton, L., Maximenko, N., McCuller, M., Murray, C., Par, J., Shcherbina, A., Wright, C., Ruiz, G.M., 2023. Extent and reproduction of coastal species on plastic debris in the North Pacific subtropical gyre. Nat. Ecol. Evol. 7 (5), 687–697. https://doi.org/10.1038/s41559-023-01997-y.

Hédouin, L.S., Wolf, R.E., Phillips, J., Gates, R.D., 2016. Improving the ecological relevance of toxicity tests on scleractinian corals: influence of season, life stage, and seawater temperature. Environ. Pollut. 213, 240–253. https://doi.org/10.1016/j.envpol.2016.01.086.

Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., Duflos, G., 2017. Occurrence and effects of plastic additives on marine environments and organisms: a review. Chemosphere 182, 781–793. https://doi.org/10.1016/j.chemosphere.2017.05.096.

His, E., Heyvang, I., Geffard, O., De Montaudouin, X., 1999. A comparison between oyster (*Crassostrea gigas*) and sea urchin (*Paracentrotus lividus*) larval bioassays for toxicological studies. Water Res. 33 (7), 1706–1718. https://doi.org/10.1016/S0043-1354(98)00381-9.

Hong, Y., Li, H., Feng, C., Liu, D., Yan, Z., Qiao, Y., Wu, F., 2022. A review on the water quality criteria of nonylphenol and the methodological construction for reproduction toxicity endocrine disrupting chemicals. Rev. Environ. Contam. Toxicol. 260 (1), 5.

International Organization for Standardization, 2015. ISO 17244:2015 - Water quality — Determination of the Toxicity of Water Samples on the Embryo-Larval Development of Japanese Oyster (*Crassostrea gigas*) and Mussel (*Mytilus edulis* or *Mytilus galloprovincialis*).

Intes, A., 1984. L'huître nacrière en Polynésie Française: Mutation de l'exploitation. Pêche Maritime 1272, 161–166.

Jambeck, J., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. The ocean: the ocean. Mar. Pollut. 347 (6223), 768. https://science.sciencemag.org/CONTENT/347/6223/768.abstract.

Kaandorp, M.L.A., Lobelle, D., Kehl, C., Dijkstra, H.A., van Sebille, E., 2023. Global mass of buoyant marine plastics dominated by large long-lived debris. Nat. Geosci. 16 (8), 689–694. https://doi.org/10.1038/s41561-023-01216-0.

Kinnberg, K., Korsgaard, B., Bjerregaard, P., Jespersen, Å., 2000. Effects of nonylphenol and 17β-estradiol on vitellogenin synthesis and testis morphology in male platyfish *Xiphophorus maculatus*. J. Exp. Biol. 203 (2), 171–181. https://doi.org/10.1242/jeb.203.2.171.

Kwak, H.I., Bae, M.O., Lee, M.H., Lee, Y.S., Lee, B.J., Kang, K.S., Chae, C.H., Sung, H.J., Shin, J.S., Kim, J.H., Mar, W.C., Sheen, Y.Y., Cho, M.H., 2001. Effects of nonylphenol, bisphenol A, and their mixture on the viviparous swordtail fish (*Xiphophorus helleri*). Environ. Toxicol. Chem. 20 (4), 787–795. https://doi.org/10.1002/etc.5620200414.

Ky, C.L., Lau, C., Koua, M.S., Lo, C., 2015. Growth performance comparison of *Pinctada margaritifera* juveniles produced by thermal shock or gonad scarification spawning procedures. J. Shellfish Res. 34 (3), 811–817. https://doi.org/10.2983/035.034.0310.

Laist, D.W., 1997. Impacts of Marine Debris: Entanglement of Marine Life in Marine Debris Including a Comprehensive List of Species With Entanglement and Ingestion Records, pp. 99–139. https://doi.org/10.1007/978-1-4613-8486-1_10.

Lee, P.C., Lee, W., 1996. In vivo estrogenic action of nonylphenol in immature female rats. Bull. Environ. Contam. Toxicol. 57 (3), 341–348. https://doi.org/10.1007/s001289900196.

Lee, H.J., Chattopadhyay, S., Gong, E.Y., Ahn, R.S., Lee, K., 2003. Antiandrogenic effects of bisphenol A and nonylphenol on the function of androgen receptor. Toxicol. Sci. 75 (1), 40–46. https://doi.org/10.1093/toxsci/kfg150.

Legat, J.F.A., Puchnick-Legat, A., Sühnel, S., Pereira, A.L.M., Magalhães, A.R.M., de Melo, C.M.R., 2021. Reproductive cycle of the mangrove oyster, *Crassostrea gasar* (Adanson, 1757), in tropical and temperate climates. Aquac. Res. 52 (3), 991–1000. https://doi.org/10.1111/are.14954.

Leistenschneider, D., Wolinski, A., Cheng, J., ter Halle, A., Duflos, G., Huvet, A., Paul-Pont, I., Lartaud, F., Galgani, F., Lavergne, É., Meistertzheim, A.-L., Ghiglione, J.-F., 2023. A critical review on the evaluation of toxicity and ecological risk assessment of plastics in the marine environment. Sci. Total Environ. 896 (March), 164955 https://doi.org/10.1016/j.scitotenv.2023.164955.

Li, J., Lusher, A.L., Rotchell, J.M., Deudero, S., Turra, A., Bråte, I.L.N., Sun, C., Shahadat Hossain, M., Li, Q., Kolandhasamy, P., Shi, H., 2019. Using mussel as a global bioindicator of coastal microplastic pollution. Environ. Pollut. 244, 522–533. https://doi.org/10.1016/j.envpol.2018.10.032.

Li, Y., Liu, C., Yang, H., He, W., Li, B., Zhu, X., Liu, S., Jia, S., Li, R., Tang, K.H.D., 2024. Leaching of chemicals from microplastics: a review of chemical types, leaching mechanisms and influencing factors. Sci. Total Environ. 906 (October 2023), 167666 https://doi.org/10.1016/j.scitotenv.2023.167666.

Lithner, D., Larsson, A., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409 (18), 3309–3324. https://doi.org/10.1016/j.scitotenv.2011.04.038.

Luo, H., Xiang, Y., He, D., Li, Y., Zhao, Y., Wang, S., Pan, X., 2019. Leaching behavior of fluorescent additives from microplastics and the toxicity of leachate to *Chlorella vulgaris*. Sci. Total Environ. 678, 1–9. https://doi.org/10.1016/j.scitotenv.2019.04.401.

Luo, H., Liu, C., He, D., Sun, J., Li, J., Pan, X., 2022. Effects of aging on environmental behavior of plastic additives: migration, leaching, and ecotoxicity. Sci. Total Environ. 849 (July), 157951 https://doi.org/10.1016/j.scitotenv.2022.157951.

Mafambissa, M., Rodrigues, M., Taimo, T., Andrade, C., Lindegart, M., Macia, A., 2023. Gametogenic cycle of the oysters *Pinctada capensis* (Sowerby III, 1890) and *Saccostrea cucullata* (Born, 1778) (Class Bivalvia) in Inhaca Island, Southern Mozambique: a subsidy for bivalve culture in the region. Diversity 15 (3). https://doi.org/10.3390/d15030361.

Mahmood, Y., Ghaffar, A., Hussain, R., 2021. New insights into Hemato-biochemical and histopathological effects of Acetochlor in bighead carp (*Aristichthys nobilis*). Pak. Vet. J. 8318 (2), 85–92. https://doi.org/10.1097/QCO.0b013e3283638104.

Mahmood, Y., Hussain, R., Ghaffar, A., Ali, F., Nawaz, S., Mehmood, K., Khan, A., 2022. Acetochlor affects bighead carp (*Aristichthys Nobilis*) by producing oxidative stress, lowering tissue proteins, and inducing genotoxicity. Biomed. Res. Int. 2022 https://doi.org/10.1155/2022/9140060.

Mai, H., Cachot, J., Brune, J., Geffard, O., Belles, A., Budzinski, H., Morin, B., 2012. Embryotoxic and genotoxic effects of heavy metals and pesticides on early life stages of Pacific oyster (*Crassostrea gigas*). Mar. Pollut. Bull. 64 (12), 2663–2670. https://doi.org/10.1016/j.marpolbul.2012.10.009.

Mai, H., Morin, B., Pardon, P., Gonzalez, P., Budzinski, H., Cachot, J.Ô., 2013. Environmental concentrations of irgarol, diuron and S-metolachlor induce deleterious effects on gametes and embryos of the Pacific oyster, *Crassostrea gigas*. Mar. Environ. Res. 89, 1–8. https://doi.org/10.1016/j.marenvres.2013.04.003.

Markich, S.J., 2021. Comparative embryo/larval sensitivity of Australian marine bivalves to ten metals: a disjunct between physiology and phylogeny. Sci. Total Environ. 789, 147988 https://doi.org/10.1016/j.scitotenv.2021.147988.

Meijer, L.J.J., van Emmerik, T., van der Ent, R., Schmidt, C., Lebreton, L., 2021. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. Sci. Adv. 7 (18), 1–14. https://doi.org/10.1126/sciadv.aaz5803.

Meng, F., Ni, Z., Tan, L., Cai, P., Wang, J., 2024. Oxidative stress and energy metabolic response of *Isochrysis galbana* induced by different types of pristine and aging microplastics and their leachates. Chemosphere 348 (238), 140755. https://doi.org/10.1016/j.chemosphere.2023.140755.

Morroni, L., Pinsino, A., Pellegrini, D., Regoli, F., 2018. Reversibility of trace metals effects on sea urchin embryonic development. Ecotoxicol. Environ. Saf. 148 (November 2017), 923–929. https://doi.org/10.1016/j.ecoenv.2017.11.013.

Morroni, L., Sartori, D., Costantini, M., Genovesi, L., Magliocco, T., Ruocco, N., Buttino, I., 2019. First molecular evidence of the toxicogenetic effects of copper on sea urchin *Paracentrotus lividus* embryo development. Water Res. 160, 415–423. https://doi.org/10.1016/j.watres.2019.05.062.

Mu, J., Wang, J., Jin, F., Wang, X., Hong, H., 2014. Comparative embryotoxicity of phenanthrene and alkyl-phenanthrene to marine medaka (*Oryzias melastigma*). Mar. Pollut. Bull. 85 (2), 505–515. https://doi.org/10.1016/j.marpolbul.2014.01.040.

Nadella, S.R., Fitzpatrick, J.L., Franklin, N., Bucking, C., Smith, S., Wood, C.M., 2009. Toxicity of dissolved Cu, Zn, Ni and Cd to developing embryos of the blue mussel (*Mytilus trossolus*) and the protective effect of dissolved organic carbon. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 149 (3), 340–348. https://doi.org/10.1016/j.cbpc.2008.09.001.

Net, S., Delmont, A., Sempéré, R., Paluselli, A., Ouddane, B., 2015. Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): a review. Sci. Total Environ. 515–516, 162–180. https://doi.org/10.1016/j.scitotenv.2015.02.013.

Noorimotlagh, Z., Mirzaee, S.A., Martinez, S.S., Rachoń, D., Hoseinzadeh, M., Jaafarzadeh, N., 2020. Environmental exposure to nonylphenol and cancer progression risk–a systematic review. Environ. Res. 184 (February) https://doi.org/10.1016/j.envres.2020.109263.

Nowland, S.J., O'Connor, W.A., Elizur, A., Southgate, P.C., 2021. Evaluating spawning induction methods for the tropical black-lip rock oyster, *Saccostrea echinata*. Aquac. Rep. 20, 100676 https://doi.org/10.1016/j.aqrep.2021.100676.

Ortiz-Zarragoitia, M., Trant, J.M., Cajaraville, M.P., 2006. Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and development of zebrafish (*Danio rerio*). Environ. Toxicol. Chem. 25 (9), 2394–2404. https://doi.org/10.1897/05-456R.1.

Osunde, I.M., Coyle, S., Tidwell, J., 2004. Acute toxicity of copper to juvenile freshwater prawns, *Macrobrachium rosenbergii*. J. Appl. Aquac. 14 (3-4), 71–79.

Pagenkopp Lohan, K.M., Hill-Spanik, K.M., Torchin, M.E., Strong, E.E., Fleischer, R.C., Ruiz, G.M., 2015. Molecular phylogenetics reveals first record and invasion of Saccostrea species in the Caribbean. Mar. Biol. 162 (5), 957–968. https://doi.org/10.1007/s00227-015-2637-5.

Pagès, J., Andréfouët, S., 2001. A reconnaissance approach for hydrology of atoll lagoons. Coral Reefs 20 (4), 409–414. https://doi.org/10.1007/s00338-001-0192-7.

Paluselli, A., Fauvelle, V., Galgani, F., Sempéré, R., 2019. Phthalate release from plastic fragments and degradation in seawater. Environ. Sci. Technol. 53 (1), 166–175. https://doi.org/10.1021/acs.est.8b05083.

Pétinay, S., Chataigner, C., Basuyaux, O., 2009. Standardisation du développement larvaire de l'oursin, *Paracentrotus lividus*, pour l'évaluation de la qualité d'une eau de mer. C. R. Biol. 332 (12), 1104–1114. https://doi.org/10.1016/j.crvi.2009.08.002.

Plastics Europe, 2022. Plastics – the facts 2022. https://plasticseurope.org/fr/knowledge-hub/plastics-the-facts-2022/.

Rajapakse, N., Silva, E., Kortenkamp, A., 2002. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. Environ. Health Perspect. 110 (9), 917–921. https://doi.org/10.1289/ehp.02110917.

Rakaj, A., Morroni, L., Grosso, L., Fianchini, A., Pensa, D., Pellegrini, D., Regoli, F., 2021. Towards sea cucumbers as a new model in embryo-larval bioassays: *Holothuria tubulosa* as test species for the assessment of marine pollution. Sci. Total Environ. 787, 147593 https://doi.org/10.1016/j.scitotenv.2021.147593.

Ramadhaniaty, M., Setyobudiandi, I., Madduppa, H.H., 2018. Morphogenetic and population structure of two species marine bivalve (Ostreidae: *Saccostrea cucullata* and *Crassostrea iredalei*) in aceh, Indonesia. Biodiversitas 19 (3), 978–988. https://doi.org/10.13057/biodiv/d190329.

Rangel-Buitrago, N., Neal, W.J., Galgani, F., 2023. Plastics in the Anthropocene: a multifaceted approach to marine pollution management. Mar. Pollut. Bull. 194 (PA), 115359 https://doi.org/10.1016/j.marpolbul.2023.115359.

Rosen, G., Rivera-Duarte, I., Bart Chadwick, D., Ryan, A., Santore, R.C., Paquin, P.R., 2008. Critical tissue copper residues for marine bivalve (*Mytilus galloprovincialis*) and echinoderm (*Strongylocentrotus purpuratus*) embryonic development: conceptual, regulatory and environmental implications. Mar. Environ. Res. 66 (3), 327–336. https://doi.org/10.1016/j.marenvres.2008.05.006.

Silva, E., Rajapakse, N., Kortenkamp, A., 2002. Something from "nothing" - eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ. Sci. Technol. 36 (8), 1751–1756. https://doi.org/10.1021/es0101227.

Sol Dourdin, T., Rivière, G., Cormier, A., Di Poi, C., Guyomard, K., Rabiller, M., Akcha, F., Bah Sadialiou, T., Le Monier, P., Sussarellu, R., 2023. Molecular and phenotypic effects of early exposure to an environmentally relevant pesticide mixture in the Pacific oyster, *Crassostrea gigas*. Environ. Pollut. 326 (March) https://doi.org/10.1016/j.envpol.2023.121472.

Staple, C.A., Adams, W.J., Parkerton, T.F., Gorsuch, J.W., 1997. Aquatic toxicity of eighteen phthalate esters. Environ. Toxicol. Chem. 16 (5), 875–891. https://doi.org/10.1002/etc.5620160507.

Stara, A., Kubec, J., Zuskova, E., Buric, M., Faggio, C., Kouba, A., Velisek, J., 2019. Effects of S-metolachlor and its degradation product metolachlor OA on marbled crayfish (*Procambarus virginalis*). Chemosphere 224, 616–625. https://doi.org/10.1016/j.chemosphere.2019.02.187.

Su, W., Zha, S., Wang, Y., Shi, W., Xiao, G., Chai, X., Wu, H., Liu, G., 2017. Benzo[a]pyrene exposure under future ocean acidification scenarios weakens the immune responses of blood clam, *Tegillarca granosa*. Fish Shellfish Immunol. 63, 465–470. https://doi.org/10.1016/j.fsi.2017.02.046.

Tabata, A., Kashiwada, S., Ohnishi, Y., Ishikawa, H., Miyamoto, N., Itoh, M., 2018. Estrogenic influences of estradiol-17β, p-nonylphenol and bis-phenol-A on Japanese Medaka (*Oryzias latipes*) at detected environmental concentrations. Water Sci. Technol. 43 (2), 109–116. https://iwaponline.com/wst/article-pdf/43/2/109/39387/109.pdf.

Ulman, A., Ferrario, J., Occhpinti-Ambrogi, A., Arvanitidis, C., Bandi, A., Bertolino, M., Bogi, C., Chatzigeorgiou, G., Çiçek, B.A., Deidun, A., Ramos-Esplá, A., Koçak, C., Lorenti, M., Martinez-Laiz, G., Merlo, G., Princisgh, E., Scribano, G., Marchini, A., 2017. A massive update of non-indigenous species records in Mediterranean marinas. PeerJ 2017 (10), 1–59. https://doi.org/10.7717/peerj.3954.

Van Metre, P.C., Alvarez, D.A., Mahler, B.J., Nowell, L., Sandstrom, M., Moran, P., 2017. Complex mixtures of pesticides in Midwest U.S. streams indicated by POCIS time-integrating samplers. Environ. Pollut. 220, 431–440. https://doi.org/10.1016/j.envpol.2016.09.085.

Wagner, M., Monclús, L., Arp, H.P.H., Groh, K.J., Løseth, M.E., Muncke, J., Wang, Z., Wolf, R., Zimmermann, L., 2024. State of the science on plastic chemicals - identifying and addressing chemicals and polymers of concern. PlastChem 181.

Wang, J., Tan, Z., Peng, J., Qiu, Q., Li, M., 2016. The behaviors of microplastics in the marine environment. Mar. Environ. Res. 113, 7–17. https://doi.org/10.1016/j.marenvres.2015.10.014.

Wessel, N., Santos, R., Menard, D., Le Menach, K., Buchet, V., Lebayon, N., Loizeau, V., Burgeot, T., Budzinski, H., Akcha, F., 2010. Relationship between PAH biotransformation as measured by biliary metabolites and EROD activity, and genotoxicity in juveniles of sole (*Solea solea*). Mar. Environ. Res. 69 (Suppl. 1), S71–S73. https://doi.org/10.1016/j.marenvres.2010.03.004.

Zalasiewicz, J., Waters, C.N., Ivar do Sul, J.A., Corcoran, P.L., Barnosky, A.D., Cearreta, A., Edgeworth, M., Gałuszka, A., Jeandel, C., Leinfelder, R., McNeill, J.R., Steffen, W., Summerhayes, C., Wagreich, M., Williams, M., Wolfe, A.P., Yonan, Y., 2016. The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. Anthropocene 13, 4–17. https://doi.org/10.1016/j.ancene.2016.01.002.

Zhang, Z., Chen, Q., Chen, B., Dong, T., Chen, M., 2023. Toxic effects of pesticides on the marine microalga *Skeletonema costatum* and their biological degradation. Sci. China Earth Sci. 66 (3), 663–674. https://doi.org/10.1007/s11430-022-1064-7.

Zhou, J., Chen, B., Cai, Z., 2015. Metabolomics-based approach for assessing the toxicity mechanisms of dibutyl phthalate to abalone (*Haliotis diversicolor supertexta*). Environ. Sci. Pollut. Res. 22 (7), 5092–5099. https://doi.org/10.1007/s11356-014-3859-7.