



## Toxicity of tire particle leachates on early life stages of keystone sea urchin species<sup>☆</sup>

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### ABSTRACT

Particles from tires are a major fraction of microplastic pollution. They contain a wide range of chemical additives that can leach into the water and be harmful to aquatic organisms. In this study, we investigated the acute toxicity of tire particle leachates in early life stages of three keystone echinoderm species (*Paracentrotus lividus*, *Arbacia lixula*, *Diadema africanum*). Embryos were exposed for 72 h to a range of leachate dilutions, prepared using a concentration of 1 g L<sup>-1</sup>. Larval growth, abnormal development, and mortality were the measured endpoints. Furthermore, we estimated the activity of glutathione S transferase (GST) and the electron transport system (ETS) in *P. lividus*. Strong concentration-dependent responses were observed in all species, though with differing sensitivity. The median effect concentrations for abnormal development in *P. lividus* and *A. lixula* were 0.16 and 0.35 g L<sup>-1</sup>, respectively. In *D. africanum*, mortality overshadowed abnormal development and the median lethal concentration was 0.46 g L<sup>-1</sup>. Larvae of *P. lividus* were significantly smaller than the control from 0.125 g L<sup>-1</sup>, while the other two species were affected from 0.5 g L<sup>-1</sup>. ETS activity did not change but there was a non-significant trend of increasing GST activity with leachate concentration in *P. lividus*. Seven organic chemicals and eight metals were detected at elevated concentrations in the leachates. While we regard zinc as a strong candidate to explain some of the observed toxicity, it can be expected that tire particle leachates exhibit a cocktail effect and other leached additives may also contribute to their toxicity. Our results emphasize the importance of multi-species studies as they differ in their susceptibility to tire particle pollution. We found negative effects at concentrations close to projections in the environment, which calls for more research and mitigation actions on these pollutants.

### 1. Introduction

Global microplastic (MP) pollution has been researched extensively in the past decade, with most studies focusing on MP ingestion. However, recent research suggests that leached additives are the primary cause of MP toxicity in aquatic organisms (Beiras et al., 2021; Manzo and Schiavo, 2022). In plastic production, thousands of chemicals are used as monomers, additives, and processing aids, with the number and amount of additives varying depending on the type and application of the plastics. Wiesinger et al. (2021) identified more than 10,000 relevant

chemicals, including over 2,400 of potential concern and around 4,100 for which no hazard classification is available. These substances are weakly bound to plastic polymers and can leach into the environment, where organisms may be exposed to them (Wiesinger et al., 2021). However, the effects of plastic-leached additives on biota and ecosystems remain poorly understood. Therefore, more research on the ecological impact of additives from different plastic materials is needed.

Tire wear particles (TWP) have recently been identified as a major source of MPs (Baensch-Baltrusch et al., 2020; Rørdland, 2019) with a potentially high risk of environmental impact (Tian et al., 2021). TWPs

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are generated by abrasion of tires against the road surface (Kole et al., 2017). Despite being mainly composed of natural and synthetic rubber polymers, elastomers like rubber have been largely ignored in MP research due to unclear definitions (Hartmann et al., 2019) and challenging characterization (Baensch-Baltruschat et al., 2020). Estimates suggest that almost six million tons of TWPs were released globally in 2016, constituting 5–10% of the global mass of plastics entering the oceans (Kole et al., 2017). Other studies indicate that TWPs are the major or one of the biggest sources of MPs in the environment (Hann et al., 2018; Lassen et al., 2015; Rødland, 2019). Besides rubber, TWPs are typically composed of carbon black, oils, plasticizers, vulcanizing agents and other additives (Kreider et al., 2010; Rodgers and Waddell, 2013; Wagner et al., 2018). Müller et al. (2022), identified 214 organic compounds in tire wear of which 145 were classified as leachable. Also, several studies found high concentrations of metals in TWP leachates (Capolupo et al., 2020; Halsband et al., 2020). While acute toxicity of TWP leachates as well as sub-lethal effects on behavior, development, feeding, reproduction, and biomarkers have been studied in copepods, rotifers, and bivalves (Capolupo et al., 2020; Halsband et al., 2020; Lehtiniemi et al., 2021; Shin et al., 2022; Tallec et al., 2022b, 2022a; Yang et al., 2022), the number of studied invertebrate species is still limited. Adverse effects of TWP leachates or the tire-derived compound 6PPD-quinone have also been reported for several fish species, resulting in impaired eye development, changes in swimming behavior and mortality (Brinkmann et al., 2022; Chang et al., 2023; Kim et al., 2023; McIntyre et al., 2021; Tian et al., 2021). However, it has also been demonstrated that sensitivity to tire-derived additives can be highly species-specific, such as for 6PPD-quinone (Brinkmann et al., 2022; Foldvik et al., 2022; Tian et al., 2021).

Sea urchins are crucial to coastal benthic communities, contributing to the resilience of coral reefs and shaping benthic rocky communities in temperate habitats through their grazing activity (Agnetta et al., 2013; Dang et al., 2020; Pearse, 2006). It can be assumed that they are exposed to plastics and associated chemicals throughout their lifecycle, since coastal areas are among the most polluted marine habitats and close to the main entry points for plastics to the ocean (Manzo and Schiavo, 2022; Thushari and Senevirathna, 2020). Early life stages of sea urchins are most sensitive to stress (Pandori and Sorte, 2019; Przeslawski et al., 2015), while being decisive for population dynamics (Thorson, 1966). Recently, a review concluded that the main impact on sea urchins stems from released additives and sorbed chemicals rather than MP particles themselves (Manzo and Schiavo, 2022). The impact of plastic leachates on sea urchins has been studied for several materials (Beiras et al., 2021, 2019; Cormier et al., 2021; Martínez-Gómez et al., 2017; Nobre et al., 2015; Oliviero et al., 2019; Piccardo et al., 2021; Rendell-Bhatti et al., 2021; Uribe-Echeverría and Beiras, 2022), but not for tire particles.

This study aims to investigate the effects of tire particle leachates on early developmental stages of three keystone sea urchin species (*Paracentrotus lividus*, *Arbacia lixula* and *Diadema africanum*). These species are dominant in coastal areas of the Canary Islands and other Macaronesian islands, and they play important ecological roles, such as grazing control of benthic algae. This can strongly affect the benthic community structure of coastal habitats since the abundance of sea urchins determines the shift between the two major habitat states: erect algal forests and coralline barrens (Agnetta et al., 2013; García-Sanz et al., 2014; Gizzi et al., 2020; Wangensteen et al., 2011). Although co-occurring, the three species differ in their traits and ecological role. While *P. lividus* and *A. lixula* inhabit the intertidal and subtidal, *D. africanum* only inhabits the subtidal and generally occurs at greater depths (Tuya et al., 2007). *P. lividus* feeds more on flashy algae, while *A. lixula* with its superior Aristotle's lantern (thus greater grazing ability) feeds on encrusting coralline algae as well as invertebrates (Agnetta et al., 2013). *D. africanum* feeds both on macroalgae and encrusting algae (Sangil et al., 2014; Tuya et al., 2001). Of the three studied species, *D. africanum* is described as the dominant herbivore, able to generate big barrens with relatively low diversity (García-Sanz et al., 2014;

Hernández et al., 2008). *P. lividus* can easily move into erect algal forests and create barren patches, which are then colonized by encrusting algae and cryptic fauna. Since this species is often characterized by cryptic behavior (meaning that it hides in crevices and stays within small patches), and more gentle grazing, it is weaker in maintaining the barren state. In contrast, *A. lixula*, less capable in creating barren patches, maintains these open areas through its more intense grazing and movement (Agnetta et al., 2013; Bonaviri et al., 2011). In the Canary Islands, *D. africanum* was for many decades the most abundant sea urchin species, creating extensive barrens, named locally “blanquiales” (white barren grounds). However, after two mass mortality events in 2009/2010 and 2018 its abundance decreased by 94–98% and many areas have shifted back to algal forests (Sangil and Hernández, 2022).

The overall hypothesis of this study was that the early developmental stages of sea urchins are negatively affected by tire leachates. Specifically, our research questions were: 1) Do tire particle leachates impair the growth, morphological development and survival of early life stages in sea urchins? 2) Does the sensitivity towards tire particle leachates differ between the three dominant sea urchin species of the Macaronesian islands? 3) Does the activity of selected biomarkers (the electron transport system (ETS) and glutathione S transferase (GST)) change under exposure to tire particle leachates?

## 2. Materials and methods

### 2.1. Animal collection and spawning

Adult sea urchins (*P. lividus*, *A. lixula*, *D. africanum*) were collected by snorkeling in Arinaga, Gran Canaria, on the day of a given exposure experiment. The animals were transported to the laboratory in cool boxes with *in situ* seawater. Spawning was triggered by injecting 1 mL of a 0.55 M potassium chloride solution through the mouth. Immediately after, individuals were placed upside down on beakers filled with filtered seawater (FSW). All FSW used in this study had a salinity of 35 psu, 20 °C, pH 8.01 and an oxygen saturation of >90%. In case of a spawning male, strands of released sperm were immediately collected from the water column with a Pasteur pipette and transferred to a glass vial on ice. In case of a spawning female, oocytes were allowed to deposit in the beaker and once spawning diminished, they were transferred to a new beaker with FSW. The active swimming of sperm was checked under a microscope (DMI1 Leica) and the quality of the oocytes (evenness of size and spherical shape) was evaluated using a stereo microscope (Leica S APO). For fertilization, the oocytes of one to three females were mixed to create an oocyte suspension of roughly 2000 mL<sup>-1</sup>. Diluted sperm of up to three males was added to the suspension and fertilization success was confirmed by observing the development of a fertilization membrane around >90% of the eggs.

### 2.2. Preparation of tire particle leachates

The leachates were obtained as described in Almeda et al. (2023) with minor modifications in the micronization process (Page et al., 2022). Briefly, micronized tire particles (<250 µm, new Imperial 145/70–13 71T-Snowdragon HP tires) were kept frozen at –20 °C until use. A suspension of 1 g L<sup>-1</sup> was prepared with FSW in a 500 mL glass bottle with a screw cap with a polytetrafluoroethylene protected seal. The bottle was completely filled and closed air-free. It was placed on a roller (LLG-UniROLLER 6 easy) at 15 rpm in an incubator at 20 °C in darkness. After 72 h, the suspension was filtered over a 0.7 µm glass fiber filter to remove the particles. The filtered liquid constituted the leachate stock solution (i.e., 100%) of which the following dilutions were prepared: 75%, 50%, 25%, 12.5% and 6.25% (corresponding to 0.75, 0.5, 0.25, 0.125 and 0.0625 g L<sup>-1</sup>). Throughout the paper, we refer to these dilutions in their equivalent mass concentrations. The pH of the leachate stock, as well as the FSW, was checked in triplicates to ensure that the pH did not differ between treatments (pH 7.87 ± 0.1 in leachates, pH

7.89 ± 0.1 in FSW, Crison GLP21).

### 2.3. Chemical analysis of leachates

Undiluted leachates were analyzed for 52 organic compounds (Table S1) and 50 elements (Table S3). Details on the used reagents are available in the supplementary material.

#### 2.3.1. Organic compounds analysis

After the preparation of the leachates, subsamples of the leachate stock solution (1 g L<sup>-1</sup>) for organic analysis were immediately frozen at -20 °C. For the analysis, leachate samples were thawed and placed on an orbital shaker for 2 h. An aliquot of 50 mL was transferred into another vessel, and 10 µL of the procedural internal standard (P-IS) solution (containing acenaphthalene-d10, chrysene-d12, phenanthrene-d10, and PCB 200) was added. The solution was left in darkness for 1 h. All samples were extracted and analyzed in triplicate. Then, they were submitted to solid-phase extraction using 200 mg (3 mL) Chromabond® C18ec columns (Macherey-Nagel, Germany) mounted in a vacuum manifold (Waters Corporation, USA). Before applying the samples, the columns were cleaned and conditioned with 2 mL of methanol and 2 mL of water/n-propanol (85:15, v/v). Next, 50 mL of the leachates were passed through the cartridge under vacuum at a flow rate of 1.5 mL min<sup>-1</sup>. The column was washed with 1 mL of the water/n-propanol solution, and dried under high vacuum for 30 min. Finally, the compounds were eluted from the column with 1 mL of dichloromethane.

Quality controls and blanks were prepared in autoclaved seawater using the same methodology. They were spiked 1 h before the extraction with the mix standard solution and/or with the P-IS solution, respectively. A 10-point calibration curve in dichloromethane ranging between 0.195 and 100 ng mL<sup>-1</sup> was used for quantification of the organic compounds.

The analysis was performed using a 7890B gas chromatography (GC) system coupled to a Triple Quad 7010 mass spectrometer (MS) (Agilent Technologies, USA). All GC parameters have been described previously (Camacho et al., 2019) and retention times, precursor, fragment ions, and collision energies for each compound are listed in the supplementary material.

#### 2.3.2. Elemental analysis

An aliquot of 40 mL of leachates from the stock solution (1 g L<sup>-1</sup>) was transferred into a 50 mL falcon tube, vortexed, and placed in an ultrasonic bath for 30 min. Once sonicated, the samples were centrifuged at 2500 rpm for 3 min. A direct dilution of the sample was made at 1:10, using a solution of nitric acid (HNO<sub>3</sub>) (2%). For this purpose, 1800 µL of 2% HNO<sub>3</sub> and an aliquot of 200 µL of the sample were added to the vial and shaken.

Two complementary calibration curves covering the range 0.03–300 ng mL<sup>-1</sup> were used. The elemental analysis was performed using an Agilent 7900 inductively coupled plasma mass spectrometer (ICP-MS) equipped with standard nickel cones and a crossflow nebulizer with a make-up gas port (×400 nebulizer, Saville Corporation, USA) for all measurements. Daily, a tuning solution consisting of a mixture of cesium, cobalt, lithium, magnesium, thallium and yttrium (Agilent Technologies) was used to optimize the ICP-MS instrument. The working conditions were assessed by considering the sensitivity of the six isotopes in counts per second, the associated relative standard deviation, oxide ratio, and double ion charges. The entire procedure has been described in detail previously (Rodríguez-Hernández et al., 2019).

### 2.4. Exposure experiment

A 72 h exposure of developing embryo/larvae to the six leachate dilutions plus a control (FSW) was conducted. Small (68 mL) glass bottles were filled with 30 mL of the respective dilution or FSW and fertilized oocytes were added to reach a density of 30 mL<sup>-1</sup>. All

treatments were done in triplicate. The bottles were loosely covered with aluminum foil and placed in an incubator at 20 °C in darkness.

For *P. lividus*, additional exposure bottles of four treatments (25%, 12.5%, 6.25% and control) were prepared for enzymatic assays (see below). In this case, the volume per bottle was 200 mL with a density of 50 fertilized oocytes per mL to obtain enough biomass for later analysis. Except for this difference, the bottles were prepared and treated in the same way as the small ones.

After 72 h, we quantified the percentage of mortality and abnormal development (developmental arrest, strong skeletal deformations, missing arms; see Rendell-Bhatti et al., 2021). In a subsample of 1 mL, the total number of larvae, and the number of dead and abnormally developed larvae was determined under a stereo microscope. In parallel, 12 mL of each bottle were transferred to a falcon tube with the addition of four drops of 10% formalin. After the larvae had settled, they were transferred to a glass slide and images were taken on a microscope with a connected camera (Leica Flexacam C3). For each replicate, the length (from the apical end to the connection between the anterolateral arms) of 20 randomly selected larvae was measured using the software ImageJ.

The total content of the 200 mL bottles was filtered on a 50 µm plankton mesh and the larvae were transferred to a pre-weighed 2 mL Eppendorf tube using a fine spatula. Immediately, the wet weight was recorded and the tube was frozen at -80 °C.

### 2.5. Biochemical analysis of GST and ETS activity

Proteins were extracted by homogenizing the whole filtrate of larvae in phosphate buffer (0.1 M, pH 7.8) with 0.2% Triton X100 using an ultrasonic tissue homogenizer. The oxidative stress marker glutathione S transferase (GST) was assayed in protein extracts at 340 nm on a BMG-FLUOstar omega microplate reader following the microplate-adapted procedure (Frasco and Guilhermino, 2002) from the original work of Habig et al. (1974). The activity of the electron transport system (ETS) was measured in larvae homogenates following the production of INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride)-formazan at 490 nm using a Cary series UV-VIS spectrophotometer, according to Owens and King (1975) and the modified procedure of Gómez et al. (1996).

Enzyme activity was calculated with a molar extinction coefficient of 9.6 mM<sup>-1</sup> cm<sup>-1</sup> and 14.9 mM<sup>-1</sup> cm<sup>-1</sup> for GST and ETS activity, respectively. The activity of ETS was then stoichiometrically related to oxygen consumption (Hernández-León and Gómez, 1996). The Pierce BCA protein assay kit (ThermoFisher Scientific) was used to measure the protein concentration of larvae homogenates, and results were expressed as specific activity (µmol mL<sup>-1</sup> mg of protein<sup>-1</sup> for GST and µL O<sub>2</sub> h<sup>-1</sup> mg of protein<sup>-1</sup> for ETS activity).

### 2.6. Data analysis

All statistical analyses were performed using the software R (version 3.6.3, R Core Team, 2022). Analysis of variance (ANOVA) was used to test for differences between means of treatments in the size of larvae, enzymatic activity, and mortality (for *P. lividus* and *A. lixula*). The assumptions of normality and homogeneity of variances were tested with the Shapiro-Wilks and Fligner-Killeen test, respectively. As a posthoc test Tukeys HSD was used. Dose-response curves were modeled with a four-parameter log-logistic function (drc R package, Ritz et al., 2015) with their associated 95% interval calculated with Delta's method, using abnormal development data of *P. lividus* and *A. lixula* and mortality data of *D. africanum*. The absolute half maximal effective dilution (ED<sub>50</sub>, *P. lividus* and *A. lixula*) and absolute half maximal lethal dilution (LD<sub>50</sub>, *D. africanum*) were calculated using respective models. Based on this, the estimated LD<sub>50</sub> and ED<sub>50</sub> (%) are expressed in their equivalent mass concentrations in g of tire particles L<sup>-1</sup> (LC<sub>50</sub> and EC<sub>50</sub>) to facilitate the comparison with other toxicity studies and the evaluation of the risk considering the environmental concentrations of TWPs. LC<sub>50</sub> and EC<sub>50</sub>

values were also used to determine and rank species sensitivity. Lethal effects (reflected in a  $LC_{50}$ ) were ranked as more severe, i.e. implying a higher sensitivity, than sub-lethal effects (reflected in an  $EC_{50}$ ).

### 3. Results and discussion

#### 3.1. Tire particle leachates affect normal development and survival of sea urchin larvae

We found clear concentration-dependent effects of tire particle leachates on all species. In the controls, all individuals were alive at the end of the exposure and very few showed signs of abnormal development (2.2% of *P. lividus*, 3.8% of *A. lixula* and 4.5% of *D. africanum*, respectively) (Fig. 1). In *P. lividus*, the fraction of normally developed larvae steadily decreased with increasing leachate concentration with a minimal percentage of 26.7% normally developed larvae at  $0.25 \text{ g L}^{-1}$ . From a leachate concentration of  $0.5 \text{ g L}^{-1}$  on, no normally developed larvae were found. At the same time, mortality was observed from this concentration and up, with a maximum level of 36.9% mortality in the undiluted leachate ( $1 \text{ g L}^{-1}$ ) (Fig. 1A). In contrast to *P. lividus*, both *A. lixula* and *D. africanum* showed high levels of normal development (79–96% and 89–96%, respectively) until a leachate concentration of  $0.25 \text{ g L}^{-1}$ . However, from  $0.25$  to  $0.5 \text{ g L}^{-1}$  both species displayed a marked increase in abnormal development and mortality. For *A. lixula*, the majority of larvae was abnormally developed from a leachate concentration of  $0.5 \text{ g L}^{-1}$  on and there was a marked increase in dead larvae (14.3% at  $0.5 \text{ g L}^{-1}$  to 24.7% at  $1 \text{ g L}^{-1}$ ) (Fig. 1B). Mortality of larvae started appearing at lower leachate concentrations for *D. africanum* (from  $0.06 \text{ g L}^{-1}$ ) but increased dramatically from 6.7 to 63.7% dead larvae at  $0.25$  and  $0.5 \text{ g L}^{-1}$ , respectively. In the two highest concentrations, all larvae were dead at the end of the exposure (Fig. 1C).

The percentage of abnormally developed larvae was used to plot dose-response curves and calculate  $EC_{50}$  values for *P. lividus* and *A. lixula* (Fig. 2). After 72 h, normally developed larvae had reached a four-arm pluteus stage (Fig. 2B). Malformations were often very pronounced, including malformed or missing arms, which indicates impaired skeleton development. Similar phenotypes were observed when *P. lividus* was exposed to leachates of PVC, beached nurdles and biobeads (Rendell-Bhatti et al., 2021). The  $EC_{50}$  values for *P. lividus* and *A. lixula* were  $0.158 \text{ g L}^{-1}$  and  $0.345 \text{ g L}^{-1}$ , respectively, indicating a higher sensitivity of the former.

The effects of tire leachates on early development of marine invertebrates have so far only been studied in bivalves. Capolupo et al. (2020) observed abnormal development in the Mediterranean mussel *M. galloprovincialis* after 48 h with an  $EC_{50}$  of  $1.8 \text{ g L}^{-1}$ . After 36 h exposure, the embryonic development of oysters (*C. gigas*) was

significantly impaired at a concentration of  $10 \text{ g L}^{-1}$  (Tallec et al., 2022b). These effect concentrations are one to two orders of magnitude higher than our findings, which suggests that sea urchin larvae are more sensitive to tire particle leachates than bivalve larvae. Differences can, however, also be related to the individual composition and concentrations of chemicals in the leachates. This depends on the used tire material and may also be influenced by the used protocol for preparing the leachates (e.g., tire particle size, particle concentration, incubation time). Thus, standardizing the protocols in leachate studies as proposed by Almeda et al. (2023) is an important step to increase comparability.

For *D. africanum*, effects on development were overridden by mortality at higher leachate concentrations. Since dead larvae had usually started degrading by the end of the experiment, it was not possible to determine their phenotype (normally vs. abnormally developed) with certainty. Instead of an  $EC_{50}$ , we therefore calculated a  $LC_{50}$ , which was  $0.456 \text{ g L}^{-1}$  (Fig. 2D). As mentioned above, some degree of mortality was also observed for the other two species, but nowhere near those of *D. africanum*. Since lethal effects are more severe than sub-lethal effects, *D. africanum* was the most sensitive of the tested species. The observed differences between species clearly demonstrate that responses are highly species-specific and it could indicate differing stress response mechanisms or uptake of chemicals among species.

Effects of tire particle leachates on survival have also been found for other marine zooplankton. Reported  $LC_{50}$  values are  $47.5 \text{ g L}^{-1}$  for larvae of *M. galloprovincialis* (Capolupo et al., 2020),  $5.34 \text{ g L}^{-1}$  for the copepod *Tigriopus japonicus* (Yang et al., 2022),  $35 \text{ g L}^{-1}$  and  $<5 \text{ g L}^{-1}$  for the copepods *Calanus* and *Acartia* respectively (Halsband et al., 2020), and  $0.6 \text{ g L}^{-1}$  for the rotifer *Brachionus plicatilis* (Shin et al., 2022). Similar to our  $EC_{50}$  findings, the obtained  $LC_{50}$  value is considerably lower than most reported ones in the literature. Although differences in  $LC_{50}/EC_{50}$  can be due to differences in the methodology as described above, our results indicate that early life stages of sea urchins are very sensitive to tire particle leachates compared to other zooplankton. This is in accordance with sea urchin embryos and larvae generally being acknowledged as highly sensitive to pollutants and therefore being used as standard test organisms in ecotoxicology (Environment Canada, 2011; Morrioni et al., 2016). However, the most sensitive species will depend on the pollutant and it is important to study effects in a range of organisms (Bellas et al., 2005). The importance of multi-species studies has also been demonstrated in the work with tire leachates and fish. For instance, McIntyre et al. (2021) found acute toxicity of TWP leachates to coho (*Oncorhynchus kisutch*) but not closely related chum salmon (*O. keta*). Also, the tire-derived chemical 6PPD-quinone resulted in mortality of some fish species, but not others (Tian et al., 2021; Brinkmann et al., 2022; Foldvik et al., 2022). In line with our observations, this highlights the high species-specificity towards tire leachates and

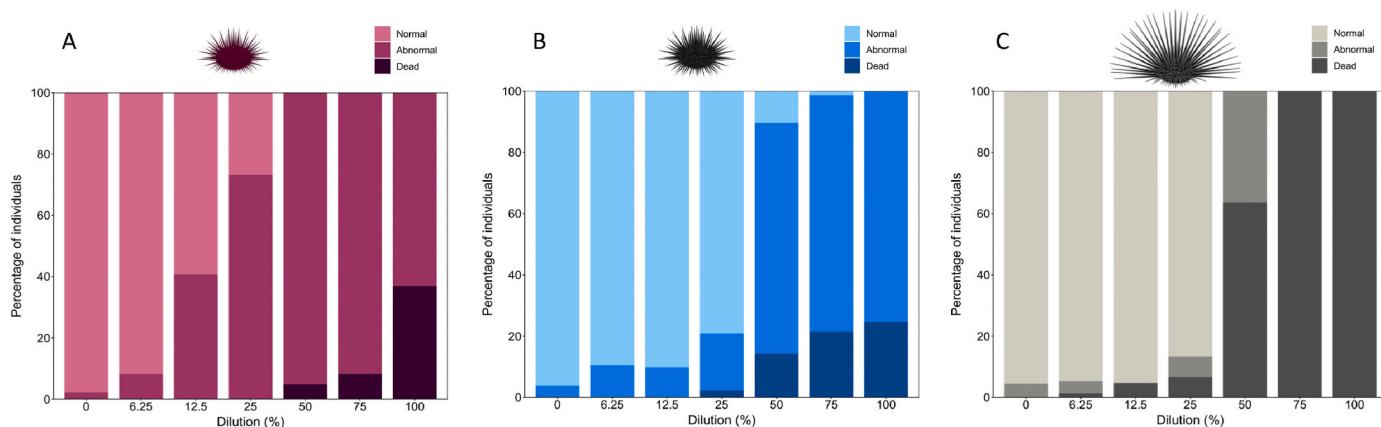
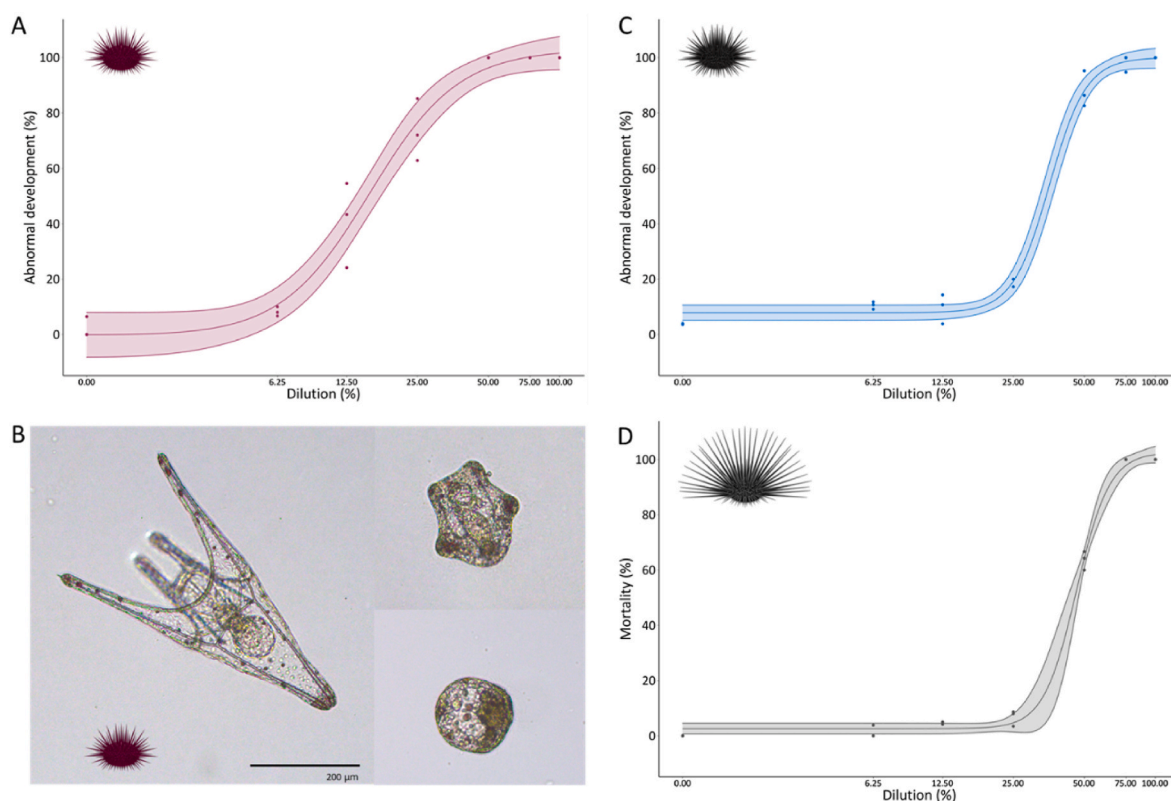


Fig. 1. Percentage of larvae of *Paracentrotus lividus* (A), *Arbacia lixula* (B) and *Diadema africanum* (C) that were normally developed, abnormally developed or dead at the end of 72 h exposure to a range of dilutions of tire particle leachates (100% =  $1 \text{ g L}^{-1}$ ).



**Fig. 2.** Dose-response curve of the percentage of abnormally developed larvae (out of all live larvae at the end of 72 h exposure) of *Paracentrotus lividus* (A) and *Arbacia lixula* (C) at different dilutions of tire particle leachates (100% = 1 g L<sup>-1</sup>). B. Microscope images of a normally developed larva (left, control treatment) and abnormally developed larvae (right, 25% treatment) of *P. lividus* after 72 h of exposure. D. Dose-response curve of the percentage of dead larvae of *Diadema africanum* at different dilutions of car tire leachates. All curves (A, C, D) represent the mean and the 95% confidence interval.

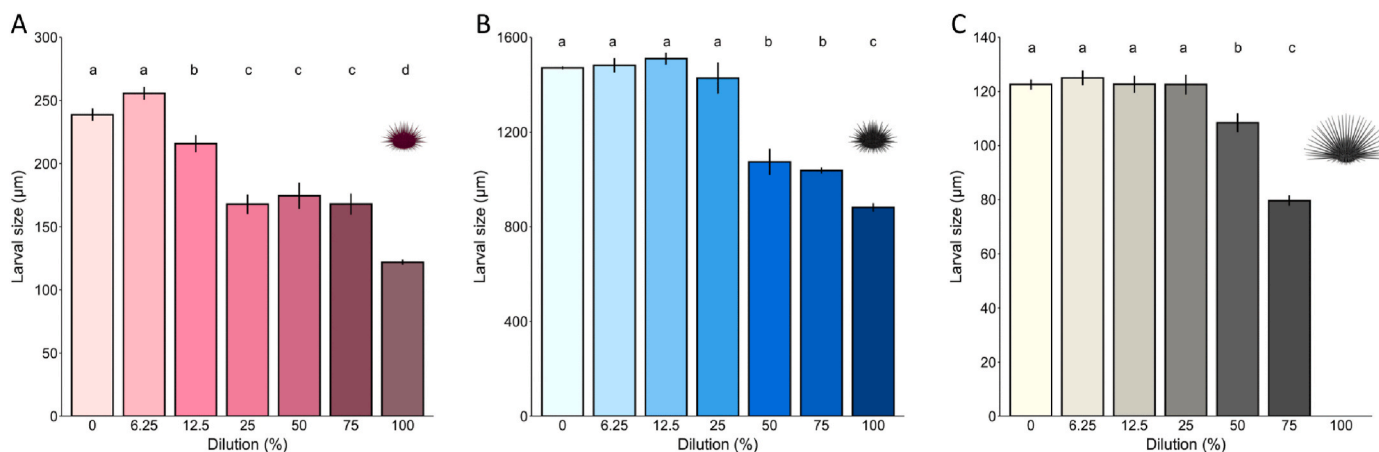
associated chemicals.

### 3.2. Impact of tire particle leachates on larval growth

The larvae of all species exhibited a concentration-dependent decrease in body size (Fig. 3). This was most pronounced for *P. lividus*, for which the reduction in comparison to the control was up to 49% (Fig. 3A), while this was 44% for *A. lixula* (Figs. 3B) and 35% for *D. africanum* (at 0.75 g L<sup>-1</sup> since larvae in the undiluted leachate (1 g L<sup>-1</sup>) had fully degraded by the end of the experiment, Fig. 3C). For both

*A. lixula* and *D. africanum*, larvae were significantly smaller than the control from a concentration of 0.5 g L<sup>-1</sup> on. These are the same leachate concentrations at which marked changes in abnormal development and survival were observed (Fig. 1B and C). In contrast, larval size of *P. lividus* already decreased significantly by 10% at 0.125 g L<sup>-1</sup>. The high sensitivity of *P. lividus* here is in line with the findings of abnormal development.

A decrease in larval size of *P. lividus* was also found with leachates of environmental MPs, though from a concentration of 3.3 g L<sup>-1</sup> (Cormier et al., 2021). Furthermore, leachates of PVC plastic toys reduced larval



**Fig. 3.** Mean size  $\pm$  sd of the larvae of *Paracentrotus lividus* (A), *Arbacia lixula* (B) and *Diadema africanum* (C) after 72 h exposure to different dilutions of tire particle leachates (100% = 1 g L<sup>-1</sup>). 20 individuals were measured per replicate. Small letters indicate the results of Tukey's HSD posthoc test. Groups that do not share the same letter are significantly different from each other.

growth, with EC<sub>50</sub> values between 3.3 and 14.3 g L<sup>-1</sup> (Oliviero et al., 2019). In juvenile oysters it was found that exposure to TWP leachates reduced their scope for growth by 57% at a concentration as low as 1 mg L<sup>-1</sup> due to reductions in clearance and respiration rates (Tallec et al., 2022a). Since we performed this experiment in the absence of food, differential feeding cannot be a cause for the observed decrease in larval growth.

### 3.3. Enzymatic responses to tire particle leachates

Glutathione S transferase (GST) activity tended to increase in the leachate-exposed larvae of *P. lividus* compared to the control (Fig. 4A), reaching approximately twice the activity at a leachate concentration of 0.25 g L<sup>-1</sup>. This well-known enzyme involved in detoxification is often used as a biomarker of environmental contamination as it facilitates the elimination of xenobiotic compounds through catalyzing their conjugation to the antioxidant glutathione (Cunha et al., 2005). Thus, the observed trend could indicate that a detoxification response involving GST was induced by tire particle leachates, although the increase was not statistically significant ( $p = 0.07$ ). Other studies report that the GST activity of *P. lividus* larvae can be induced, inhibited, or unaffected depending on the nature and the intensity of the chemical stress (Bellas et al., 2022; el Idrissi et al., 2022). Taking together our observations and reports in the literature, GST can serve as a sub-lethal endpoint in exposure studies with pollutants and may give insights into molecular processes. However, more work is needed to confirm the response of GST to tire particle leachates and other plastic-associated chemicals in echinoderm larvae.

The activity of the electron transport system (ETS) (index of respiratory activity) ranged from 0.4 to 0.9  $\mu\text{mol O}_2 \text{ h}^{-1} \text{ mg}^{-1}$  in larvae of *P. lividus*, with no clear trend in relation to exposure condition (Fig. 4B). No significant effect of the leachate was found. Values were in accordance with those reported by Tomšić et al. (2011) in early stages of *P. lividus*. The absence of a response in this parameter could indicate that the respiration rate remained constant while maintenance costs might have increased due to detoxification processes (e.g., GST induction). Therefore, larvae may either have reduced energy invested in maturation and structural growth to favor maintenance cost (Jager et al., 2016), or increased the energy produced by anaerobic means. The former could partly explain the observed decrease in larval growth and impairments in development.

### 3.4. Potential drivers of chemical toxicity

Leachates are highly complex mixtures of chemicals (Müller et al., 2022). Thus, it is very challenging to determine which compound(s) cause the observed effects. Most likely, the effects are a result of the presence and interactions between multiple chemicals, which hampers interpretation. For instance, Yang et al. (2022) observed an antagonistic effect of zinc and benzothiazole (a common organic chemical in tires) on copepods. Furthermore, the results from our chemical analysis cannot be generalized since the chemical composition of the tire material and its leachate depends on many factors, including tire age, brand, and the lixiviation protocol.

The chemical analysis of our leachates revealed elevated concentrations of seven organic chemicals and eight metals (Table 1). Among the organics, pyrene (0.48  $\mu\text{g L}^{-1}$ ), tris (2-chloroisopropyl)phosphate (0.43  $\mu\text{g L}^{-1}$ , TCPP) and naphthalene (0.22  $\mu\text{g L}^{-1}$ ) exhibited the highest concentrations. In a previous study, pyrene and naphthalene were found to have a low toxicity to larvae of *P. lividus* with an EC<sub>10</sub> and EC<sub>50</sub> value of 68.8  $\mu\text{g L}^{-1}$  and 4.7 mg L<sup>-1</sup>, respectively (Bellas et al., 2008). These are two and four orders of magnitude above our detected concentrations. Our observed effects are therefore unlikely to be caused by pyrene or naphthalene, at least for this species. TCPP is a common additive (flame retardant) in plastics and considered a chemical of emerging concern. Very little toxicity data on marine invertebrates is available to date. In adults of *P. lividus*, TCPP was found to affect the gonad development at 1  $\mu\text{g L}^{-1}$  and above (Campoy-López et al., 2020). This is more than double the concentration in our tire particle leachate. However, early life stages are often more sensitive than adults and the toxicity of TCPP to sea urchin embryos and larvae remains to be studied.

The analysis of metals revealed elevated concentrations of barium and zinc in high concentrations (88.43 and 43.98  $\mu\text{g L}^{-1}$ , respectively), nickel, manganese, and iron in low concentrations (2–8  $\mu\text{g L}^{-1}$ ), chromium, lead, and copper in very low concentrations (<1  $\mu\text{g L}^{-1}$ ). The elevated concentration of zinc is in accordance with other studies that found zinc as the most prominent metal in tire particle leachates (Capolupo et al., 2020; Halsband et al., 2020; Tallec et al., 2022b). This is not surprising as zinc is a common additive in tires, serving as a vulcanization activator (Wik and Dave, 2009). Zinc was identified as the main toxicant of TWP leachates for the copepod *T. japonicus* (Yang et al., 2022). It is also known to be toxic to sea urchin larvae, which is likely due to a disruption of calcium homeostasis (Tellis et al., 2014). Radenac

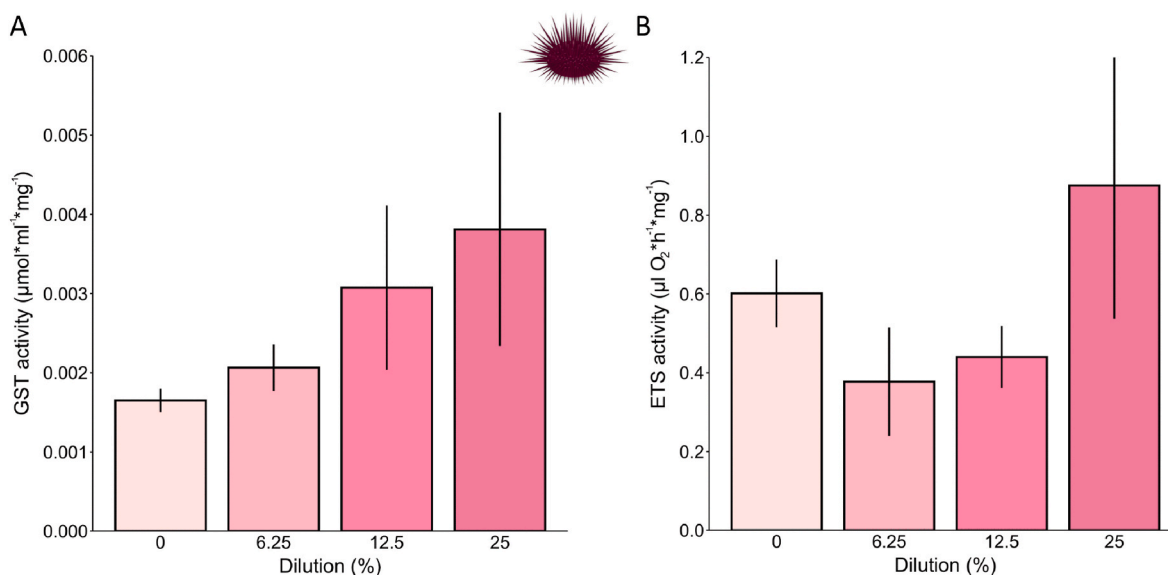


Fig. 4. Mean activity  $\pm$  sd of glutathione S transferase (GST, A) and the electron transport system (ETS, B) in larvae of *Paracentrotus lividus* after 72 h exposure to different dilutions of tire particle leachates (100% = 1 g L<sup>-1</sup>). Enzymatic activities are standardized by the mass of protein in each sample.

**Table 1**

Chemical composition of the undiluted tire particle leachates (100%, 1 g L<sup>-1</sup>) and of the filtered seawater. All organic compounds and metals are shown for which concentrations in the leachate exceeded those of the blank. All values in µg L<sup>-1</sup>. a, b, c indicate replicates.

Compound/element	Blank Seawater		Tire particle leachate			
	a	b	a	b	c	mean ± sd
<b>Organic compounds</b>						
Triethylphosphate	n.d.	n.d.	0.03	0.03	0.04	0.03 ± 0.00
Triisobutylphosphate	0.02	n.d.	0.03	0.03	0.03	0.03 ± 0.00
Tris (2-chloroethyl) phosphate	n.d.	n.d.	0.10	0.09	0.10	0.10 ± 0.01
Tris (2-chloroisopropyl) phosphate	0.02	n.d.	0.42	0.43	0.45	0.43 ± 0.02
Acenaphthene	n.d.	n.d.	0.14	0.11	0.13	0.13 ± 0.01
Naphthalene	0.04	n.d.	0.21	0.21	0.25	0.22 ± 0.02
Pyrene	n.d.	n.d.	0.37	0.48	0.58	0.48 ± 0.10
<b>Metals</b>						
52 Cr	0.00	0.12	0.57	0.77	0.88	0.74 ± 0.16
55 Mn	0.00	0.00	3.45	4.80	4.04	4.10 ± 0.68
56 Fe	2.10	0.00	1.89	1.81	4.16	2.62 ± 1.33
60 Ni	0.00	0.00	7.51	8.45	8.86	8.27 ± 0.69
63 Cu	0.00	0.00	0.62	0.54	0.50	0.56 ± 0.06
66 Zn	11.20	4.71	25.67	57.55	48.73	43.98 ± 16.46
137 Ba	9.36	9.66	76.90	97.12	91.28	88.43 ± 10.41
208 Pb	0.00	0.08	0.80	0.75	0.62	0.72 ± 0.09

et al. (2001) found substantial bioaccumulation of zinc in larvae of *P. lividus* and observed 100% abnormal development at 250 µg L<sup>-1</sup>. This roughly corresponds to the zinc concentration in our 25% (0.25 g L<sup>-1</sup>) treatment. Novelli et al. (2003) reported an EC<sub>50</sub> of 49 µg L<sup>-1</sup> for the 72 h development of *P. lividus*. Zinc is therefore very likely to explain some of our observations. Reported EC<sub>50</sub> values for other sea urchin larvae are 96.9 µg L<sup>-1</sup> (Phillips et al., 2003) and 151 µg L<sup>-1</sup> (Nadella et al., 2013) for *Strongylocentrotus purpuratus*, and 27.7 µg L<sup>-1</sup> for *Evechinus chloroticus* (Rouchon and Phillips, 2017). These results again highlight the species-specific sensitivity of sea urchin larvae. The effect of zinc has also been found to differ in mixtures with other metals, showing that both antagonistic and synergistic effects are possible (Nogueira et al., 2021; Phillips et al., 2003; Rouchon and Phillips, 2017). The binary mixture of zinc and nickel for instance strongly affected Ca<sup>2+</sup> influx in *S. purpuratus* (Nogueira et al., 2021). The same study also reported strong effects of nickel and the zinc-nickel mixture on larval development. This was observed at a nickel concentration of 5 µg L<sup>-1</sup>, which roughly corresponds to our 50% (0.5 g L<sup>-1</sup>) treatment. Therefore, nickel as well is a strong candidate to have caused some of the observed effects. Barium, the most highly concentrated metal in our leachates, is known to be deposited in the skeleton (Moore, 1991) but few studies have investigated the toxicity on marine invertebrate larvae. Spangenberg and Cherr (1996) observed abnormal larval development of *Mytilus californianus* at concentrations between 200 and 900 µg L<sup>-1</sup>, more than twice our concentration. Lethal effects on embryos of the yellow crab (*Cancer anthonyi*) were observed at 100 mg L<sup>-1</sup> (Macdonald et al., 1988). Although barium therefore seems unlikely to be a driver for the observed toxicity, its effect on sea urchin larvae remains to be studied.

As the tire particle leachates are a complex mixture of chemicals, it is almost certain that more compounds were present in our leachates than those we detected with target analysis, resulting in potentially unknown effect mechanisms. As a next step we therefore suggest using a method coupling chemical fractionation associated with bio-tests, such as Effect-Directed Analysis, to identify chemicals that individually or associated with others are responsible for the observed toxicity (Burgess et al., 2013).

### 3.5. Ecological implications

Planktonic larvae are decisive for the population dynamics of benthic invertebrate populations. They already face high levels of mortality due to predation and transport to unsuitable habitats (Thorson, 1950). If their development is additionally impaired by chemical pollution, this will decrease the chance of successful recruitment. The three studied species of echinoids play key roles in the dynamics of benthic coastal rocky ecosystems as major grazers of benthic algae. Negative impacts on their abundance through a decrease in recruitment would directly affect the degree of herbivory in those coastal benthic ecosystems, potentially changing the dynamics of algal forests and coralline barrens (Agnetta et al., 2013). This would have consequences for the whole benthic community which differs between these two states. At low concentrations, *P. lividus* was the most affected species as it was already significantly impaired at a leachate concentration of 0.125 g L<sup>-1</sup>. *A. lixula* and *D. africanum* were not affected at this level. A reduction of *P. lividus* could result in fewer barren patches within algal forests, decreasing heterogeneity and the habitat for associated encrusting and cryptic organisms. At higher concentrations of leachates (from 0.5 g L<sup>-1</sup>), *D. africanum* was most susceptible, showing high levels of larval mortality. Such a 'high pollution scenario' could threaten the occurrence of this species locally, especially after the recent mass mortalities (Sangil and Hernández, 2022). However, these leachate concentrations are unlikely in the water column based on current knowledge.

The cause for the observed difference in sensitivity between species is difficult to determine. Although not quantified, we observed a marked difference in egg size and color. While those of *P. lividus* and *A. lixula* only differed in color (orange vs. purple), *D. africanum* had substantially smaller and paler eggs, even though similar egg sizes for all three species have been reported (Byrne, 1990; George et al., 1990; Hernández et al., 2020). Intraspecific variation can be related to genetic differences or environmental influences (Moran and McAlister, 2009). Since egg size/volume is related to energy content (McAlister and Moran, 2012; Moran and McAlister, 2009), the larvae of *D. africanum* may have had lower energy reserves, potentially explaining the stronger response in mortality. The coloration of eggs is related to different pigments, which can have antioxidant properties such as astaxanthin in *A. lixula* (Galasso et al., 2018; Limatola et al., 2021). However, the role of pigments in the stress tolerance of sea urchin embryos and larvae is unclear. Different sensitivity of species that live in the same habitat could ultimately lead to changes in the community structure in polluted areas.

Field data on the concentration of tire particles in coastal environments is still very scarce, making it difficult to evaluate their ecological risk. Wik and Dave (2009) estimated a predicted environmental TWP concentration in surface waters of up to 56 mg L<sup>-1</sup>. Based on these data, the range of equivalent concentrations used in our exposure concentrations (0.06–1.0 g L<sup>-1</sup>) are environmentally relevant, particularly after runoffs events in coastal shallow waters. Concentrations of TWPs of 125 mg L<sup>-1</sup> and above are currently unlikely in marine waters. However, it is important to identify local point sources. For instance, coastal areas with heavy traffic and/or the release of untreated storm runoff water can be major entry sources for TWPs. Furthermore, the growing evidence for detrimental effects of leachates from tires questions their intentional deployment, for instance, to build artificial reefs and to be used in ports (Collins, 2021). As with other MPs, TWPs are expected to sink to the seafloor reaching higher concentrations than in the water column.

There, they may continue leaching their toxic chemicals into the water. Leads and Weinstein (2019) found up to 2.3 mg TWP<sub>s</sub> kg<sup>-1</sup> in subtidal sediments, 0.29 mg m<sup>-2</sup> in intertidal sediments and 0.01 mg L<sup>-1</sup> in the sea surface microlayer of the Charleston Harbor Estuary, USA. Other studies reported TWP mass concentrations of up to 14.5 g L<sup>-1</sup> in snow meltwater (Rødland et al., 2022), 150 mg g<sup>-1</sup> in gully pot sediment (Mengistu et al., 2021), 155 mg g<sup>-1</sup> in river sediments (Wik and Dave, 2009) and 280 mg L<sup>-1</sup> in stormwater runoff (2–125 μm TWP<sub>s</sub>, Järnskog et al., 2022).

There is still a high level of uncertainty concerning the impact of TWP<sub>s</sub> on marine ecosystems. This is complicated by the fact that chemical composition differs between brands and depends on the use time and weathering state of the material (Halsband et al., 2020; Kim et al., 2023). As mentioned above, this hampers the comparability between leachate studies and making predictions for natural ecosystems based on their results. Furthermore, leached chemicals can be transformed in the environment, resulting in enhanced toxicity (Tian et al., 2021). Therefore, data on the concentration, persistence and fate of TWP<sub>s</sub> as well as their associated chemicals in the environment is urgently needed to better evaluate the potential impacts of these pollutants on aquatic biota.

#### 4. Conclusions

Early life stages of three sea urchin species were negatively affected by acute exposure to tire particle leachates, resulting in reduced growth, abnormal development, and mortality. The sensitivity towards the leachates differed among species. Overall, *D. africanum* was the most sensitive species with high levels of mortality (LC<sub>50</sub> 0.46 g L<sup>-1</sup>), followed by *P. lividus* (EC<sub>50</sub> 0.16 g L<sup>-1</sup>) and *A. lixula* (EC<sub>50</sub> 0.35 g L<sup>-1</sup>) based on the abnormal development of the latter two. There was however a threshold for *D. africanum* at a leachate concentration of around 0.5 g L<sup>-1</sup>, below which this species did not show effects. At these low concentrations, *P. lividus* was most strongly impacted. This reflects the importance of conducting multi-species studies to determine the toxic effects of contaminants on natural marine communities. The three species play different roles in shaping coastal benthic habitats through their grazing activity and changes in their fitness or abundance can have profound consequences for the entire benthic community. The responses were observed at concentrations that are slightly above currently measured and predicted levels. However, data on TWP<sub>s</sub> and their leached chemicals in the marine environment is still extremely limited, and more research is urgently needed to evaluate the impacts of TWP pollution on marine ecosystems.

#### Author statement

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#### 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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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2023.122453>.

#### References

- Agnetta, D., Bonaviri, C., Badalamenti, F., Scianna, C., Vizzini, S., Gianguzza, P., 2013. Functional traits of two co-occurring sea urchins across a barren/forest patch system. *J. Sea Res.* 76, 170–177. <https://doi.org/10.1016/j.seares.2012.08.009>.
- Almeda, R., Gunaalan, K., Alonso-López, O., Vilas, A., Clérandeau, C., Loisel, T., Nielsen, T.G., Cachot, J., Beiras, R., 2023. A protocol for lixiviation of micronized plastics for aquatic toxicity testing. *Chemosphere* 333, 138894. <https://doi.org/10.1016/j.chemosphere.2023.138894>.
- Baensch-Baltruschat, B., Kocher, B., Stock, F., Reifferscheid, G., 2020. Tyre and Road Wear Particles (TRWP) - A Review of Generation, Properties, Emissions, Human Health Risk, Ecotoxicity, and Fate in the Environment. *Science of the Total Environment*. <https://doi.org/10.1016/j.scitotenv.2020.137823>.
- Beiras, R., Tato, T., López-Ibáñez, S., 2019. A 2-Tier standard method to test the toxicity of microplastics in marine water using *Paracentrotus lividus* and *Acartia clausi* larvae. *Environ. Toxicol. Chem.* 38, 630–637. <https://doi.org/10.1002/etc.4326>.
- Beiras, R., Verdejo, E., Campoy-López, P., Vidal-Liñán, L., 2021. Aquatic toxicity of chemically defined microplastics can be explained by functional additives. *J. Hazard Mater.* 406, 124338. <https://doi.org/10.1016/j.jhazmat.2020.124338>.
- Bellas, J., Beiras, R., Mariño-Balsa, J.C., Fernández, N., 2005. Toxicity of organic compounds to marine invertebrate embryos and larvae: a comparison between the sea urchin embryogenesis bioassay and alternative test species. *Ecotoxicology* 14, 337–353. <https://doi.org/10.1007/s10646-004-6370-y>.
- Bellas, J., Saco-Álvarez, L., Nieto, Ó., Beiras, R., 2008. Ecotoxicological evaluation of polycyclic aromatic hydrocarbons using marine invertebrate embryo-larval bioassays. *Mar. Pollut. Bull.* 57, 493–502. <https://doi.org/10.1016/j.marpolbul.2008.02.039>.
- Bellas, J., Rial, D., Valdés, J., Vidal-Liñán, L., Bertucci, J.I., Muniategui, S., León, V.M., Campillo, J.A., 2022. Linking biochemical and individual-level effects of chlorpyrifos, triphenyl phosphate, and bisphenol A on sea urchin (*Paracentrotus lividus*) larvae. *Environ. Sci. Pollut. Control Ser.* 29, 46174–46187. <https://doi.org/10.1007/s11356-022-19099-w>.
- Bonaviri, C., Vega Fernández, T., Fanelli, G., Badalamenti, F., Gianguzza, P., 2011. Leading role of the sea urchin *Arbacia lixula* in maintaining the barren state in southwestern Mediterranean. *Mar. Biol.* 158, 2505–2513. <https://doi.org/10.1007/s00227-011-1751-2>.
- Brinkmann, M., Montgomery, D., Selinger, S., Miller, J.G.P., Stock, E., Alcaraz, A.J., Challis, J.K., Weber, L., Janz, D., Hecker, M., Wiseman, S., 2022. Acute toxicity of the tire rubber-derived chemical 6PPD-quinone to four fishes of commercial, cultural, and ecological importance. *Environ. Sci. Technol. Lett.* 9, 333–338. <https://doi.org/10.1021/acs.estlett.2c00050>.
- Burgess, R.M., Ho, K.T., Brack, W., Lamoree, M., 2013. Effects-directed analysis (EDA) and toxicity identification evaluation (TIE): complementary but different approaches for diagnosing causes of environmental toxicity. *Environ. Toxicol. Chem.* 32, 1935–1945. <https://doi.org/10.1002/etc.2299>.
- Byrne, M., 1990. Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. *Mar. Biol.* 104, 275–289.
- Camacho, M., Herrera, A., Gómez, M., Acosta-Dacal, A., Martínez, I., Henríquez-Hernández, L.A., Luzzardo, O.P., 2019. Organic pollutants in marine plastic debris from Canary Islands beaches. *Sci. Total Environ.* 662, 22–31. <https://doi.org/10.1016/j.scitotenv.2018.12.422>.
- Campoy-López, P., Pereira-Pinto, E., Mantilla-Aldana, L., Beiras, R., 2020. Effects of ethinylestradiol (EE2) and an organophosphorus flame retardant (TCPP) on gonadal maturation in the sea urchin, *Paracentrotus lividus*. *J. Mar. Sci. Eng.* 8, 611. <https://doi.org/10.3390/jmse8080611>.



- Capolupo, M., Sørensen, L., Jayasena, K.D.R., Booth, A.M., Fabbri, E., 2020. Chemical composition and ecotoxicity of plastic and car tire rubber leachates to aquatic organisms. *Water Res.* 169, 115270 <https://doi.org/10.1016/j.watres.2019.115270>.
- Chang, J., Jiao, M., Zhang, Z., Liu, W., Li, W., Xu, P., Wan, B., 2023. Mechanistic insight into the adverse outcome of tire wear and road particle leachate exposure in zebrafish (*Danio rerio*) larvae. *Environ. Int.* 178, 108053 <https://doi.org/10.1016/j.envint.2023.108053>.
- Collins, K.J., 2021. Environmental impact of tires used in marine construction. In: *Tire Waste and Recycling*. Elsevier, pp. 275–296. <https://doi.org/10.1016/B978-0-12-820685-0.00017-X>.
- Cormier, B., Gambardella, C., Tato, T., Perdiat, Q., Costa, E., Vecclin, C., le Bihanic, F., Grassl, B., Dubocq, F., Kärrman, A., van Arkel, K., Lemoine, S., Lagarde, F., Morin, B., Garaventa, F., Faimali, M., Cousin, X., Bégout, M.-L., Beiras, R., Cachot, J., 2021. Chemicals sorbed to environmental microplastics are toxic to early life stages of aquatic organisms. *Ecotoxicol. Environ. Saf.* 208, 111665 <https://doi.org/10.1016/j.ecoenv.2020.111665>.
- Cunha, I., García, L.M., Guilhermino, L., 2005. Sea-urchin (*Paracentrotus lividus*) glutathione S-transferases and cholinesterase activities as biomarkers of environmental contamination. *J. Environ. Monit.* 7, 288–294. <https://doi.org/10.1039/B414773A>.
- Dang, V.D.H., Cheung, P.-Y., Fong, C.-L., Mulla, A.J., Shiu, J.-H., Lin, C.-H., Nozawa, Y., 2020. Sea urchins play an increasingly important role for coral resilience across reefs in Taiwan. *Front. Mar. Sci.* 7 <https://doi.org/10.3389/fmars.2020.581945>.
- el Idrissi, O., Santini, J., Bonnin, M., Demolliens, M., Aiello, A., Gobert, S., Pasqualini, V., Ternengo, S., 2022. Stress response to trace elements mixture of different embryonic stages of *Paracentrotus lividus*. *Mar. Pollut. Bull.* 183, 114092 <https://doi.org/10.1016/j.marpolbul.2022.114092>.
- Environment Canada. Biological Test Method: Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars). <https://www.canada.ca/en/environment-climate-change/services/wildlife-research-landscape-science/biological-test-method-publications/fertilization-assay-echinoids/chapter-6.html>.
- Foldvik, A., Kryuchkov, F., Sandodden, R., Uhlig, S., 2022. Acute toxicity testing of the tire rubber-derived chemical 6PPD-quinone on Atlantic salmon (*Salmo salar*) and Brown Trout (*Salmo trutta*). *Environ. Toxicol. Chem.* 41, 3041–3045. <https://doi.org/10.1002/etc.5487>.
- Frasco, M.F., Guilhermino, L., 2002. Effects of dimethoate and beta-naphthoflavone on selected biomarkers of *Poecilia reticulata*. *Fish Physiol. Biochem.* 26, 149–156. <https://doi.org/10.1023/A:1025457831923>.
- Galasso, C., Orefice, I., Toscano, A., Vega Fernández, T., Musco, L., Brunet, C., Sansone, C., Cirino, P., 2018. Food modulation controls astaxanthin accumulation in eggs of the sea urchin *Arbacia lixula*. *Mar. Drugs* 16, 186. <https://doi.org/10.3390/md16060186>.
- García-Sanz, S., Navarro, P.G., Tuya, F., 2014. Contrasting recruitment seasonality of sea urchin species in Gran Canaria, Canary Islands (eastern Atlantic). *Mediterr. Mar. Sci.* 15, 475–481. <https://doi.org/10.12681/mms.547>.
- George, S.B., Cellario, C., Fenaux, L., 1990. Population differences in egg quality of *Arbacia lixula* (Echinodermata: echinoidea): proximate composition of eggs and larval development. *J. Exp. Mar. Biol. Ecol.* 141, 107–118.
- Gizzi, F., Jiménez, J., Schäfer, S., Castro, N., Costa, S., Lourenço, S., José, R., Canning-Clode, J., Monteiro, J., 2020. Before and after a disease outbreak: tracking a keystone species recovery from a mass mortality event. *Mar. Environ. Res.* 156, 104905 <https://doi.org/10.1016/j.marenvres.2020.104905>.
- Gómez, M., Torres, S., Hernández-León, S., 1996. Modification of the electron transport system (ETS) method for routine measurements of respiratory rates of zooplankton. *S. Afr. J. Mar. Sci.* 17, 15–20. <https://doi.org/10.2989/025776196784158446>.
- Habig, W.H., Pabst, M.J., Jakob, W.B., 1974. Glutathione S-transferases. *J. Biol. Chem.* 249, 7130–7139. [https://doi.org/10.1016/S0021-9258\(19\)42083-8](https://doi.org/10.1016/S0021-9258(19)42083-8).
- Halsband, C., Sørensen, L., Booth, A.M., Herzke, D., 2020. Car tire crumb rubber: Does leaching produce a toxic chemical cocktail in coastal marine systems? *Front. Environ. Sci.* 8 <https://doi.org/10.3389/fenvs.2020.00125>.
- Hann, S., Darrah, C., Sherrington, C., Blacklaws, K., Horton, I., Thompson, A., 2018. Reducing Household Contributions to Marine Plastic Pollution Report for Friends of the Earth.
- Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A. E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N. P., Lusher, A.L., Wagner, M., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. *Environ. Sci. Technol.* 53, 1039–1047. <https://doi.org/10.1021/acs.est.8b05297>.
- Hernández, J.C., Clemente, S., Sangil, C., Brito, A., 2008. The key role of the sea urchin *Diadema aff. antillarum* in controlling macroalgae assemblages throughout the Canary Islands (eastern subtropical Atlantic): an spatio-temporal approach. *Mar. Environ. Res.* 66, 259–270. <https://doi.org/10.1016/j.marenvres.2008.03.002>.
- Hernández, J.C., Clemente, S., García, E., McAlister, J.S., 2020. Planktonic stages of the ecologically important sea urchin, *Diadema africanum*: larval performance under near future ocean conditions. *J. Plankton Res.* 42, 286–304. <https://doi.org/10.1093/plankt/fbaa016>.
- Hernández-León, S., Gómez, M., 1996. Factors affecting the respiration/ETS ratio in marine zooplankton. *J. Plankton Res.* 18, 239–255. <https://doi.org/10.1093/plankt/18.2.239>.
- Jager, T., Ravagnan, E., Dupont, S., 2016. Near-future ocean acidification impacts maintenance costs in sea-urchin larvae: identification of stress factors and tipping points using a DEB modelling approach. *J. Exp. Mar. Biol. Ecol.* 474, 11–17. <https://doi.org/10.1016/j.jembe.2015.09.016>.
- Järslöck, I., Jaramillo-Vogel, D., Rausch, J., Gustafsson, M., Strömval, A.-M., Andersson-Sköld, Y., 2022. Concentrations of tire wear microplastics and other traffic-derived non-exhaust particles in the road environment. *Environ. Int.* 170, 107618 <https://doi.org/10.1016/j.envint.2022.107618>.
- Kim, L., Kim, H., Lee, T.-Y., An, Y.-J., 2023. Chemical toxicity screening of tire particle leachates from vehicles and their effects on organisms across three trophic levels. *Mar. Pollut. Bull.* 192, 114999 <https://doi.org/10.1016/j.marpolbul.2023.114999>.
- Kole, P.J., Löhr, A.J., van Belleghem, F., Ragas, A., 2017. Wear and tear of tyres: a stealthy source of microplastics in the environment. *Int. J. Environ. Res. Publ. Health* 14, 1265. <https://doi.org/10.3390/ijerph14101265>.
- Kreider, M.L., Panko, J.M., McAtee, B.L., Sweet, L.I., Finley, B.L., 2010. Physical and chemical characterization of tire-related particles: comparison of particles generated using different methodologies. *Sci. Total Environ.* 408, 652–659. <https://doi.org/10.1016/j.scitotenv.2009.10.016>.
- Lassen, C., Hansen, S.F., Magnusson, K., Norén, F., Hartmann, N.B., Jensen, P.R., Nielsen, T.G., Brinch, A., 2015. *Microplastics: Occurrence, Effects and Sources of Releases to the Environment in Denmark*. Danish Environmental Protection Agency, Copenhagen K.
- Leads, R.R., Weinstein, J.E., 2019. Occurrence of tire wear particles and other microplastics within the tributaries of the Charleston Harbor Estuary, South Carolina, USA. *Mar. Pollut. Bull.* 145, 569–582. <https://doi.org/10.1016/j.marpolbul.2019.06.061>.
- Lehtiniemi, M., Hartikainen, S., Turja, R., Lehtonen, K.K., Vepsäläinen, J., Peräniemi, S., Leskinen, J., Setälä, O., 2021. Exposure to leachates from post-consumer plastic and recycled rubber causes stress responses and mortality in a copepod *Limnocalanus macrurus*. *Mar. Pollut. Bull.* 173, 113103 <https://doi.org/10.1016/j.marpolbul.2021.113103>.
- Limatola, N., Chun, J.T., Santella, L., 2021. Fertilization and development of *Arbacia lixula* eggs are affected by osmolality conditions. *Biosystems* 206, 104448. <https://doi.org/10.1016/j.biosystems.2021.104448>.
- Macdonald, J.M., Shields, J.D., Zimmer-Faust, R.K., 1988. Acute toxicities of eleven metals to early life-history stages of the yellow crab *Cancer anthonyi*. *Mar. Biol.* 98, 201–207.
- Manzo, S., Schiavo, S., 2022. Physical and chemical threats posed by micro(nano)plastic to sea urchins. *Sci. Total Environ.* 808, 152105 <https://doi.org/10.1016/j.scitotenv.2021.152105>.
- Martínez-Gómez, C., León, V.M., Calles, S., Gomáriz-Olcina, M., Vethaak, A.D., 2017. The adverse effects of virgin microplastics on the fertilization and larval development of sea urchins. *Mar. Environ. Res.* 130, 69–76. <https://doi.org/10.1016/j.marenvres.2017.06.016>.
- McAlister, J.S., Moran, A.L., 2012. Relationships among egg size, composition, and energy: a comparative study of geminate sea urchins. *PLoS One* 7, e41599. <https://doi.org/10.1371/journal.pone.0041599>.
- McIntyre, J.K., Prat, J., Cameron, J., Wetzel, J., Mudrock, E., Peter, K.T., Tian, Z., Mackenzie, C., Lundin, J., Stark, J.D., King, K., Davis, J.W., Kolodziej, E.P., Scholz, N.L., 2021. Treading water: tire wear particle leachate recreates an urban runoff mortality syndrome in coho but not chum salmon. *Environ. Sci. Technol.* 55, 11767–11774. <https://doi.org/10.1021/acs.est.1c03569>.
- Mengistu, D., Heistad, A., Coutris, C., 2021. Tire wear particles concentrations in gully pot sediments. *Sci. Total Environ.* 769, 144785 <https://doi.org/10.1016/j.scitotenv.2020.144785>.
- Moore, J.W., 1991. *Inorganic Contaminants of Surface Water: Research and Monitoring Priorities*. Springer-Verlag, New York.
- Moran, A.L., McAlister, J.S., 2009. Egg size as a life history character of marine invertebrates: is it all its cracked up to be? *Biol. Bull.* 216, 226–242.
- Morrioni, L., Pinsky, A., Pellegrini, D., Regoli, F., Matranga, V., 2016. Development of a new integrative toxicity index based on an improvement of the sea urchin embryo toxicity test. *Ecotoxicol. Environ. Saf.* 123, 2–7. <https://doi.org/10.1016/j.ecoenv.2015.09.026>.
- Müller, K., Hübner, D., Huppertsberg, S., Knepper, T.P., Zahn, D., 2022. Probing the chemical complexity of tires: identification of potential tire-borne water contaminants with high-resolution mass spectrometry. *Sci. Total Environ.* 802, 149799 <https://doi.org/10.1016/j.scitotenv.2021.149799>.
- Nadella, S.R., Tellis, M., Diamond, R., Smith, S., Bianchini, A., Wood, C.M., 2013. Toxicity of lead and zinc to developing mussel and sea urchin embryos: critical tissue residues and effects of dissolved organic matter and salinity. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 158, 72–83. <https://doi.org/10.1016/j.cbpc.2013.04.004>.
- Nobre, C.R., Santana, M.F.M., Maluf, A., Cortez, F.S., Cesar, A., Pereira, C.D.S., Turra, A., 2015. Assessment of microplastic toxicity to embryonic development of the sea urchin *Lytechinus variegatus* (Echinodermata: echinoidea). *Mar. Pollut. Bull.* 92, 99–104. <https://doi.org/10.1016/j.marpolbul.2014.12.050>.
- Nogueira, L.S., Domingos-Moreira, F.X.V., Klein, R.D., Bianchini, A., Wood, C.M., 2021. Influence of environmentally relevant concentrations of Zn, Cd and Ni and their binary mixtures on metal uptake, bioaccumulation and development in larvae of the purple sea urchin *Strongylocentrotus purpuratus*. *Aquat. Toxicol.* 230, 105709 <https://doi.org/10.1016/j.aquatox.2020.105709>.
- Novelli, A.A., Losso, C., Ghetti, P.F., Ghirardini, A.V., 2003. Toxicity of heavy metals using sperm cell and embryo toxicity bioassays with *Paracentrotus lividus* (Echinodermata: echinoidea): comparisons with exposure concentrations in the Lagoon of Venice, Italy. *Environ. Toxicol. Chem.* 22, 1295–1301. <https://doi.org/10.1002/etc.5620220616>.
- Oliviero, M., Tato, T., Schiavo, S., Fernández, V., Manzo, S., Beiras, R., 2019. Leachates of micronized plastic toys provoke embryotoxic effects upon sea urchin *Paracentrotus lividus*. *Environ. Pollut.* 247, 706–715. <https://doi.org/10.1016/j.envpol.2019.01.098>.

- Owens, T.G., King, F.D., 1975. The measurement of respiratory electron-transport-system activity in marine zooplankton. *Mar. Biol.* 30, 27–36. <https://doi.org/10.1007/BF00393750>.
- Page, T.S., Almeda, R., Koski, M., Bournaka, E., Nielsen, T.G., 2022. Toxicity of tyre wear particle leachates to marine phytoplankton. *Aquat. Toxicol.* 252, 106299. <https://doi.org/10.1016/j.aquatox.2022.106299>.
- Pandori, L.L.M., Sorte, C.J.B., 2019. The weakest link: sensitivity to climate extremes across life stages of marine invertebrates. *Oikos* 128, 621–629. <https://doi.org/10.1111/oik.05886>.
- Pearse, J.S., 2006. Ecological role of purple sea urchins. *Science* 314 (1979), 940–941. <https://doi.org/10.1126/science.1131888>.
- Phillips, B.M., Nicely, P.A., Hunt, J.W., Anderson, B.S., Tjeerdema, R.S., Palmer, S.E., Palmer, F.H., Puckett, H.M., 2003. Toxicity of cadmium-copper-nickel-zinc mixtures to larval purple sea urchins (*Strongylocentrotus purpuratus*). *Bull. Environ. Contam. Toxicol.* 70, 592–599. <https://doi.org/10.1007/s00128-003-0026-0>.
- Piccardi, M., Provenza, F., Grazioli, E., Anselmi, S., Terlizzi, A., Renzi, M., 2021. Impacts of plastic-made packaging on marine key species: effects following water acidification and ecological implications. *J. Mar. Sci. Eng.* 9, 432. <https://doi.org/10.3390/jmse9040432>.
- Przeslawski, R., Byrne, M., Mellin, C., 2015. A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Global Change Biol.* 21, 2122–2140. <https://doi.org/10.1111/gcb.12833>.
- R Core Team, 2022. *R: A Language and Environment for Statistical Computing* [Online].
- Radenac, G., Fichet, D., Miramand, P., 2001. Bioaccumulation and toxicity of four dissolved metals in *Paracentrotus lividus* sea-urchin embryo. *Mar. Environ. Res.* 51, 151–166.
- Rendell-Bhatti, F., Paganos, P., Pouch, A., Mitchell, C., D'Aniello, S., Godley, B.J., Pazzo, K., Arnone, M.I., Jimenez-Guri, E., 2021. Developmental toxicity of plastic leachates on the sea urchin *Paracentrotus lividus*. *Environ. Pollut.* 269, 115744. <https://doi.org/10.1016/j.envpol.2020.115744>.
- Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. *PLoS One* 10.
- Rodgers, B., Waddell, W., 2013. The science of rubber compounding. In: *The Science and Technology of Rubber*. Elsevier, pp. 417–471. <https://doi.org/10.1016/B978-0-12-394584-6.00009-1>.
- Rødland, A.E., 2019. *Ecotoxic Potential of Road-Associated Microplastic Particles (RAMPs)*.
- Rødland, E.S., Lind, O.C., Reid, M.J., Heier, L.S., Okoffo, E.D., Rauert, C., Thomas, K.v., Meland, S., 2022. Occurrence of tire and road wear particles in urban and peri-urban snowbanks, and their potential environmental implications. *Sci. Total Environ.* 824, 153785. <https://doi.org/10.1016/j.scitotenv.2022.153785>.
- Rodríguez-Hernández, Á., Zumbado, M., Henríquez-Hernández, L.A., Boada, L.D., Luzardo, O.P., 2019. Dietary intake of essential, toxic, and potentially toxic elements from mussels (*Mytilus* spp.) in the Spanish population: a nutritional assessment. *Nutrients* 11, 864. <https://doi.org/10.3390/nu11040864>.
- Rouchon, A.M., Phillips, N.E., 2017. Acute toxicity of copper, lead, zinc and their mixtures on the sea urchin *Evechinus chloroticus*. *N. Z. J. Mar. Freshw. Res.* 51, 333–355. <https://doi.org/10.1080/00288330.2016.1239643>.
- Sangil, C., Hernández, J.C., 2022. Recurrent large-scale sea urchin mass mortality and the establishment of a long-lasting alternative macroalgae-dominated community state. *Limnol. Oceanogr.* 67, S430–S443. <https://doi.org/10.1002/lno.11966>.
- Sangil, C., Sansón, M., Díaz-Villa, T., Hernández, J.C., Clemente, S., Afonso-Carrillo, J., 2014. Spatial variability, structure and composition of crustose algal communities in *Diadema africanum* barrens. *Helgol. Mar. Res.* 68, 451–464. <https://doi.org/10.1007/s10152-014-0401-8>.
- Shin, H., Sukumaran, V., Yeo, I.-C., Shim, K.-Y., Lee, S., Choi, H.-K., Ha, S.Y., Kim, M., Jung, J.-H., Lee, J.-S., Jeong, C.-B., 2022. Phenotypic toxicity, oxidative response, and transcriptomic deregulation of the rotifer *Brachionus plicatilis* exposed to a toxic cocktail of tire-wear particle leachate. *J. Hazard Mater.* 438, 129417. <https://doi.org/10.1016/j.jhazmat.2022.129417>.
- Spangenberg, J.V., Cherr, G.N., 1996. Developmental effects of barium exposure in a marine bivalve (*Mytilus californianus*). *Environ. Toxicol. Chem.* 15, 1769–1774. <https://doi.org/10.1002/etc.5620151018>.
- Taltec, K., Gabriele, M., Paul-Pont, I., Alunno-Bruscia, M., Huvet, A., 2022a. Tire rubber chemicals reduce juvenile oyster (*Crassostrea gigas*) filtration and respiration under experimental conditions. *Mar. Pollut. Bull.* 181, 113936. <https://doi.org/10.1016/j.marpolbul.2022.113936>.
- Taltec, K., Huvet, A., Yeuc'h, V., le Goïc, N., Paul-Pont, I., 2022b. Chemical effects of different types of rubber-based products on early life stages of Pacific oyster, *Crassostrea gigas*. *J. Hazard Mater.* 427, 127883. <https://doi.org/10.1016/j.jhazmat.2021.127883>.
- Tellis, M.S., Lauer, M.M., Nadella, S., Bianchini, A., Wood, C.M., 2014. Sublethal mechanisms of Pb and Zn toxicity to the purple sea urchin (*Strongylocentrotus purpuratus*) during early development. *Aquat. Toxicol.* 146, 220–229. <https://doi.org/10.1016/j.aquatox.2013.11.004>.
- Thorson, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* 25 (1), 1–45. <https://doi.org/10.1111/j.1469-185X.1950.tb00585.x>.
- Thorson, G., 1966. Some factors influencing the recruitment and establishment of marine benthic communities. *Neth. J. Sea Res.* 3, 267–293. [https://doi.org/10.1016/0077-7579\(66\)90015-9](https://doi.org/10.1016/0077-7579(66)90015-9).
- Thushari, G.G.N., Senevirathna, J.D.M., 2020. Plastic pollution in the marine environment. *Heliyon* 6, e04709. <https://doi.org/10.1016/j.heliyon.2020.e04709>.
- Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A.E., Biswas, R.G., Kock, F.V.C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., Gilbreath, A., Sutton, R., Scholz, N.L., Davis, J.W., Dodd, M.C., Simpson, A., McIntyre, J.K., Kolodziej, E.P., 2021. A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science* 371 (1979), 185–189. <https://doi.org/10.1126/science.abd6951>.
- Tomšić, S., Stanković, S., Lucu, Č., 2011. Oxygen consumption rate and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in early developmental stages of the sea urchin *Paracentrotus lividus* Lam. *Helgol. Mar. Res.* 65, 431–434. <https://doi.org/10.1007/s10152-011-0261-4>.
- Tuya, F., Martín, J.A., Reuss, G.M., Luque, A., 2001. Food preferences of the sea urchin *Diadema antillarum* in gran Canaria (canary islands, central-east Atlantic ocean). *J. Mar. Biol. Assoc. U. K.* 81, 845–849. <https://doi.org/10.1017/S0025315401004672>.
- Tuya, F., Cisneros-Aguirre, J., Ortega-Borges, L., Haroun, R.J., 2007. Bathymetric segregation of sea urchins on reefs of the Canarian Archipelago: role of flow-induced forces. *Estuar. Coast Shelf Sci.* 73, 481–488. <https://doi.org/10.1016/j.ecss.2007.02.007>.
- Uribe-Echeverría, T., Beiras, R., 2022. Acute toxicity of bioplastic leachates to *Paracentrotus lividus* sea urchin larvae. *Mar. Environ. Res.* 176, 105605. <https://doi.org/10.1016/j.marenvres.2022.105605>.
- Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T., Reemtsma, T., 2018. Tire wear particles in the aquatic environment - a review on generation, analysis, occurrence, fate and effects. *Water Res.* 139, 83–100. <https://doi.org/10.1016/j.watres.2018.03.051>.
- Wangensteen, O., Turon, X., García-Cisneros, A., Recasens, M., Romero, J., Palacín, C., 2011. A wolf in sheep's clothing: carnivory in dominant sea urchins in the Mediterranean. *Mar. Ecol. Prog. Ser.* 441, 117–128. <https://doi.org/10.3354/meps09359>.
- Wiesinger, H., Wang, Z., Hellweg, S., 2021. Deep dive into plastic monomers, additives, and processing aids. *Environ. Sci. Technol.* 55, 9339–9351. <https://doi.org/10.1021/acs.est.1c00976>.
- Wik, A., Dave, G., 2009. Occurrence and effects of tire wear particles in the environment – a critical review and an initial risk assessment. *Environ. Pollut.* 157, 1–11. <https://doi.org/10.1016/j.envpol.2008.09.028>.
- Yang, K., Jing, S., Liu, Yang, Zhou, H., Liu, Yan, Yan, M., Yi, X., Liu, R., 2022. Acute toxicity of tire wear particles, leachates and toxicity identification evaluation of leachates to the marine copepod, *Tigriopus japonicus*. *Chemosphere* 297, 134099. <https://doi.org/10.1016/j.chemosphere.2022.134099>.