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PVC pellet leachates affect adult immune system and embryonic development but not reproductive capacity in the sea urchin *Paracentrotus lividus*

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ABSTRACT

Microplastic pollution is a major concern of our age, eliciting a range of effects on organisms including during embryonic development. Plastic preproduction pellets stunt the development of sea urchins through the leaching of teratogenic compounds. However, the effect of these leachates on adult sea urchins and their fertility is unknown. Here we investigate the effect of PVC leachates on the capacity to produce normal embryos, and demonstrate that adults kept in contaminated water still produce viable offspring. However, we observe a cumulative negative effect by continued exposure to highly polluted water: adult animals had lower counts and disturbed morphological profiles of immune cells, were under increased oxidative stress, and produced embryos less tolerant of contaminated environments. Our findings suggest that even in highly polluted areas, sea urchins are fertile, but that sublethal effects seen in the adults may lead to transgenerational effects that reduce developmental robustness of the embryos.

1. Introduction

Plastic pollution in the marine environment is an ever-growing problem (Eriksen et al., 2014; Thushari and Senevirathna, 2020). Large plastic items such as plastic bottles or bags can break down in time by the action of waves, UV light and biological and chemical processes transforming into smaller particles, becoming micro- or even nano-plastics (Barnes et al., 2009), identified as secondary microplastics. However, plastics can already enter the environment as small particles, which are known as primary microplastics. These are the result of degradation of products during use before being released to the environment, such as tyres or paint, or plastics intentionally made small such as plastic pre-production pellets or cosmetic beads (Sundt and Per-Erik Schultze, 2014; Boucher and Friot, 2017). One of the main contributors to primary microplastic pollution by weight, along with tyre wear particles, are plastic pre-production pellets, or nurdles (Essel et al., 2015; Sherrington, 2016). These are the raw material that feed plastic factories to manufacture any type of plastic object, from plastic bottles to chairs of medical material. Plastic nurdles are supplemented at production with different chemical compounds, such as plasticisers, stabilisers or antioxidants, to give them the physical characteristics that make them malleable to produce the final products. Unfortunately, these pellets can be lost to the environment at the production site, during usage or while being transported, typically by road or sea (Essel et al., 2015; Karlsson et al., 2018). When they end up in the waterways, the chemicals present in the nurdles – and also in other types of plastics (Martínez-Gómez et al., 2017; Oliviero et al., 2019; Sarker et al., 2020) – can leach from the particles into sea water (Rendell-Bhatti et al., 2021; Paganos et al., 2023).

Embryos are an extremely vulnerable life stage in aquatic organisms. In the case of sea urchins, and many other aquatic invertebrates, fertilisation occurs in the water column and embryos remain there with the only protection of a chorion. Afterwards, larvae will also become part of the planktonic population until metamorphosis occurs. Despite development being a robust process, disturbances at this stage may result in a total failure of embryogenesis, which may jeopardise the next generation. Previous research has shown the dramatic effect that the associated chemicals in plastics can have in the development of several marine

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organisms (Nobre et al., 2015; Li et al., 2016; Oliviero et al., 2019; Gardon et al., 2020; Rendell-Bhatti et al., 2021; Paganos et al., 2023). In particular, leachates from high concentrations of new PVC as well as mixes of environmentally retrieved pre-production pellets can produce non-viable radialised larvae in *Paracentrotus lividus* (Rendell-Bhatti et al., 2021) and *Strongylocentrotus purpuratus* (Paganos et al., 2023). Moreover, previous studies have shown immune cell changes following exposure to plastic. Developing larvae pigment cells, a major component of their immune cell population, do not form when treated with PVC leachates (Paganos et al., 2023). The immune cells of adult sea urchins, the coelomocytes, are a heterogeneous cell population which are known to respond to environmental stressors, including plastic particles (Matranga et al., 2000, 2006; Pinsino and Matranga, 2015; Migliaccio et al., 2019; Milito et al., 2020; Murano et al., 2021). Despite these insights, the effects on the adults and their gametes when exposed to plastic pre-production leachates has not been studied, leaving an open question as to whether the previously observed larval defects were solely embryonic, or if they were influenced by a parental effect.

To address this, we set out to investigate if adult sea urchins were affected by the exposure to plastic pre-production leachates and, in particular, whether they are able to produce viable offspring. We used the sea urchin *Paracentrotus lividus* to compare the effect in embryos and larvae from adults kept in clean sea water compared to those from adults kept in PVC nurdle leachates, and in addition, whether there was a cumulative effect as the time of exposure increased. To understand any differences observed in the development of larvae coming from treated parents, we investigated the immune cell response and their associated stress levels in adult *P. lividus*.

2. Materials and methods

2.1. Microplastic leachate preparation

Commercial PVC nurdles were purchased from Northern Polymers and Plastics Ltd. (UK) in January 2022. Flexible tubular PVC nurdles were transparent, with a slight blue hue, and with average dimensions of 3.2 × 3.7 mm. Microplastic leachates were prepared as described in (Rendell-Bhatti et al., 2021). In brief, PVC pellets were added to filtered seawater (FSW) at a concentration of 10 % (*v*/v), which is equivalent to 6.5 g of PVC pellets per 100 ml of sea water. Serial dilutions of these plastic leachates had been used before (Rendell-Bhatti et al., 2021; Paganos et al., 2023) -ranging from 1 to 10 % PVC pellets in water- and developmental abnormalities were described for the 10 % concentration. This high concentration of nurdles also allowed us, in the case of the current work, to deal with a worse-case-scenario for adult sea urchins. Pellets were leached for 72 h at room temperature (ca 18 °C) in the dark. After that time, leachates were obtained by filtering through filter paper in order to remove the plastic particles. Previous studies show that leachates obtained from these pellets contain high amounts of Zn (1 μg/g) (Paganos et al., 2023), in the order of a thousand times higher than the concentration of Zn described in the Mediterranean sea (Middag et al., 2022). Calcium based stabilisers, including calcium-zinc molecules, are used as heat stabilisers in the production of PVC and other plastic pellets (European Stabiliser Producers Association, n.d.), and were the stabilisers used in these pellets, as described by the provider, being the source of the Zn leached from the pellets.

2.2. Animal exposure and fertilisation

Between January and March, during their natural reproductive season, adult *Paracentrotus lividus* from the bay of Naples were briefly kept in circulating seawater aquaria at 18 °C in the aquarium facility of the Stazione Zoologica Anton Dohrn, Naples (Italy), to confirm their fitness before being used for the experiments. Adults were transferred to 3 l tanks at a ratio of 3 animals per tank, and further acclimatised for a week. 15 animals were used per condition (either control in filtered sea water, or treated with 10 % PVC leachates), with a final number of 5 tanks for control animals and five tanks for PVC leachate treated animals (Supplementary Fig. 1). Animals were housed in these tanks for two weeks. At the end of every week, gametes were acquired and fertilisations performed. At least one breeding pair was used every week. Gamete acquisition and fertilisations were performed as described in (Rendell-Bhatti et al., 2021). Embryos from control and treated adults were then added to beakers with either filtered sea water or 10 % PVC leachates at a concentration of 50 embryos per ml and left to develop at 18 °C on a 12:12 light dark cycle. Each experiment was repeated three times.

2.3. Larval phenotypic observations

A fraction of the embryos and larvae were immobilised with a small amount of 4 % PFA at 24 and 48 hours post fertilisation (hpf) and imaged using a ZEISS Imager.Z2 microscope. Embryos and larvae were classified in three groups: developed, for normally developed individuals; aberrant, for a suite of phenotypes that varied from delayed to radialised animals (Supplementary Fig. 2C–E); and not developed (Supplementary Fig. 2A, B), for those embryos that had not managed to proceed to gastrulation. Statistical analysis was done performing unpaired *t*-test.

2.4. Immune cell retrieval and oxidative stress markers

Five animals per treatment, housed as described above, were used for these tests. Coelomic fluid was extracted from the peristomal area of each specimen as described in (Murano et al., 2021) at 24 h, 7, 10 and 14 days of exposure. The coelomic fluid was withdrawn using an anticoagulant solution CCM 2× (NaCl 1 M, MgCl2 10 mM, EGTA 2 mM, Hepes 40 mM, pH 7.4) at a ratio of 1:1 (anticoagulant: coelomic fluid). Coelomocytes were immediately counted using Neubauer chamber (Bright-Line Hemacytometer) under optical microscope and cells were morphologically identified according to (Pinsino and Matranga, 2015) as phagocytes, vibratile cells, white and red amoebocytes. Intracellular levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as well as total antioxidant capacity (TAC) was measured by spectrofluorometry as described in (Murano et al., 2020) (see also Supporting Information). Total proteins were measured at 595 nm according to (Bradford, 1976) using a Tecan spectrometer and bovine serum albumin as standard. Coelomocyte differences, intracellular levels of ROS and RNS, and TAC activity were analysed by two-way ANOVA followed by Bonferroni's multiple comparisons test.

3. Results and discussion

3.1. PVC pellet leachate exposure as adults does not affect fertilisation capacity of eggs and sperm or developmental success in P. lividus

Adult *P. lividus* kept for two weeks in 10 % PVC leachates were able to produce normal embryos and pluteus larvae when the fertilised eggs were left to develop in clean sea water. It is important to note that the leachates of these plastic pellets have been chemically analysed before by inductively coupled plasma – optical emission spectrometry (ICP-OES) (Paganos et al., 2023), and the most important contaminant found was Zn, in concentrations of 1 μg/ g at 10 % PVC, which constitute very high doses of Zn compared to natural sea water (Middag et al., 2022). When developing in clean sea water, both gastrulas from control (kept in filtered sea water) and PVC leachate-treated adults showed normal embryos with standard ingression of primary mesenchyme cells (PMCs), a cell population that is responsible for the formation of the larval triradiate skeleton. Moreover, we observed no effects on the progression of the archenteron towards the oral side, or the ingression of the secondary mesenchyme cells, a diverse cell population positioned at the most anterior part of the archenteron (foregut), that will give rise to distinct cell types including immune cells (e.g., pigment cells and globular cells), blastocoelar cells, larval coeloms and musculature (Fig. 1A, B, I, J). Likewise, pluteus larvae grown in clean water from both treated and control adults showed a typical four-arm phenotype with a well-formed ciliary band and a tripartite gut (Fig. 1E, F, M, N). These high concentrations of plastic pellets will very seldom be found in natural environments, and probably only achieved in the event of an accidental spill, like that occurred in Sri Lanka in 2020 (Sewwandi et al., 2022), among others. This study gives insights into the resilience of sea urchins to high levels of plastic contamination, and suggests that the current levels of plastic contamination may not negatively affect the capacity of sea urchins to produce viable offspring so long as the embryos are able to exit the polluted environment (see below).

However, when the embryos of both control and PVC leachate reared adults were left to develop in 10 % PVC leachates, larvae were not viable, as described previously for this species (Rendell-Bhatti et al., 2021) and *S. purpuratus* (Paganos et al., 2023). Gastrula stage embryos from both control and treated adults showed a disarrangement of the PMCs and a delay in the invagination of the archenteron (Fig. 1C, D, K, L). At 48 hpf, larvae from both adult treatments showed a clear radialised phenotype, as well as a lack of arms, probably due to the malformation of the skeleton, as well as not presenting ciliary bands (Fig. 1G, H, O, P). Other aberrant phenotypes were found (Supplementary Fig. 2). These go from arrest before gastrulation to radialisation as described above, or stunted and aberrant growth of the pluteus larvae. These phenotypes are discussed below in more detail. Moreover, as shown in our previous studies, treated animals are lacking pigment cells both at 24 and 48 hpf (Fig. 1G, H, O, P) (Paganos et al., 2023). The absence of pigment cells, the dominant cell type among the sea urchin larvae immune cells, is in agreement with previous findings showing that PVC exposure inhibits the specification of this cell type and therefore may leave the developing embryos and larvae immunocompromised (Paganos et al., 2023). In contrast, as previously reported, the digestive tract of the treated animals appears to be normally partitioned. This radialised phenotype is also in accordance with that shown in *S. purpuratus* larvae treated with the same type of particles (Paganos et al., 2023), which is most likely caused by the high concentration of zinc found in these leachates, as reported in Paganos et al. (2023). Radialisation of embryos and larvae exposed to Zn has been known for sea urchins for a long time (Mitsunaga and Yasumasu, 1984; Kobayashi and Okamura, 2004), and the phenotypic abnormalities seen in our larvae are similar to those seen in several species of sea urchin treated with heavy metals or heavy metal containing products (Mitsunaga and Yasumasu, 1984; Kobayashi and Okamura, 2004; Oliviero et al., 2019; Cunningham et al., 2020).

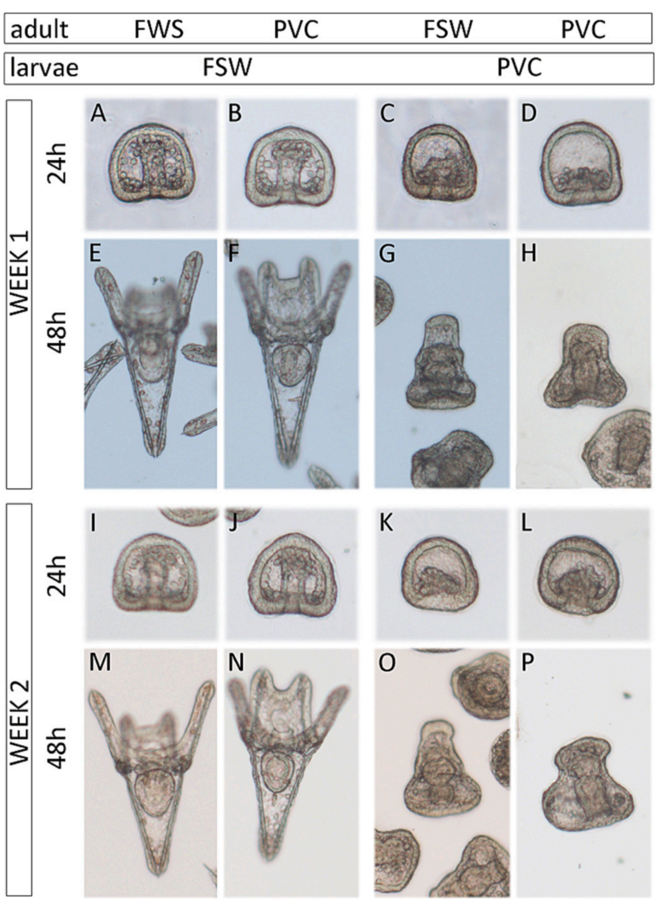

Fig. 1. Phenotypes observed for each treatment. Adult and larval treatments are depicted at the top. FSW: filtered sea water; PVC: 10 % PVC leachates. Embryo (24 h) and pluteus larvae (48 h) for each of the treatments after one week (week 1) and two weeks (week 2) of exposure. PVC treated larvae phenotype shows the most radialised phenotype obtained. For other phenotypes please see Supplementary Fig. 2.

Next, to investigate if fertilisation was altered in contaminated water, we fertilised gametes from control adults in 10 % PVC leachates. Eggs and sperm were left in 10 % PVC leachates for 20 and 5 min respectively (sperm function decreases rapidly after being activated with water) before performing the fertilisation in these leachates. After visualisation of the fertilisation membrane, we removed the fertilised eggs from the leachates at set intervals of time, starting after 5 min and then at each hour until 7 hpf (Supplementary Fig. 3). We saw a stronger disruption of developmental process when embryos were developing in contaminated water for more than 3 h. Before this, despite embryos being smaller than controls, they recovered in time to produce normal larvae, pointing at a delayed but otherwise normal development. During the very early stages of development, embryos rely on maternal transcripts, and zygotic genes only become active later. In *P. lividus*, this so called maternal-to-zygotic transition happens at 4 hpf (Gildor and Ben-Tabou de-Leon, 2015), matching the time window for the stronger effects seen in the treated embryos. Development in 10 % PVC leachates, which contain high concentration of Zn (Paganos et al., 2023), appears to slow down all processes, including transcription and translation, hence affecting zygotic genes more than maternal ones, which already are deposited as mRNA in the egg. In fact, transcriptomic data from the sea urchin *S. purpuratus* shows problems with DNA and RNA synthesis in 24 hpf larvae exposed to these leachates (Paganos et al., 2023), in support of this suggestion. In any case, fertilisation occurs, and normal pluteus larvae can be obtained (even if in lower proportion as treatment time increases), when embryos are rescued in normal sea water, even after 7 h post fertilisation and growth in contaminated water (Supplementary Fig. 3).

3.2. Embryos from PVC leachate treated adults are less robust in contaminated water

Regardless of the treatment applied to the adults, when fertilised eggs were grown in normal filtered sea water there was no differences in the phenotypes at 48 hpf, at one or two weeks of adult exposure, and typical plutei larvae were obtained (Fig. 1E, F, M, N; Fig. 2).

When fertilised eggs were grown in PVC leachates, both embryos and larvae coming from control and PVC treated adults had more aberrations than the ones grown in control water (Fig. 1C, D, G, H, K, L, O, P; Fig. 2; Supplementary Fig. 2), consistent with the phenotypes observed when embryos are treated with Zn or other heavy metals (Hardin et al., 1992; Kobayashi and Okamura, 2004). For larvae derived from control adults, both after one and two weeks of adult exposure, there was a significant increase in the number of aberrant larvae in PVC treated embryos (96 and 92 % for each week) than in the ones developed in FSW ($p < 0.001$). When PVC treated larvae were derived from PVC leachate treated adults, there was a significant increase in aberrant and not developed

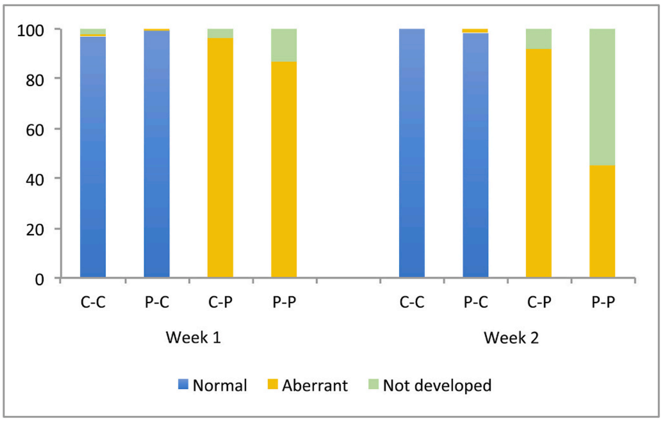

Fig. 2. Percentage larval phenotypes at 48 hpf. Percentage of observed phenotypes (Blue: normal; yellow: aberrant; green: not developed) for each treatment after one (Week 1) and two (Week 2) weeks of adult treatment. C-C: control adults, control water grown embryos; P-C: PVC leachate housed adults, control water grown embryos; C-P: control adults, PVC leachate grown embryos; P-P: PVC leachate housed adults, PVC leachate grown embryos. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

larvae (those that arrested development at or before the blastula stage, Supplementary Fig. 2A, B) at 48 hpf, even from the first week of exposure, when compared to embryos grown in normal sea water ($p < 0.005$). For these PVC treated larvae from PVC leachates treated adults, larvae from the second week of adult exposure had significative higher number of non-developed individuals ($p < 0.05$), suggesting there was a cumulative effect as the adults stayed for longer in contaminated water.

In short, larvae from either control or treated adults grown in normal sea water developed properly, while when they were grown in contaminated water they did not. However, we saw that the negative outcomes were worse when adults were treated with PVC leachates and this these outcomes increased following a longer exposure period.

3.3. Adults *P. lividus* exposed to PVC leachates show changes in the immune cells

Adult *P. lividus* kept in PVC leachates for two weeks showed no evident differences with control animals kept in normal sea water. To find possible differences that could explain the worse outcome of larvae from PVC treated adults grown in PVC contaminated water, we decided to investigate the immune response of the adults treated with PVC leachates. Cell-mediated immune response acts as the first line of defence against pathogens and against everything that the cell recognizes as "non-self", maintaining and regulating the physiological homeostasis. In sea urchins, all these responses are strictly directed by a heterogeneous population of cells including phagocytes, vibratile cells, red spherule and white amoebocytes, in conjunction known as coelomocytes. Over the years, the evidence that microplastic exposure negatively influences the immune cells functional traits of different marine organisms has become increasingly marked (Browne et al., 2013; Murano et al., 2020, 2023; Hu and Palić, 2020), and we previously showed that in PVC-leachate treated sea urchin embryos and larvae, immune cells are absent (Paganos et al., 2023).

Here, we investigated the concentration and the morphological profile of the immune cells in sea urchins at 1, 7, 10 and 14 days of exposure to PVC leachates (Fig. 3A). After one day of exposure, there was no visible difference between the number of coelomocytes found in control and PVC treated animals. However, after a week of exposure the total counts of coelomocytes statistically decreased in treated specimens in comparison to the control group (Fig. 3A, 7 days, $p < 0.01$), and this difference was more pronounced after two weeks of exposure (Fig. 3A, 14 days, $p < 0.01$). Similar findings have been already reported in animals treated with microbial colonised micro-polystyrene in which the total number of coelomocytes decreased after 24 h of exposure (Murano et al., 2021). Likewise, we observed a switch in the morphological profiling of coelomocyte types as time went by (Fig. 3B–E). While in control animals the proportion between red spherules and white amoebocytes was always lower than one, this increased in PVC leachate treated animals: at every time point the number of red cells increased in respect of the white cells. This increased red/white cell ratio has been previously linked to a reaction in the immune system and increased stress in the organism (Pinsino and Matranga, 2015; Milito et al., 2020),

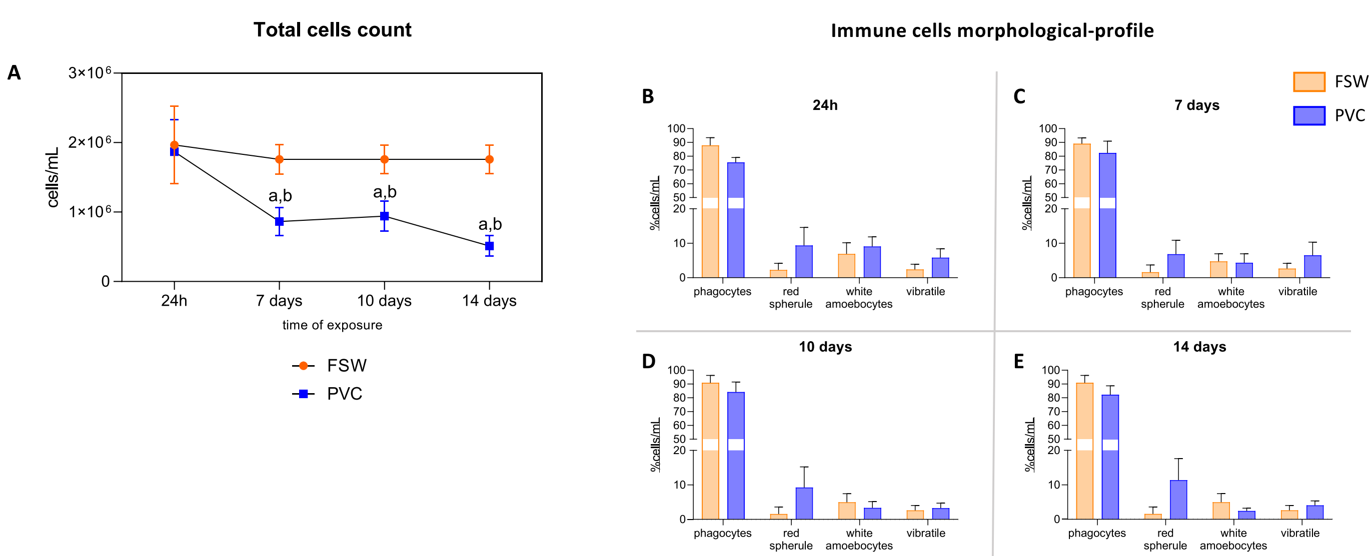

Fig. 3. Immune cell characterisation in PVC-leachate exposed animals. (A) Total count of immune cells of sea urchins at 24 h, 7, 10, and 14 days after exposure to PVC leachates. All data were analysed by Two-way ANOVA followed by Bonferroni post-test compared with the control (FSW exposed). Bars represent mean ± SD. "a" indicate values that are significantly different from the control, ($P < 0.01$); "b" indicate values that are significantly different from the PVC exposed at 24 h. (B-E) Morphological profile of sea urchin immune cells at 24 h (B), 7 (C), 10 (D) and 14 (E) days after exposure to PVC leachates. Bars represent mean ± SD.

and previous literature has demonstrated that acute (one day) treatment with $ZnCl_2$ (2.5 mg l^{-1}) increases the proportion of red spherule within the coelomocytes (Pagliara and Stabili, 2012). This concentration of Zn is similar to the one our treated sea urchins were exposed to, since previous literature confirmed a concentration of Zn of nearly 1 mg l^{-1} (Paganos et al., 2023) and hence it is very possible that the Zn leached from the PVC particles is causing this increased proportion of red cells, which we also observed from 24 h of exposure (Fig. 3B–E).

Oxidative stress and its responding pathways are a key driver underlying the toxicity of microplastics (Hu and Palić, 2020). Hence, we explored the oxidative stress status of the coelomocytes through the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as well as the total antioxidant capacity (TAC) (Fig. 4). The ROS and RNS molecules exhibit a kind of duality as part of their basal metabolism as well as in response to harmful stimuli and therefore their homeostasis is crucial to maintain at optimal levels to prevent damage (Donaghy et al., 2015). In fact, higher concentration of ROS and RNS enforce harsh oxidative stress damaging cell proteins, lipids, and nucleic acids (Sinha et al., 2013). On the other hand, low concentrations act as signalling molecules for a wide range of physiological functions (Nathan and Cunningham-Bussel, 2013; Schieber and Chandel, 2014). In this study, the intracellular levels of ROS showed a significant increase starting from 10-days of exposure to PVC leachates in treated specimens compared to the control group (Fig. 4A). Concomitantly, we observed a higher negative effect in development of larvae from adults exposed to leachates after two weeks than one week (Fig. 2). In contrast, treated animals clearly displayed a time-dependent decrease of the intracellular levels of RNS up to 14-days compared to the control levels (Fig. 4B). Interestingly, previous literature has already described an increase of ROS coupled with a decrease of RNS, despite a general increase of oxidative stress, suggesting the sequestering of nitric oxide when reacting to superoxide anions, forming peroxynitrite (Kirsch and de Groot, 2000; Tafalla et al., 2003; Gallina et al., 2014). This same trend of increase of ROS concomitant with a decrease of RNS has been previously observed in sea urchin coelomocytes exposed to microplastic particles (Murano et al., 2021). Finally, the negative effect of the increase of ROS in PVC leachate treated animals can be confirmed by the reduced total antioxidant capacity of their coelomocytes after 10 days of exposure (Fig. 4C), indicating that initially immune cells cope with oxidative stress but after 10 days their number is reduced and the ones that remain cannot compensate the increased oxidative stress.

Microplastic toxicity has previously been correlated with the formation of ROS in larvae, tissues and immune cells of several marine invertebrates (Lu et al., 2016; Jeong et al., 2016, 2017; Hu and Palić, 2020), including studies in sea urchin where increased oxidative stress was found in coelomocytes of animals exposed to microplastics (Murano et al., 2021, 2023). All these studies investigated the effects of commercial particles with no known chemical additives. In contrast, our microplastic leachates lack the presence of the plastics themselves, but instead only contain the chemical additives that were in the plastic particle that has leached in the water (Rendell-Bhatti et al., 2021; Paganos et al., 2023). In this case, the main contaminant leached from the PVC nurdles is zinc, as we demonstrated in a previous study (Paganos et al., 2023). Heavy metals, such as zinc, are known to generate oxidative stress (Kim et al., 2014). It is thought that heavy metals can alter mitochondrial membrane permeability and once inside can inhibit the function of antioxidant enzymes, increasing the reactive oxygen species present in the cell (Sun et al., 2022). Given the high concentration of Zn released from these particles (Paganos et al., 2023), it is safe to assume that this is the cause of the increased oxidative stress in the adult sea urchins. It is worth noting that both micro- and nanoplastic particles and leachates from microplastics generated similar effects on redox homeostasis in the immune cells of sea urchin. Other types of anthropogenic stressors such as fuel oil or proximity of human activity are also known to increase oxidative stress in sea urchins (Duan et al., 2018; Quetglas-Llabrés et al., 2020), and it could be that this is the general mechanism by which adult sea urchins are affected under a range of human-influenced environments. Moreover, we have previously shown the absence of differentiated immune cells, and especially the predominant immune cell population comprised by the pigment cells in embryos and larvae upon acute exposure (Paganos et al., 2023). Since coelomocytes are continuously produced in adults by hematopoietic-like organs – the pharynx and axial organ (Golconda et al., 2019), we believe that the decrease in the number of coelomocytes upon PVC leachate exposure could be due to either specification or differentiation issues of this lineage in such organs, though this requires further investigation.

Despite adults treated with PVC leachates being able produce normal embryos and larvae when they are subsequently transferred to clean conditions, adults living in PVC leachates have worse outcomes than controls when embryos are grown in contaminated water: embryos from treated adults show higher rates of early developmental arrest. We also see a cumulative effect during time; the longer the adult is exposed to PVC leachates, the worse the outcome for the embryos grown in contaminated water. This is correlated with a clear decrease in the number of coelomocytes and an increase in the oxidative stress in adults, which is shown by the increase in ROS as the time of exposure of the adults increases, starting after a week of exposure (Fig. 4A). In the case of *P. lividus*, the final outcome of PVC leachate treated embryos, both from adults kept in clean or PVC-treated waters, is the same: the larvae grown in PVC leachates are not viable. Hence, both conditions prevent the creation of a new generation. However, our results point to a transgenerational effect, probably mediated by the increased oxidative stress suffered by the leachate-treated adult animals, that could be important in lower contamination concentrations which may not be lethal, but could be affecting larvae from adults exposed to contaminants, since treated adults have embryos that are more susceptible to contaminated waters than control adults. If this was a direct effect of the leachates on the embryos, unrelated to the adults, we would not expect

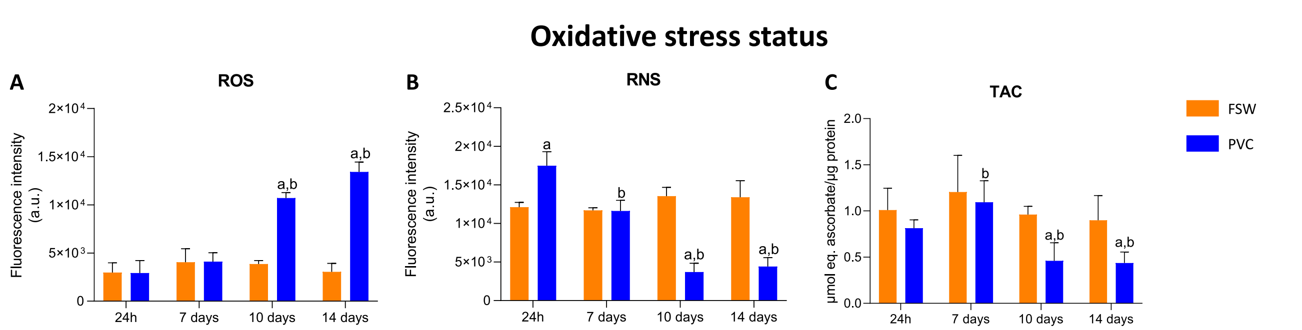

Fig. 4. Oxidative stress status of sea urchin immune cells at 24 h, 7, 10 and 14 days after exposure to PVC leachates. (A) Intracellular levels of ROS; (B) intracellular levels of RNS; (C) Total antioxidant capacity. All data were analysed by Two-way ANOVA followed by Bonferroni post-test compared with the control (FSW exposed). Bars represent mean ± SD. "a" indicate values that are significantly different from the control, ($P < 0.01$); "b" indicate values that are significantly different from the PVC exposed at 24 h.

to see a difference between treated and control adult offspring. Therefore, we think that the treated adults produce eggs and/or sperm that produce embryos that are more susceptible to contamination, and the subsequent stronger effect on the embryos come from weaker gametes. The immune defence against external factors such as microplastics and their leachates includes the involvement of cellular detoxification processes which have a significant metabolic cost (as described in (Murano et al., 2023)). Generally, organisms alter energetic trade-offs between different physiological and behavioural processes to meet the increased energetic demands, allocating more energy in one process rather than another. Therefore, our results suggest an "expensive budget" due to the physiological stress induced by leachates which may lead to a reduction in the energy budget for obtaining high quality gametes. Epigenetic effects derived from contamination has been determined in other systems, including transgenerational effects from plastic contamination (Martins and Guilhermino, 2018; Jimenez-Guri et al., 2021; Sobhani et al., 2021). Furthermore, oxidative stress is known to mediate epigenetic modifications (Lewandowska and Bartoszek, 2011; Chia et al., 2011; Simpson et al., 2012; Guillaumet-Adkins et al., 2017), and it is known that environmental stressors can be triggering this cascade (Baccarelli and Bollati, 2009). It would be interesting to explore, in further studies, whether plastic pre-production pellet leachates have a role in transgenerational outcomes and what type of epigenetic mechanisms are involved.

4. Conclusions

Plastic contamination is known to exert effects in the development of several invertebrate species. How this translates to current environmental conditions is not known, however most tested exposure concentrations are in general higher than concentrations of plastic contamination found in the ocean today. One possible source of high plastic pollution concentration is in the event of spills of plastic pre-production nurdles (Essel et al., 2015; Sewwandi et al., 2022). These nurdles are known to cause developmental abnormalities in sea urchin embryos (Nobre et al., 2015; Rendell-Bhatti et al., 2021; Paganos et al., 2023). Sea urchin embryos develop very quickly, consequently only a short exposure time is needed to eliminate a whole cohort of embryos (Rendell-Bhatti et al., 2021; Paganos et al., 2023). However, what would happen to the adult sea urchin in the same conditions is not known. Here, continuous exposure of adult *P. lividus* to high concentrations of PVC leachates, containing high amounts of zinc (1 μg/g), for two weeks does not affect their capacity to produce embryos and larvae, and fertilisation of eggs can also happen in these conditions. However, embryos grown in contaminated water have higher levels of arrested development at very early stages (before gastrulation) when they are derived from adults that were in contaminated water. Our research shows that, in contaminated conditions, adults show reduced immune cell counts and increased oxidative stress. Nevertheless, their production of gametes (but not embryogenesis) appears resilient to contaminated water, which is a welcome observation if the formed embryos can escape contaminated areas. However, sublethal effects seen in the adults may lead to transgenerational effects that reduce developmental robustness of the embryos, a feature worthy of further study to predict the consequences of rising levels of contamination.

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CRediT authorship contribution statement

Eva Jimenez-Guri: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Carola Murano:** Conceptualization, Investigation, Data curation, Formal analysis, Methodology, Resources, Validation, Visualization, Writing – review & editing. **Periklis Paganos:** Formal analysis, Methodology, Writing – review & editing. **Maria Ina Arnone:** Conceptualization, Methodology, Resources, Writing – review & editing.

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

Our data is supplied in the figures

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