Sea urchin chronicles. The effect of oxygen super-saturation and marine polluted sediments from Bagnoli-Coroglio Bay on different life stages of the sea urchin *Paracentrotus lividus*

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Abstract

In marinas and harbours the accumulation of pollutants in sediments, combined with poor exchange of water with the open sea, poses a major environmental threat. The presence of photosynthetic organisms and the related oxygen production, however, may alleviate the negative effects of environmental contamination on heterotrophic organisms, enhancing their physiological defences. Furthermore, possible transgenerational buffer effects may increase the ability of natural populations to face environmental stress. Here we tested the occurrence of transgenerational effects on larvae of the sea urchin *Paracentrotus lividus*, whose parents were exposed during the gametogenesis to contaminated sediments, two temporal patterns of water re-suspension events and normal- (90%) vs. super-saturated (200%) levels of O_2. The study site was Bagnoli-Coroglio (Gulf of Naples, South Tyrrhenian Sea), a historically polluted brownfield and Site of National Interest for which environmental restoration options are currently under exploration. Larvae from different adult populations were significantly, although not linearly, affected by the interaction of all factors to which parents were exposed, at both 24h and 48h post fertilization. Specifically, the exposure of larvae to elutriates from contaminated sediments determined a developmental delay, a reduction in size and an increased percentage of abnormalities in all larval populations independently of their parental exposure. On the contrary, larvae coming from parents exposed to contaminated sediments, when reared in clean filtered sea water, succeed in developing until the echinopluteus stage after 48h, with size and abundance comparable to those of larvae from control parents. Pre-exposure of parents to contaminated sediments did not successfully buffer the negative effects of elutriates on their offspring, and no positive effects of 'super-saturated' levels of O_2 in response to contaminants were observed, suggesting that the Bagnoli-Coroglio area is currently not suitable for the re-stocking or re-introduction of this species.

1 Introduction

Human activities have heavily modified marine environments, especially in coastal areas where increasing urbanization and associated activities are responsible for the release of a range of contaminants that ultimately accumulate in marine sediments and contribute to habitat alteration (Agardy et al. 2005; Arizzi Novelli et al. 2006; Bertocci et al. 2019; Vitousek et al. 1997). Disused industrial plants are often significant sources of a wide range of chemicals that crucially threaten

both environmental and human health. Furthermore, inshore areas are particularly vulnerable to ongoing climate change, including the current and predicted increase in the intensity and frequency of severe storms and associated water turbulence, which can promote the resuspension of polluted sediments and availability of toxic chemicals in the water column and nearby habitats (Neumann et al. 2015; Spalding et al. 2014). The Bagnoli-Coroglio brownfield site (Southern Tyrrhenian Sea, Italy) is an infamous example of a heavily polluted marine area. All industrial activity at the site ceased in the mid 1990s, after almost a century of concrete, steel, chemical and asbestos production resulting in heavy contamination of both the soil and marine sediments. In particular, high concentrations of heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) occur in this area (Albanese et al. 2010; Arienzo et al. 2017; De Vivo and Lima 2008; Trifuoggi et al. 2017). Consequently, in 2014 Bagnoli-Coroglio bay was designated as a Site of National Interest (SNI) requiring environmental rehabilitation.

Several studies have found that exposure to polluted sediments can have severe consequences on marine organisms, including changes in biodiversity and ecosystem functioning (Bertocci et al. 2019; Martínez-Lladó et al. 2007; Ryu et al. 2011). These effects may be exacerbated in combination with those of global climate change, such as warming and ocean acidification, which can further contribute to dramatically alter the development of marine organisms (Bressan et al. 2014; Matozzo et al. 2012; 2013; Munari et al. 2016; 2018; 2019; Range et al. 2014). Additional concerns arise from the current and predicted modification of patterns of intensity and temporal occurrence of extreme weather conditions (Aumann et al. 2008; Burge et al. 2014; Easterling et al. 2000; Trapp et al. 2007; Wolff et al. 2016), which can result in more severe and frequent exposure of marine organisms to the re-suspension of chemical substances accumulated in sediments. Thus, it is pivotal to assess the combined effects of sediment pollution and changing patterns of water turbulence on marine organisms, to better understand and predict wide anthropogenic impacts on marine systems and to effectively direct environmental restoration plans.

The effects of environmental chemical contamination can be potentially buffered by oxygen release in coastal habitats, due to the activity of photosynthetic organisms that may enhance animal aerobic performance under stressful conditions, as reported for several marine taxa in response to ocean warming (Giomi et al. 2019). It must also be considered that, under natural conditions, marine organisms are typically adapted to respond to environmental changes within a tolerance

range set by internal physiological regulation (Pörtner et al. 2004; Zhang et al. 2012). While the maintenance of homeostasis allows acclimatization, it may result in a reallocation of resources away from growth and reproduction. Furthermore, it is widely recognized that early life stages of many organisms are more sensitive than adults to environmental stress (Byrne 2012; Moran 1994), representing a possible bottle-neck for natural populations. However, negative effects on early life stages imposed by environmental perturbations can be mitigated by anticipatory parental effects (Agrawal et al. 1999; Marshall and Uller 2007; Thor and Dupont 2015). This type of heritable plasticity, being a source of phenotypic individual variation within populations, has important ecological and evolutionary consequences (Uller 2008), and may allow populations to adapt to new environmental conditions (Miller et al. 2012; Parker et al. 2012; Reed et al. 2011).

In this study we decided to use the purple sea urchin *Paracentrotus lividus* (Lamarck, 1816) since it is widely used as a model species in many ecotoxicological studies, being considered a good bioindicator of environmental change (Bellas 2008; Bonaventura et al. 2011; Bošnjak et al. 2011; Dinnel et al. 1988; Kobayashi and Okamura 2004; Matranga et al. 2000; Morale et al. 1998; Pinsino et al. 2010; Sconzo et al. 1995; Zito et al. 2005). Furthermore, the species has a key role in shaping benthic marine communities in the Mediterranean Sea and Atlantic Ocean (Guidetti et al. 2003; Guidetti 2004; Sala and Zabala 1996), where it also represents an important commercial resource (Bertocci et al. 2014). *P. lividus* is a gonochoric species lacking sexual dimorphism (Gianguzza et al. 2009). The reproductive cycle of *P. lividus* is annual, with three main phases: the growing phase (late autumn and winter) when gonads accumulate reserve material; the maturation phase (spring and early summer) in which gametogenesis and spawning take place; and the spent/regenerating phase in which relict gametes are resorbed by the nutritive phagocytes (Spirlet et al. 1998). Here, we first exposed adult sea urchins during their gametogenesis phase to experimental combinations of two water turbulence patterns ('aggregated' vs. 'spaced' events of re-suspension of contaminated sediments) and two levels of dissolved oxygen ('normal' vs. 'super-saturated'), in order to simulate oxygen production by photosynthetic organisms, (Viaroli and Christian 2003). Larval populations were obtained from adults exposed to each of these combinations of factors and reared either in clean filtered sea water (FSW) or in the presence of elutriates from contaminated sediments. The aims were (i) to assess the simultaneous and potential interactive effects of patterns of turbulence associated with the resuspension of contaminated sediments and oxygen availability on larval development after the exposure of adults during

gametogenesis; (ii) to quantify the parental effect on offspring performance after the pre-exposure of adults to stressful conditions.

2 Materials & Methods

2.1 Study Site

The study site was the coastal area of Bagnoli-Coroglio, an industrially contaminated region located in the South-Eastern part of the Pozzuoli Bay (Gulf of Naples – southern Tyrrhenian Sea, Figure 1), characterized by sandy and clay substrates protected by artificial rocky reef (Bertocci et al. 2019; Cocco et al. 1988; De Pippo et al. 1988). Industrial activity was ongoing in this area from 1905 to 1990, predominantly in the form of steel production, asbestos manufacturing, concrete and chemicals production. In 1994, the Italian Government implemented a remediation plan for this brownfield site, which was classified as a SNI in 2000. Previous studies on the chemical composition of coastal marine sediments in the Bagnoli area highlighted heavy contamination by metals, PAHs and PCBs (Albanese et al. 2010; Arienzo et al. 2017; Trifuoggi et al. 2017). High sediment concentrations of heavy metals such as Cu, Fe, Hg, Mn, Ni, Pb and Zn, were reported by Romano et al. (2018), although they could also be related to local hydrothermal activity and other anthropogenic sources (De Vivo and Lima 2008). Environmental data as temperature, conductivity/salinity, dissolved oxygen and fluorescence profiles were collected during five cruises from February to October 2019 in seven stations within the Pozzuoli Bay, and results are shown in Margiotta et al. (2020).

Analyses of the historical data on the benthic diversity in the Bagnoli-Coroglio site, reported the occurrence of the species *Paracentrotus lividus* in this area in the past (Gaglioti et al. 2020), however further studies are needed to assess the current state of the local *P. lividus* population.

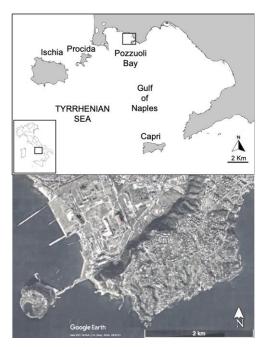Figure 1: Map of the Bagnoli-Coroglio study area.

2.2 Field oxygen data collection

To evaluate natural fluctuations in water oxygenation, dissolved oxygen was monitored by mooring an oxygen probe equipped with calibrated dissolved oxygen concentration (optical) sensors (MiniDOT sensors, PME, Vista, Ca, USA). The probe, with a logging frequency of five minutes, was deployed within a seagrass (*Posidonia oceanica*) meadow in an uncontaminated area in the Gulf of Naples (40°43'58"N 13°57'59"E; temperature 15.00±0.50°C, salinity 38.00±0.03 psu), in order to reflect the expected conditions at the study site once the ecosystem has been successfully restored.

2.3 Animal collection

Two hundred adults *P. lividus* individuals were collected in March 2019 at San Pancrazio (40°42'15.0"N 013°57'22.3"E), Ischia Island (Gulf of Naples), at a maximum depth of 5 m with the authorization of the Marine Protected Area 'Regno di Nettuno'. Animals were kept in a cool box after collection and in the laboratory prior to experimental procedures. *P. lividus* is not protected or endangered and all experimental procedures on animals were in compliance with the guidelines of the European Union (Directive 609/86).

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2.4 Adult sea urchin rearing mesocosms

'*Ad hoc*' mesocosms comprising twelve independent, closed recirculating tanks (~ 50 liters each), filled with natural sea water, were set up in the Stazione Zoologica Anton Dohrn facility of Villa Comunale (Naples) for the maintenance of marine organisms. Each tank had an internal filter divided into three sequential compartments. The first compartment was filled with filtering wool and foam sponges, ensuring mechanical filtration by collecting particulate matter and debris. The second compartment housed SUBSTRATpro (Ehiem GmbH & Co. KG, Deizisau, Germany), a pearl-shaped sintered glass specifically designed for the colonization of aerobic chemoautotrophic bacteria belonging to genus *Nitrosomonas*, *Nitrosococcus* and *Nitrospira* (Schreier et al. 2010) as well as several ammonia-oxidizing archaea (Hatzenpichler 2012), to ensure biological filtration. The third compartment contained a Syncra Silent recirculating pump (pump flow rate 700 L/h, pump wattage 8 Watt; SICCE S.r.l, Pozzoleone, Italy) to ensure water recirculation in the internal filter. The sea water level was checked on a daily basis and manually adjusted to balance evaporation. Chemical-physical variables of both sea water and sediments are available in Ruocco et al. (2020).

2.5 Adult sea urchin experimental design

The bottom of eight tanks, randomly chosen of the twelve, was covered with approximately 5 centimeters of sediment collected from a sampling station located within the Bagnoli-Coroglio area (40°48'25.9''N, 014°09'33.2''E; depth: 3.8 meters). The sediment used to cover the tanks came from the same sampling core, however before being used, it was uniformly mixed. Data regarding the composition and quantification of the contaminants within the sediment are described in Morroni et al. (2020).

The remaining four tanks were maintained with sea water without sediment. Two of these tanks without sediment, and four of the tanks with sediment were randomly assigned to exposure to the 'spaced' turbulence pattern (two events, 48h duration, separated by two weeks within one month of exposure). The remaining tanks were assigned to exposure to the 'aggregated' turbulence pattern (two events, 48h duration, separated by three days within one month of exposure). This allowed the manipulation of the temporal variance of turbulence events, controlling the total frequency and intensity of events established over the course of the experiment (Benedetti-Cecchi

2003; Benedetti-Cecchi et al. 2006; Bertocci et al. 2005). The application of water turbulence events in tanks without sediment allowed us to test potential effects of hydrodynamics, independently of the presence of sediments and associated chemical and physical effects. A detailed illustration of the mesocosm experimental design was provided by Ruocco et al. (2020). To produce water turbulence, and re-suspension of the sediment, an electric pump (flow rate 4500L/h, power: 10 watt) was placed in each experimental tank. Duration and frequency of experimental re-suspension events were set up on the measures of the natural storm events in the study area. The environmental data such as wind speed, the wave height and period were collected daily from an oceanographic buoy within a monitoring program of the Stazione Zoologica Anton Dohrn as reported by Ruocco et al. (2020). Water and suspended particles were collected on the second day following each event of turbulence and analyzed for heavy metals and polycyclic aromatic hydrocarbons (PAHs) content. Chemical analyses were performed by Bioscience Research Center SRL (Orbetello, Italy). Details of the analyses are reported in Ruocco et al. (2020).

Oxygen was provided by bubbling it into four tanks randomly chosen out of the eight tanks with sediment using an ACQ140 automatic control system (Aquatronica, Reggio Emilia, Italy) connected to submersed dissolved oxygen probes (Aquatronica, model ACQ310N-O2) via digital oxygen interfaces (Aquatronica, model ACQ210N-O2). In addition, in order to ensure a constant dissolved oxygen concentration, the automatic control system digitally controlled eight solenoid valves (Aquatronica, model ACQ421) connected to a 40-liter oxygen tank. Specifically, a value of 90% of oxygen saturation served as reference while a value of 200% was chosen as representative of the daily super-saturation (Table 1, Figure 2). Ten adult sea urchins per tank (seven females and three males) were then exposed to each experimental condition for a month, during gonad development, under a 12h:12h light: dark photoperiod setting. Adults were daily checked for mortality and fed with 'sea lettuce', *Ulva lactuca* Linnaeus *ad libitum*.

Table 1: List of abbreviation of adult sea urchin experimental design

ID	Sediment	O₂ saturation	Pattern of turbulence
POP 1	NO	90%	Aggregated
POP 2	NO	90%	Spaced
POP 3	YES	90%	Aggregated
POP 4	YES	90%	Spaced
POP 5	YES	200%	Aggregated
POP 6	YES	200%	Spaced

Figure 2: Adult experimental design. See M&M section for details.

2.6 Larval experimental design

At the end of the exposure period, adults from each tank were weighed and the test diameter of each individual was measured. A minimum of three females and three males from each experimental condition (n = 6) were separated to constitute pools of gametes in order to establish six unique laboratory populations of embryos. Sperms were collected with a micropipette and kept dry on ice in a plastic tube until use. Eggs were collected in 500 mL beakers filled with 0.22 μm FSW. Prior to use, egg and sperm quality and quantity were evaluated. Pools of eggs and sperms were established using the same number of gametes from each individual, in order to have the same contribution from every parent during fertilization. A constant sperm: egg ratio (1250:1) was used (Moschino and Marin 2002), and fertilization was checked after 30 minutes by observing the elevation of the jelly coat. Embryo populations were then maintained in 25 ml vials, three replicates for each experimental condition, for 48h of exposure at 22°C. To test parental effect in

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response to the selected factors, offspring from each parental population were exposed to the conditions that parents experienced during gametogenesis. Embryos coming from parents exposed to sediments were reared in elutriates. Elutriates were prepared as reported by Gallo et al. (2020) from the same sediment core used in the adult tank. Specifically, POP 1 and POP 2 embryos were exposed to FSW at O_2 90%-saturation level (FSW_90% O_2), POP 3 and POP 4 to elutriate at O_2 90%-saturation level (ELU_90% O_2), and POP 5 and POP 6 to elutriate at O_2 200%-saturation level (ELU_200% O_2). Furthermore, POP 1 and POP 2 embryos were transplanted into FSW at 'super-saturated' O_2 level (FSW_200% O_2), into elutriates at 'normal-saturated' O_2 level (ELU_90% O_2) and elutriates at ''super-saturated'' O_2 level (ELU_200% O_2), while POP 3, 4, 5 and 6 embryos were transplanted into FSW at 'normal-saturated' O_2 level (FSW_90% O_2). This approach has the potential to highlight: (a) specific effects of parental exposure on embryonic and larval development comparing larval populations reared in FSW; (b) selective pressure acting during gametogenesis and resulting in a better response of offspring from adults reared in stressful conditions to stress (in this case adults with the highest phenotypic plasticity are most likely to spawn and pass down their genotypes to the next generation).

2.7 Larval developmental traits

The effects of the three combined factors on larval growth were examined in two developmental stages (24h and 48h post-fertilization). Embryos at 24h and 48h post-fertilization (hereafter as hpf) were observed with an optical macroscope (Leica Z16 APO), equipped with a Leica DFC 300FX camera connected to a computer with the Leica LAS program (Leica Application Suite, Version 4.5). A minimum of 100 embryos per replicate were photographed. Different developmental stages were distinguished to evaluate any delay after 24 and 48 hours of exposure, and their frequencies were calculated. Five main classes were considered: arrested that corresponds to undeveloped embryos, gastrulae distinguished by the onset of invagination, prism characterized by the early development of calcareous rods and the typical cuboid shape of the embryos, early pluteus is distinguished by the beginning development of the four appendixes, echinopluteus represents the 48 hpf stage characterized by the total development of oral and aboral rods. (Figure 3).

For echinoplutei, the frequency of abnormal larvae (Figure 4) was evaluated and the length of somatic (SS) and oral (OS) spicules (Figure 5) was assessed using image analysis software (IMAQ^{TM} Vision, National Instrument^{TM}, Version 6.0).

Figure 3: *P. lividus* developmental stages.

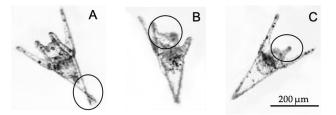From left: arrested embryos, gastrulae, prism, early pluteus and echinopluteus.

Figure 4: Examples of anomalous 4-arm pluteus at 48 ==hpf.== ==A: crossed spicules,==
==B and C: asymmetrical or completely degenerated arms==
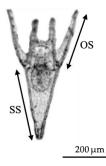

Figure 5: Somatic (SS) and oral (OS) spicules at 48 hpf.

2.8   Statistical analysis

A non-parametric PERMutational multivariate ANalysis Of Variance, PERMANOVA (Anderson and Braak 2003) applied on the Euclidean distance matrix of raw data was chosen to test differences on larval growth among experimental conditions. A one-way PERMANOVA model was used to test for differences among larval populations reared in FSW_90% O_2 only, as well as to test differences among original populations. In cases where results were significant, PERMANOVA was used to test for the interactive effect of the factors, i.e., turbulence pattern, sediment presence and oxygenation, to which parents had been exposed on larval development. Furthermore, PERMANOVA was used to run relevant pair-wise comparisons among and within larval populations. In cases where the number of unique values from permutations was too low,

the Monte-Carlo procedure was used to calculate p values. The software package PRIMER 6 PERMANOVA Plus (PRIMER-E Ltd, Plymouth, UK) was used for all statistical analyses.

3 Results

3.1 Natural variation of seawater oxygenation

Data collected during the mooring of the probe revealed that oxygen levels in the *P. oceanica* meadow fluctuated during the day; in particular, peaks of oxygen production occurred at mid-day causing local conditions of oxygen super-saturation. Diel (i.e. a 24-hour period) seawater oxygen fluctuations are shown in Figure 6.
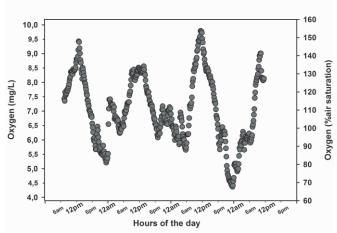

Figure 6: Diel seawater dissolved oxygen fluctuations (five minutes logging frequency).

3.2 Larval developmental stages

3.2.1 Effects of parental exposure on larval developmental stage in FSW

After 24 and 48 hpf, a significant effect of parental pre-exposure on larval development was evident on larvae reared in FSW (Table 2). At 24 hpf, all the parental experimental factors and their interactions were significant (Table 2). Overall, the adults who have experienced a spaced pattern of turbulence gave birth to a higher percentage of early plutei than those who have experienced an aggregated one. In general, an average percentage of 65% of early plutei from parents exposed to a spaced pattern of turbulence was recorded, while an average percentage of

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41% of early plutei from parents exposed to an aggregated pattern of turbulence was observed (Figure 7).

Oxygen super-saturation had a negative effect on the average percentage of early plutei as well. In particular an average percentage of 78% of early plutei between POP 3 and POP 4 was observed, while the average percentage between POP 5 and POP 6 was around 41% (Figure 7).

At 48 hpf, all the parental experimental factors and their interactions were significant, except for the sediment alone and the interaction between the three factors (Table 2). Overall, the average percentages of 4-arms echinoplutei from both parents exposed to spaced and aggregated pattern of turbulence, were comparable (89% and 94% respectively) (Figure 8). As observed at 24 hpf, the oxygen super-saturation had a negative effect on the average percentages of echinoplutei. In particular almost all (99%) larvae from POP 3 and POP 4 succeed to develop in echinoplutei, while the average percentage of echinoplutei between POP 5 and POP 6 was around 87% (Figure 8). However, these results take into account the total percentage of echinoplutei without distinguish among normal and abnormal ones.

3.2.2   Transgenerational effects of parental exposure on larval developmental stages

In general, after 24 and 48 hpf, a buffer effect of the adults' pre-exposure to contaminated sediments was not observed in larvae reared in the presence of elutriates. In fact, larvae grew better in FSW regardless the parents of origin. For each experimental larval population, the percentages of developmental stages of larvae reared in FSW and those reared in the presence of elutriates, were always significantly different (Table 2). At 24 hpf, all the parental experimental factors and their interactions were significant (Table 2). Overall, as observed in all larval populations reared in FSW, at 24 hpf a negative effect of the pre-exposure of adults to the aggregated pattern of turbulence was recorded in the larvae reared in the presence of elutriates (Figure 7). Oxygen super-saturation had a negative effect on the average percentage of early plutei as well in all the larval populations exposed to elutriates (Figure 7).

At 48 hpf, all the parental experimental factors and their interactions were significant, except for the turbulence alone (Table 2). The same trend described for larvae at 24 hpf has been recorded in larval development at 48 hpf, for what concern the negative effects of adults' pre-exposure to both aggregated pattern of turbulence and oxygen super-saturation (Figure 8).
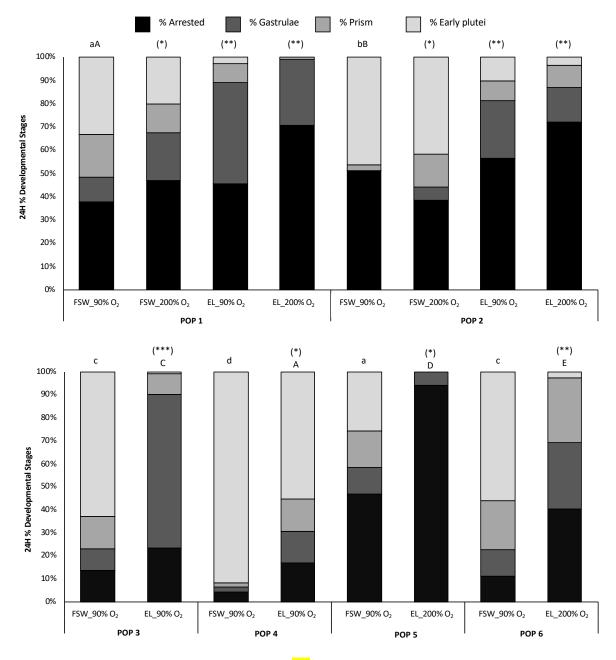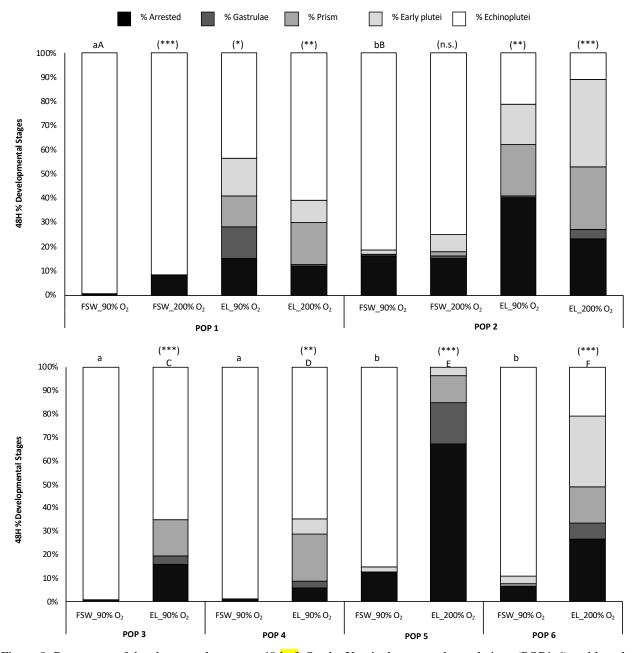
Figure 7: Percentage of developmental stages at 24 hpf. On the X axis the parental populations (POP1-6) and larval exposure conditions are shown. Significant differences ($p < 0.05$) among larval populations reared in FSW for each experimental parental condition are represented with lower-case letters (a-d), while capital letters (A-E) represent the significant differences among the larval populations reared under similar parental conditions. Asterisks represent the intra-group (FSW_90% O_2 vs. all other larval conditions) significant differences (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$), n.s. = not-significant ($p > 0.05$)

Figure 8: Percentage of developmental stages at 48 hpf. On the X axis the parental populations (POP1-6) and larval exposure conditions are shown. Significant differences (p < 0.05) among larval populations reared in FSW for each experimental parental condition are represented with lower-case letters (a-b), while capital letters (A-F) represent the significant differences among the larval populations reared under similar parental condition. Asterisks represent the intra-group (FSW_90% O_2 vs. all the other larval conditions) significant differences (*: p < 0.05, **: p < 0.01, ***: p < 0.001), n.s. = not-significant (p > 0.05)

3.3 Larval skeletal abnormalities and growth

3.3.1 Effects of parental exposure on larval skeletal development in FSW

After 48 hpf, the percentage of normally developed echinoplutei was dependent on the parental pre- exposure for larvae reared in FSW (Table 2).

The interaction between turbulence pattern and sediments influenced the normal formation of the calcareous structures in larvae maintained in FSW (Table 2). Overall, the percentage of normally developed echinoplutei both from parents who experienced a spaced pattern of turbulence and an aggregated one was comparable (77%) (Figure 9). Oxygen super-saturation had a negative effect on the average percentage of normally developed echinoplutei. In particular an average percentage of 88% of normal echinoplutei between POP 3 and POP 4 was observed, while the average percentage between POP 5 and POP 6 was around 65% (Figure 9).

The length of both SS (Figure 10 A) and OS (Figure 10 B) was significantly affected by the parental population of origin in larvae reared in FSW (Table 2). In general, larvae reared in FSW had longer SS and OS compared to their siblings reared in elutriates (Figure 10 A-B), regardless the parent of origin.

3.3.2 Transgenerational effects of parental exposure on larval skeletal development

After 48 hpf, the percentage of normally developed echinoplutei was dependent on the parental pre- exposure for larvae reared in the presence of elutriates (Table 2).

In general, it is possible to observe a negative effect of both aggregated pattern of turbulence and super-saturated oxygen level which parents were subjected (Figure 9). Furthermore, no normal echinoplutei were observed in both POP 2 and POP 5 when exposed to elutriates (Figure 9).

The length of both types of spicules was affected by parental exposure as well (Table 2). Specifically, larvae maintained in elutriates had shorter spicules compared to their siblings reared in FSW, regardless the parent of origin, with a mean length reduction of 40% for both somatic and oral spicules (Figure 10 A-B).
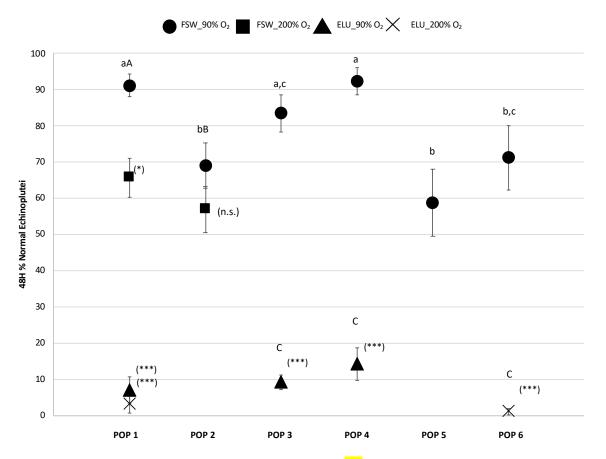
Figure 9: Percentage ± SD (n = 3) of normal echinoplutei at 48 hpf. On the X axis parental populations (POP1-6) are shown. Larval exposure conditions are shown in the legend. Significant differences (p < 0.05) among larval populations reared in FSW for each experimental parental condition are represented with lower-case letters (a-d), while the capital letters represent the significant differences among the larval populations reared at the same parental condition. Asterisks represent the intra-group significant differences (*: p < 0.05, ***: p < 0.001, n.s. not-significant; n = 3).
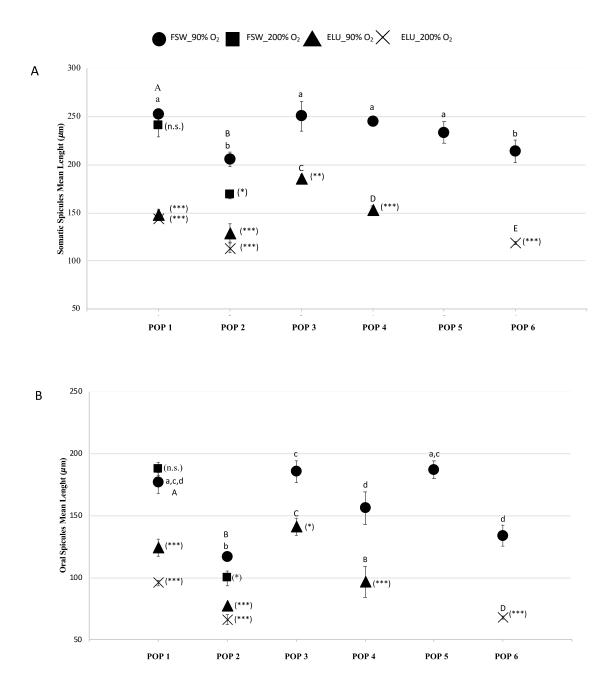
Figure 10 A-B: Mean length ± SD (n = 3) of somatic (A) and oral (B) spicules at 48 hpf. On the X axis parental populations (POP1-6) are shown. Larval exposure conditions are shown in the legend. Significant differences (p < 0.05) among larval populations reared in FSW for each experimental parental condition are represented with lower-case letters (a-b), while capital letters (A-E) represent the significant differences among the larval populations reared under the same parental conditions. Asterisks represent the intra-group (FSW_N-O_2 vs. all other larval conditions) significant differences (*: p < 0.05, **: p < 0.01, ***: p < 0.001), n.s. = not-significant.

Table 2: PERMANOVA results. Pseudo--F values (indicated as F) and Monte Carlo p-values for all the parameters measured for larvae reared in both filtered sea water FSW and under parental conditions (PC).

	Developmental stages 24h		Developmental stages 48h		Anomalies 48h		Somatic Spicules (SS) 48h		Oral Spicules (OS) 48h	
	FSW rearing	PC - rearing	FSW - rearing	PC - rearing	FSW - rearing	PC - rearing	FSW - rearing	PC - rearing	FSW - rearing	PC - rearing
Larval population	$F_{(5,17)}=22.551$ $p_{(MC)}<0.001$	$F_{(5,17)}=17.248$ $p_{(MC)}<0.001$	$F_{(5,17)}=11.999$ $p_{(MC)}<0.001$	$F_{(5,17)}=41.511$ $p_{(MC)}<0.001$	$F_{(5,17)}=11.417$ $p_{(MC)}<0.001$	$F_{(5,17)}=190.4$ $p_{(MC)}<0.001$	$F_{(5,17)}=11.260$ $p_{(MC)}<0.001$	$F_{(5,17)}=1043.8$ $p_{(MC)}<0.001$	$F_{(5,17)}=33.995$ $p_{(MC)}<0.001$	$F_{(5,17)}=246.920$ $p_{(MC)}<0.001$
Turbulence, T	$F_{(1,17)}=19.303$ $p_{(MC)}<0.001$	$F_{(1,17)}=8.133$ $p_{(MC)}=0.011$	$F_{(1,17)}=15.787$ $p_{(MC)}<0.001$	$F_{(1,17)}=3.405$ $p_{(MC)}=0.067$	$F_{(1,17)}=2.655$ $p_{(MC)}=0.116$	$F_{(1,17)}=14.360$ $p_{(MC)}<0.001$	$F_{(1,17)}=34.023$ $p_{(MC)}<0.001$	$F_{(1,17)}=0.421$ $p_{(MC)}=0.529$	$F_{(1,17)}=137.52$ $p_{(MC)}<0.001$	$F_{(1,17)}=50.987$ $p_{(MC)}<0.001$
Sediment, S	$F_{(1,17)}=31.161$ $p_{(MC)}<0.001$	$F_{(1,17)}=15.245$ $p_{(MC)}<0.001$	$F_{(1,17)}=2.651$ $p_{(MC)}=0.121$	$F_{(1,17)}=88.150$ $p_{(MC)}<0.001$	$F_{(1,17)}=3.928$ $p_{(MC)}=0.063$	$F_{(1,17)}=726.17$ $p_{(MC)}<0.001$	$F_{(1,17)}=1.865$ $p_{(MC)}=0.196$	$F_{(1,17)}=2389.7$ $p_{(MC)}<0.001$	$F_{(1,17)}=19,239$ $p_{(MC)}=0.001$	$F_{(1,17)}=429.680$ $p_{(MC)}<0.001$
O$_2$ (S)	$F_{(1,17)}=40.853$ $p_{(MC)}<0.001$	$F_{(1,17)}=26.045$ $p_{(MC)}<0.001$	$F_{(1,17)}=23.206$ $p_{(MC)}<0.001$	$F_{(1,17)}=84.888$ $p_{(MC)}<0.001$	$F_{(1,17)}=31.554$ $p_{(MC)}<0.001$	$F_{(1,17)}=182.86$ $p_{(MC)}<0.001$	$F_{(1,17)}=16.886$ $p_{(MC)}=0.001$	$F_{(1,17)}=1653.2$ $p_{(MC)}<0.001$	$F_{(1,17)}=4.328$ $p_{(MC)}=0.057$	$F_{(1,17)}=472.850$ $p_{(MC)}<0.001$
TxS	$F_{(1,17)}=10.559$ $p_{(MC)}=0.0014$	$F_{(1,17)}=7.864$ $p_{(MC)}<0.001$	$F_{(1,17)}=26.779$ $p_{(MC)}<0.001$	$F_{(1,17)}=17.435$ $p_{(MC)}<0.001$	$F_{(1,17)}=19.808$ $p_{(MC)}<0.001$	$F_{(1,17)}=19.840$ $p_{(MC)}<0.001$	$F_{(1,17)}=11.190$ $p_{(MC)}=0.007$	$F_{(1,17)}=364.79$ $p_{(MC)}<0.001$	$F_{(1,17)}=4.654$ $p_{(MC)}=0.049$	$F_{(1,17)}=112.050$ $p_{(MC)}<0.001$
TxO2 (S)	$F_{(1,17)}=4.086$ $p_{(MC)}=0.0271$	$F_{(1,17)}=24.294$ $p_{(MC)}<0.001$	$F_{(1,17)}=1.595$ $p_{(MC)}=0.229$	$F_{(1,17)}=8.8705$ $p_{(MC)}=0.004$	$F_{(1,17)}=0.170$ $p_{(MC)}=0.749$	$F_{(1,17)}=14.588$ $p_{(MC)}=0.749$	$F_{(1,17)}=1.321$ $p_{(MC)}=0.272$	$F_{(1,17)}=776.57$ $p_{(MC)}<0.001$	$F_{(1,17)}=5.433$ $p_{(MC)}=0.035$	$F_{(1,17)}=205.34$ $p_{(MC)}<0.001$

4   Discussion

In this study, the combined effect of contaminated sediment and its re-suspension associated with different turbulence patterns at both normal and 'super-saturated' levels of oxygen were studied for the first time. In general, a negative effect of the pre-exposure of adults to the examined experimental factors on larvae was observed. Furthermore, larvae derived from parents maintained in the presence of sediments did not perform better when exposed to elutriates, even when reared at a 'super-saturated' level of oxygen.

Echinoderms, including the sea urchin *P. lividus*, have been widely used as marine model organisms to study the effect of different anthropogenic stressors such as heavy metals, personal hygiene products, PAHs as well as environmental variability (Byrne 2012; Carballeira et al. 2012; Corinaldesi et al. 2017; Morroni et al. 2016; 2018). Marine sediments close to marinas, harbors and industrial plants are likely to be enriched, in particular, with heavy metals and PAHs, as recently documented in the Bagnoli-Coroglio area (Morroni et al. 2020; Ruocco et al. 2020). Exposure to these substances can jeopardize the normal development of early life stages of echinoderms, and make their populations more sensitive to environmental perturbations (Byrne 2012). Despite the large number of studies that have addressed the effects of single stressors, such as heavy metals and PAHs, on benthic organisms, those that experimentally examine the interaction between different types of physical, chemical and mechanical variables are scarce and are often focused on a single life stage.

Furthermore, it should be considered that in the natural environment increased water oxygenation due to the diel photosynthetic activity of algae and macrophytes can have a positive effect on the physiological performance of the organisms living associated with them or close by.

Giomi et al. (2019), found that maintenance at a 'super-saturated' level of oxygen (140 ± 3%) increased the LT_{50} of six Red Sea species (1 decapod, 1 echinoderm, 1 holothurian, 1 bivalve, 2 teleosts) by 2°C compared to those maintained at normal levels of oxygen saturation (97 ± 2%). Migliaccio et al. (2015) found that the exposure of *P. lividus* adults for nine days to cadmium (Cd) and manganese (Mn) during gametogenesis resulted in a higher percentage of abnormal larvae and delayed larval development when compared with larvae from control parents, despite all larval populations being reared in control sea water. Conversely, in our study, when all larval populations

were exposed to elutriates to mimic the effect of re-suspended sediments, there was a significant difference in larval development when compared to their siblings reared in FSW_90% O_2.

These findings are in accordance with several studies that found the exposure to a mix of heavy metals present in marine sediment to cause a negative effect on larval development in echinoderms, such as *P. lividus*, *Asterias rubens*, *Psammechinus miliaris* and *Strongylocentrotus intermedius* (Coteur et al. 2003; Marin et al. 2001; Xu et al. 2011). It is widely known that the combination of different heavy metals is often more toxic than one alone, due to additive and/or synergistic effects (Kobayashi and Fujinaga 1976; Pagano et al. 1996). Xu et al. (2011), for example, highlighted that the combination of different heavy metals such as Cd, Cu, Pb, Zn had a negative synergistic effect on the larval development of the sea urchin *S. intermedius*. Based on existing literature, the authors concluded that the presence of Cd could have led to oxidative damage by causing accumulation of reactive oxygen species (ROS) in larval tissue (Hassoun and Stohs 1996), that can intensify the sensitivity of cells to other toxicants present (Brzóska et al. 2008; Chowdhury et al. 1987). Furthermore, abnormal larval development could be due to the reduction of acetylcholinesterase (AChE) activity in plutei, as observed by Maisano et al. (2015) after larval exposure to increasing concentrations of CuO nanoparticles for 24h. AChE plays a pivotal role in the migration of primary mesenchyme cells (PMCs) and spicule elongation (Ohta et al. (2009). PMCs are responsible for the formation of skeletal spicules, which migrate into the blastocoel and produce these calcareous structures. Moreover, PAHs can interfere with echinoderm larval development as observed by Suzuki et al. (2015) in sea urchin *Hemicentrotus pulcherrimus* larvae exposed to two PAHs, benz[a]anthracene (BaA) and 4-hydroxybenz[a]anthracene (4-OHBaA), for 53h post-fertilization. This was due to a significant reduction of the expression of several genes involved in the formation of spicules, which the authors attributed to suppression of the transcription factor for spicule formation by 4-OHBaA. Furthermore, Pillai et al. (2003) suggest that several PAHs (phenanthrene, fluorene, and pyrene) can cause exogastrulation in exposed embryos, causing a vegetative state and death. This is corroborated by the high percentage of dead embryos in larval populations exposed to elutriates at both 24h and 48h post fertilization in our study.

Contrary to the expectation that adults experiencing more stressful conditions would produce more resistant offspring, due to anticipatory parental effect or the selection of resistant phenotypes (Thor and Dupont 2015; Weston et al. 2013), larvae from parents reared with sediments at both O_2 levels

and turbulence patterns were less resistant to elutriates than those from control parents, as highlighted by the total absence of echinoplutei in POP 5.

Nevertheless, it may be possible that one month of exposure during the gametogenesis was not enough to trigger a transgenerational buffer effect against sea water pollution.

Furthermore, no positive effects of higher oxygen levels on either of the life stages was observed and, in fact, larvae exposed to oxygen super-saturation showed a higher percentage of abnormal larvae and echinoplutei compared to larval populations reared in FSW_90% O_2. Notably, the accumulation and toxicity of Ag, As, Co and Cr in the freshwater species, *Daphnia magna* after 53 days of exposure was enhanced at 90% oxygen saturation compared to exposure at 40% oxygen saturation (De Jonge et al. (2012). The authors suggested that elevated oxygen levels resulted in the increased leaching of sediment-bound metals and their bio-availability (De Jonge et al. 2012). In addition, it might be possible that increased oxygen availability may fuel higher metabolic activity, resulting in a more efficient accumulation of contaminants. Our study provides the first evidence that the natural occurrence of oxygen super-saturation may exacerbate the bioaccumulation of contaminants, but at the same time raises questions about an increased efficiency of detoxification mechanisms and excretion processes.

These results constitute an important baseline in the understanding of the ability of marine species to adapt, in a relatively short time and through few generations, to current and predicted marine environmental stress. The study of different traits such as gametogenesis in adults during exposure to stress, and consequently the quality of gametes, as well as embryo/larval physiological performance under different experimental conditions can contribute to better predict if species or populations could be successfully re-introduced or re-stocked into a perturbed environment. According to our study, unless considerable decontamination of marine sediments is achieved, we suggest that it would be unlikely to have a permanent and healthy population of *P. lividus* in Bagnoli-Coroglio bay.

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Author contibution

References

Agardy, T., Alder, J., Dayton, P., Curran, S., Kitchingman, A., Wilson, M., Catenazzi, A., Restrepo, J., Birkeland, C. and Blaber, S. (2005). Coastal systems. In *Ecosystems and human well-being: current state and trends* (pp. 513-550). Washington, DC: Island Press.

Agrawal, A. A., Laforsch, C. and Tollrian, R. (1999). Transgenerational induction of defences in animals and plants. *Nature*, 401(**6748**), 60.

Albanese, S., De Vivo, B., Lima, A., Cicchella, D., Civitillo, D. and Cosenza, A. (2010). Geochemical baselines and risk assessment of the Bagnoli brownfield site coastal sea sediments (Naples, Italy). *Journal of Geochemical Exploration*, 105(**1**), 19-33. doi:https://doi.org/10.1016/j.gexplo.2010.01.007

Anderson, M. and Braak, C. T. (2003). Permutation tests for multi-factorial analysis of variance. *Journal of statistical computation and simulation*, 73(**2**), 85-113.

Arienzo, M., Donadio, C., Mangoni, O., Bolinesi, F., Stanislao, C., Trifuoggi, M., Toscanesi, M., Di Natale, G. and Ferrara, L. (2017). Characterization and source apportionment of polycyclic aromatic hydrocarbons (PAHs) in the sediments of gulf of Pozzuoli (Campania, Italy). *Marine Pollution Bulletin*, 124(**1**), 480-487.

Arizzi Novelli, A., Losso, C., Libralato, G., Tagliapietra, D., Pantani, C. and Ghirardini, A. (2006). Is the 1: 4 elutriation ratio reliable? Ecotoxicological comparison of four different sediment: water proportions. *Ecotoxicology and Environmental Safety*, 65(**3**), 306-313.

Aumann, H. H., Ruzmaikin, A. and Teixeira, J. (2008). Frequency of severe storms and global warming. *Geophysical Research Letters*, 35(**19**).

Bellas, J. (2008). Prediction and assessment of mixture toxicity of compounds in antifouling paints using the sea-urchin embryo-larval bioassay. *Journal of Aquatic toxicology*, 88(**4**), 308-315.

Benedetti-Cecchi, L. (2003). The importance of the variance around the mean effect size of ecological processes. *Ecology*, 84(**9**), 2335-2346.

Benedetti-Cecchi, L., Bertocci, I., Vaselli, S. and Maggi, E. (2006). Temporal variance reverses the impact of high mean intensity of stress in climate change experiments. *Ecology*, 87(**10**), 2489-2499.

Bertocci, I., Maggi, E., Vaselli, S. and Benedetti-Cecchi, L. (2005). Contrasting effects of mean intensity and temporal variation of disturbance on a rocky seashore. *Ecology*, 86(**8**), 2061-2067.

Bertocci, I., Dominguez, R., Machado, I., Freitas, C., Godino, J. D., Sousa-Pinto, I., Gonçalves, M. and Gaspar, M. (2014). Multiple effects of harvesting on populations of the purple sea urchin *Paracentrotus lividus* in north Portugal. *Fisheries Research*, 150, 60-65.

Bertocci, I., Dell'Anno, A., Musco, L., Gambi, C., Saggiomo, V., Cannavacciuolo, M., Lo Martire, M., Passarelli, A., Zazo, G. and Danovaro, R. (2019). Multiple human pressures in coastal habitats: variation of meiofaunal assemblages associated with sewage discharge in a post-industrial area. *Science of The Total Environment*, 655, 1218-1231.

Bonaventura, R., Costa, A., Pinsino, A., Russo, R., Zito, F. and Matranga, V. (2011). Echinoderms: model organisms for marine environmental monitoring and development of new emerging technologies. In *Marine Research at CNR* (pp. 1967-1978). Department of Earth and Environment.

Bošnjak, I., Šegvić, T., Smital, T., Franekić, J. and Mladineo, I. (2011). Sea urchin embryotoxicity test for environmental contaminants—potential role of the MRP proteins. *Water, Air, & Soil Pollution*, 217(**1-4**), 627-636.

Bressan, M., Chinellato, A., Munari, M., Matozzo, V., Manci, A., Marčeta, T., Finos, L., Moro, I., Pastore, P. and Badocco, D. (2014). Does seawater acidification affect survival, growth and shell integrity in bivalve juveniles? *Marine environmental research*, 99, 136-148.

Brzóska, M. M., Galażyn-Sidorczuk, M., Rogalska, J., Roszczenko, A., Jurczuk, M., Majewska, K. and Moniuszko-Jakoniuk, J. (2008). Beneficial effect of zinc supplementation on biomechanical properties of femoral distal end and femoral diaphysis of male rats chronically exposed to cadmium. *Chemico-biological interactions*, 171(**3**), 312-324.

Burge, C. A., Mark Eakin, C., Friedman, C. S., Froelich, B., Hershberger, P. K., Hofmann, E. E., Petes, L. E., Prager, K. C., Weil, E. and Willis, B. L. (2014). Climate change influences on marine infectious diseases: implications for management and society. *Annual Review of Marine Science*, 6, 249-277.

Byrne, M. (2012). Global change ecotoxicology: identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches. *Marine environmental research*, 76, 3-15.

Carballeira, C., Ramos-Gómez, J., Martín-Díaz, L. and DelValls, T. (2012). Identification of specific malformations of sea urchin larvae for toxicity assessment: application to marine pisciculture effluents. *Marine environmental research*, 77, 12-22.

Chowdhury, B. A., Friel, J. K. and Chandra, R. K. (1987). Cadmium-induced immunopathology is prevented by zinc administration in mice. *The Journal of nutrition*, 117(**10**), 1788-1794.

Cocco, E., De Pippo, T., Efaicchio, M. T. and Tarallo, F. (1988). Caratteri morfologici della piattaforma costiera del Golfo di Pozzuoli. *Memorie della Società Geologica Italiana*, 41, 995-1004.

Corinaldesi, C., Damiani, E., Marcellini, F., Falugi, C., Tiano, L., Brugè, F. and Danovaro, R. (2017). Sunscreen products impair the early developmental stages of the sea urchin Paracentrotus lividus. *Scientific Reports*, 7(**1**), 7815.

Coteur, G., Gosselin, P., Wantier, P., Chambost-Manciet, Y., Danis, B., Pernet, P., Warnau, M. and Dubois, P. (2003). Echinoderms as bioindicators, bioassays, and impact assessment tools of sediment-associated metals and PCBs in the North Sea. *Archives of Environmental Contamination and Toxicology*, 45(**2**), 190-202.

De Jonge, M., Teuchies, J., Meire, P., Blust, R. and Bervoets, L. (2012). The impact of increased oxygen conditions on metal-contaminated sediments part II: effects on metal accumulation and toxicity in aquatic invertebrates. *Water Research*, 46(**10**), 3387-3397.

De Pippo, T., Pescatore, T. and Vecchione, C. (1988). Caratteri granulometrici dei sedimenti dei terrazzi del Golfo di Pozzuoli. *Memorie della Società Geologica Italiana*, 41, 1005-1014.

De Vivo, B. and Lima, A. (2008). Characterization and remediation of a brownfield site: the Bagnoli case in Italy. In *Environmental Geochemistry* (pp. 355-385): Elsevier.

Dinnel, P. A., Pagano, G. G. and Oshida, P. S. (1988). A sea urchin test system for marine environmental monitoring. *Echinoderm Biology*, 611-619.

Easterling, D. R., Meehl, G. A., Parmesan, C., Changnon, S. A., Karl, T. R. and Mearns, L. O. (2000). Climate extremes: observations, modeling, and impacts. *Science*, 289(**5487**), 2068-2074.

Gaglioti, M., Vega Fernández, T., Musco, L. and Gambi, M. C. (2020). Habitat and benthic diversity in the bay of Bagnoli and surrounding areas (Gulf of Naples, Italy): A historical baseline for environmental restoration. *Marine environmental research*, 157, 104925.

Gallo, A., Guida, M., Armiento, G., Siciliano, A., Carraturo, F., Mormile, N., Pellegrini, D., Morroni, L., Tosti, E., Ferrante, M., Montresor, M., Molisso, F., Sacchi, M., Danovaro, R. and Libralato, G. (2020). Species-specific sensitivity of three microalgae to sediment elutriates. . *Marine environmental research*, This issue.

Gianguzza, P., Badalamenti, F., Gianguzza, F., Bonaviri, C. and Riggio, S. (2009). The operational sex ratio of the sea urchin *Paracentrotus lividus* populations: the case of the Mediterranean marine protected area of Ustica Island (Tyrrhenian Sea, Italy). *Marine Ecology*, 30(**1**), 125-132.

Giomi, F., Barausse, A., Duarte, C. M., Booth, J., Agusti, S., Saderne, V., Anton, A., Daffonchio, D. and Fusi, M. (2019). Oxygen supersaturation protects coastal marine fauna from ocean warming. *Science advances*, 5(**9**), eaax1814.

Guidetti, P., Fraschetti, S., Terlizzi, A. and Boero, F. (2003). Distribution patterns of sea urchins and barrens in shallow Mediterranean rocky reefs impacted by the illegal fishery of the rock-boring mollusc *Lithophaga lithophaga*. *Marine Biology*, 143(**6**), 1135-1142.

Guidetti, P. (2004). Consumers of sea urchins, *Paracentrotus lividus* and *Arbacia lixula*, in shallow Mediterranean rocky reefs. *Helgoland Marine Research*, 58(**2**), 110.

Hassoun, E. A. and Stohs, S. (1996). Cadmium-induced production of superoxide anion and nitric oxide, DNA single strand breaks and lactate dehydrogenase leakage in J774A. 1 cell cultures. *Toxicology*, 112(**3**), 219-226.

Hatzenpichler, R. (2012). Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. *Applied and Environmental Microbiology*, 78(**21**), 7501-7510.

Kobayashi, N. and Fujinaga, K. (1976). Synergism of inhibiting actions of heavy metals upon the fertilization and development of sea urchin eggs.[Cu, Zn, Ni, Cd]. *The Science and Engineering Review of Doshisha University*, 17(**1**), 54-69.

Kobayashi, N. and Okamura, H. (2004). Effects of heavy metals on sea urchin embryo development. 1. Tracing the cause by the effects. *Chemosphere*, 55(**10**), 1403-1412.

Maisano, M., Cappello, T., Catanese, E., Vitale, V., Natalotto, A., Giannetto, A., Barreca, D., Brunelli, E., Mauceri, A. and Fasulo, S. (2015). Developmental abnormalities and neurotoxicological effects of CuO NPs on the black sea urchin *Arbacia lixula* by embryotoxicity assay. *Marine environmental research*, 111, 121-127.

Margiotta, F., Balestra, C., Buondonno, A., Casotti, R., D'Ambra, I., Di Capua, I., Gallia, R., Mazzocchi, M. G., Merquiol, L., Pepi, M., Percopo, I., Saggiomo, M., Sarno, D. and Zingone, A. (2020). Do plankton reflect the environmental quality status? The case of a postindustrial Mediterranean bay. *Marine environmental research*, This Isuue.

Marin, M., Da Ros, L., Moschino, V. and Campesan, G. (2001). Sediment elutriate toxicity testing with embryos of sea urchin (Paracentrotus lividus). *Aquatic Ecosystem Health & Management*, 4(**2**), 215-221.

Marshall, J. D. and Uller, T. (2007). When is a maternal effect adaptive? *Oikos*, 116(**12**), 1957-1963.

Martínez-Lladó, X., Gibert, O., Martí, V., Díez, S., Romo, J., Bayona, J. M. and de Pablo, J. (2007). Distribution of polycyclic aromatic hydrocarbons (PAHs) and tributyltin (TBT) in Barcelona harbour sediments and their impact on benthic communities. *Environmental pollution*, 149(**1**), 104-113.

Matozzo, V., Chinellato, A., Munari, M., Finos, L., Bressan, M. and Marin, M. G. (2012). First evidence of immunomodulation in bivalves under seawater acidification and increased temperature. *PloS one*, 7(**3**), e33820.

Matozzo, V., Chinellato, A., Munari, M., Bressan, M. and Marin, M. G. (2013). Can the combination of decreased pH and increased temperature values induce oxidative stress in the clam Chamelea gallina and the mussel *Mytilus galloprovincialis*? *Marine Pollution Bulletin*, 72(**1**), 34-40.

Matranga, V., Toia, G., Bonaventura, R. and Müller, W. E. G. (2000). Cellular and biochemical responses to environmental and experimentally induced stress in sea urchin coelomocytes. *Cell stress & chaperones*, 5(**2**), 113.

Migliaccio, O., Castellano, I., Cirino, P., Romano, G. and Palumbo, A. (2015). Maternal exposure to cadmium and manganese impairs reproduction and progeny fitness in the sea urchin *Paracentrotus lividus. PloS one*, 10(**6**), e0131815.

Miller, G. M., Watson, S.-A., Donelson, J. M., McCormick, M. I. and Munday, P. L. (2012). Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. *Nature Climate Change*, 2(**12**), 858.

Morale, A., Coniglio, L., Angelini, G., Cimoli, G., Bolla, A., Alleteo, D., Russo, P. and Falugi, C. (1998). Biological effects of a neurotoxic pesticide at low concentrations on sea urchin early development. A teratogenic assay. *Chemosphere*, 37(**14-15**), 3001-3010.

Moran, N. A. (1994). Adaptation and constraint in the complex life cycles of animals. *Annual Review of Ecology and Systematics*, 25(**1**), 573-600. doi:10.1146/annurev.es.25.110194.003041

Morroni, L., Pinsino, A., Pellegrini, D., Regoli, F. and Matranga, V. (2016). Development of a new integrative toxicity index based on an improvement of the sea urchin embryo toxicity test. *Ecotoxicology and evironmental safety*, 123, 2-7.

Morroni, L., Pinsino, A., Pellegrini, D. and Regoli, F. (2018). Reversibility of trace metals effects on sea urchin embryonic development. *Ecotoxicology and Environmental Safety*, 148, 923-929.

Morroni, L., D'Errico, G., Sacchi, M., Molisso, F., Armiento, G., Chiavarini, S., Rimauro, J., Guida, M., Siciliano, A., Ceparano, M. T., Aliberti, F., Gallo, A., Libralato, G., Patti, F. P., Gorbi, S., Fattorini, D., Nardi, A., Di Carlo, M., Mezzelani, M., Benedetti, M., Pellegrini, D., Musco, L., Danovaro, R., Dell'Anno, A. and Regoli, F. (2020). Integrated characterization and risk management of marine sediments: the case study of the industrialized Bagnoli area (Naples, Italy). *Marine environmental research*, This issue.

Moschino, V. and Marin, M. G. (2002). Spermiotoxicity and embryotoxicity of triphenyltin in the sea urchin *Paracentrotus lividus* Lmk. *Applied Organometallic Chemistry*, 16(**4**), 175-181.

Munari, M., Chemello, G., Finos, L., Ingrosso, G., Giani, M. and Marin, M. G. (2016). Coping with seawater acidification and the emerging contaminant diclofenac at the larval stage: A tale from the clam *Ruditapes philippinarum*. *Chemosphere*, 160, 293-302.

Munari, M., Matozzo, V., Gagné, F., Chemello, G., Riedl, V., Finos, L., Pastore, P., Badocco, D. and Marin, M. G. (2018). Does exposure to reduced pH and diclofenac induce oxidative stress in marine bivalves? A comparative study with the mussel *Mytilus galloprovincialis* and the clam *Ruditapes philippinarum*. *Environmental pollution*, 240, 925-937.

Munari, M., Matozzo, V., Chemello, G., Riedl, V., Pastore, P., Badocco, D. and Marin, M. G. (2019). Seawater acidification and emerging contaminants: A dangerous marriage for haemocytes of marine bivalves. *Environmental Research*, 175, 11-21.

Neumann, B., Vafeidis, A. T., Zimmermann, J. and Nicholls, R. J. (2015). Future coastal population growth and exposure to sea-level rise and coastal flooding-a global assessment. *PloS one*, 10(**3**), e0118571.

Ohta, K., Takahashi, C. and Tosuji, H. (2009). Inhibition of spicule elongation in sea urchin embryos by the acetylcholinesterase inhibitor eserine. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 153(**4**), 310-316.

Pagano, G., His, E., Beiras, R., De Biase, A., Korkina, L., Iaccarino, M., Oral, R., Quiniou, F., Warnau, M. and Trieff, N. (1996). Cytogenetic, developmental, and biochemical effects of aluminum, iron, and their mixture in sea urchins and mussels. *Archives of environmental contamination toxicology*, 31(**4**), 466-474.

Parker, L. M., Ross, P. M., O'Connor, W. A., Borysko, L., Raftos, D. A. and Pörtner, H. O. (2012). Adult exposure influences offspring response to ocean acidification in oysters. *Global Change Biology*, 18(**1**), 82-92.

Pillai, M. C., Vines, C. A., Wikramanayake, A. H. and Cherr, G. N. (2003). Polycyclic aromatic hydrocarbons disrupt axial development in sea urchin embryos through a β-catenin dependent pathway. *Toxicology*, 186(**1-2**), 93-108.

Pinsino, A., Matranga, V., Trinchella, F. and Roccheri, M. C. (2010). Sea urchin embryos as an in vivo model for the assessment of manganese toxicity: developmental and stress response effects. *Ecotoxicology*, 19(**3**), 555-562.

Pörtner, H. O., Langenbuch, M. and Reipschläger, A. (2004). Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. *Journal of Oceanography*, 60(**4**), 705-718.

Range, P., Chícharo, M., Ben-Hamadou, R., Piló, D., Fernandez-Reiriz, M., Labarta, U., Marin, M. G., Bressan, M., Matozzo, V. and Chinellato, A. (2014). Impacts of CO_2-induced seawater acidification on coastal Mediterranean bivalves and interactions with other climatic stressors. *Regional Environmental Change*, 14(**1**), 19-30.

Reed, T. E., Schindler, D. E. and Waples, R. S. (2011). Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conservation Biology*, 25(**1**), 56-63.

Romano, E., Bergamin, L., Celia Magno, M., Pierfranceschi, G. and Ausili, A. (2018). Temporal changes of metal and trace element contamination in marine sediments due to a steel plant: The case study of Bagnoli (Naples, Italy). *Applied Geochemistry*, 88, 85-94. doi:https://doi.org/10.1016/j.apgeochem.2017.05.012

Ruocco, N., Bertocci, I., Munari, M., Musco, L., Caramiello, D., Danovaro, R., Zupo, V. and Costanitini, M. (2020). Morphological and molecular responses of the sea urchin *Paracentrotus lividus* to highly contaminated marine sediments: the case study of Bagnoli-Coroglio brownfield (Mediterranean Sea). *Marine environmental research*, This issue.

Ryu, J., Khim, J. S., Kang, S.-G., Kang, D., Lee, C.-h. and Koh, C.-h. (2011). The impact of heavy metal pollution gradients in sediments on benthic macrofauna at population and community levels. *Environmental pollution*, 159(**10**), 2622-2629.

Sala, E. and Zabala, M. (1996). Fish predation and the structure of the sea urchin *Paracentrotus lividus* populations in the NW Mediterranean. *Marine Ecology Progress Series*, 140, 71-81.

Schreier, H. J., Mirzoyan, N. and Saito, K. (2010). Microbial diversity of biological filters in recirculating aquaculture systems. *Current opinion in biotechnology*, 21(**3**), 318-325.

Sconzo, G., Romancino, D., Fasulo, G., Cascino, D. and Giudice, G. (1995). Effect of doxorubicin and phenytoin on sea urchin development. *Pharmazie*, 50(**9**), 616-618.

Spalding, M. D., Ruffo, S., Lacambra, C., Meliane, I., Hale, L. Z., Shepard, C. C. and Beck, M. W. (2014). The role of ecosystems in coastal protection: Adapting to climate change and coastal hazards. *Ocean & Coastal Management*, 90, 50-57.

Spirlet, C., Grosjean, P. and Jangoux, M. (1998). Reproductive cycle of the echinoid *Paracentrotus lividus*: analysis by means of the maturity index. *Invertebrate reproduction & development*, 34(**1**), 69-81.

Suzuki, N., Ogiso, S., Yachiguchi, K., Kawabe, K., Makino, F., Toriba, A., Kiyomoto, M., Sekiguchi, T., Tabuchi, Y. and Kondo, T. (2015). Monohydroxylated polycyclic aromatic hydrocarbons influence spicule formation in the early development of sea urchins (*Hemicentrotus pulcherrimus*). *Comparative Biochemistry and Physiology Part C: Toxicology Pharmacology*, 171, 55-60.

Thor, P. and Dupont, S. (2015). Transgenerational effects alleviate severe fecundity loss during ocean acidification in a ubiquitous planktonic copepod. *Global Change Biology*, 21(**6**), 2261-2271. doi:10.1111/gcb.12815

Trapp, R. J., Diffenbaugh, N. S., Brooks, H. E., Baldwin, M. E., Robinson, E. D. and Pal, J. S. (2007). Changes in severe thunderstorm environment frequency during the 21st century caused by anthropogenically enhanced global radiative forcing. *Proceedings of the National Academy of Sciences*, 104(**50**), 19719-19723.

Trifuoggi, M., Donadio, C., Mangoni, O., Ferrara, L., Bolinesi, F., Nastro, R. A., Stanislao, C., Toscanesi, M., Di Natale, G. and Arienzo, M. (2017). Distribution and enrichment of trace metals in surface marine sediments in the Gulf of Pozzuoli and off the coast of the brownfield metallurgical site of Ilva of Bagnoli (Campania, Italy). *Marine Pollution Bulletin*, 124(**1**), 502-511. doi:https://doi.org/10.1016/j.marpolbul.2017.07.033

Uller, T. (2008). Developmental plasticity and the evolution of parental effects. *Trends in ecology Evolutionary Applications*, 23(**8**), 432-438.

Viaroli, P. and Christian, R. J. E. I. (2003). Description of trophic status of an eutrophic coastal lagoon through potential oxygen production and consumption: dening hyperautotrophy and dystrophy. 3, 237250.

Vitousek, P. M., Mooney, H. A., Lubchenco, J. and Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science*, 277(**5325**), 494-499.

Weston, D. P., Poynton, H. C., Wellborn, G. A., Lydy, M. J., Blalock, B. J., Sepulveda, M. S. and Colbourne, J. K. (2013). Multiple origins of pyrethroid insecticide resistance across the species complex of a nontarget aquatic crustacean, *Hyalella azteca. Proceedings of the National Academy of Sciences*, 110(**41**), 16532-16537.

Wolff, N. H., Wong, A., Vitolo, R., Stolberg, K., Anthony, K. R. and Mumby, P. J. (2016). Temporal clustering of tropical cyclones on the Great Barrier Reef and its ecological importance. *Coral Reefs*, 35(**2**), 613-623.

Xu, X., Li, Y., Wang, Y. and Wang, Y. (2011). Assessment of toxic interactions of heavy metals in multi-component mixtures using sea urchin embryo-larval bioassay. *Toxicology in Vitro*, 25(**1**), 294-300.

Zhang, D., Li, S., Wang, G., Guo, D., Xing, K. and Zhang, S. (2012). Biochemical responses of the copepod *Centropages tenuiremis* to CO_2-driven acidified seawater. *Water Science Technology*, 65(**1**), 30-37.

Zito, F., Costa, C., Sciarrino, S., Cavalcante, C., Poma, V. and Matranga, V. (2005). Cell adhesion and communication: a lesson from echinoderm embryos for the exploitation of new therapeutic tools. In *Echinodermata* (pp. 7-44): Springer.