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 subject to 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, southern 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 from parents exposed to contaminated sediments, when reared in clean filtered sea water, succeeded 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.

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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 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 (Southern Tyrrhenian Sea, Italy) is an infamous example of a heavily polluted marine area. All industrial activities 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). In 2001 Bagnoli-Coroglio bay was designated as a Site of National Interest (SNI) requiring environmental rehabilitation.

Several studies reported that the 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 by 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 focused on 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). *Paracentrotus 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 reabsorbed by 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'). Oxygen super-saturation was suited 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

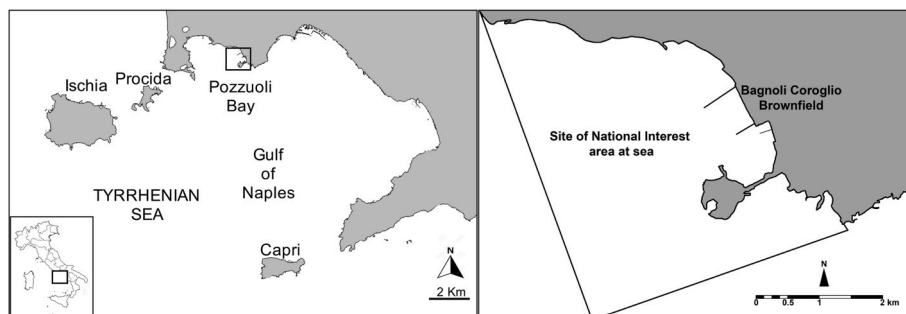The study site was the coastal area of Bagnoli-Coroglio, an

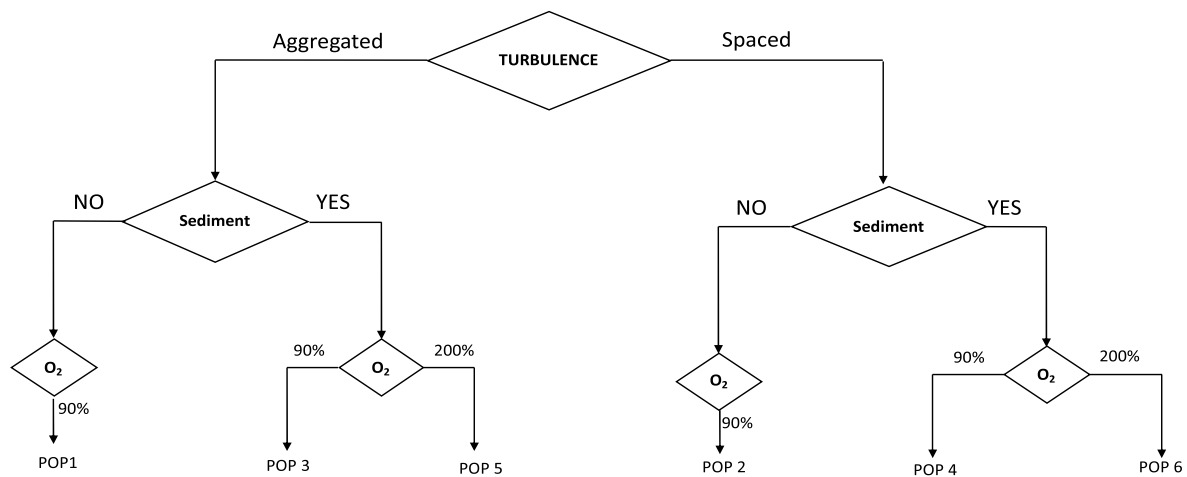**Fig. 1.** Map of the Bagnoli-Coroglio study area.

Fig. 2. Adult experimental design. See M&M section for details. POP 1 and POP 2: control adults without sediment, 90% oxygen saturation, aggregated and spaced pattern of turbulence respectively; POP 3 and POP 4: adults with sediment, 90% oxygen saturation, aggregated and spaced pattern of turbulence respectively; POP 5 and POP 6: adults with sediment, 200% oxygen saturation, aggregated and spaced pattern of turbulence respectively.

industrially contaminated region located in the south-eastern part of the Pozzuoli Bay (Gulf of Naples – southern Tyrrhenian Sea, Fig. 1), characterized by sandy and clay substrates protected by artificial rocky reefs (Bertocci et al., 2019; Cocco et al., 1988; De Pippo et al., 1988). Industrial activities were developed 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, which was classified as a SNI in 2001. 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 including 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 historical data on the benthic diversity in the Bagnoli-Coroglio site reported the occurrence of *P. lividus* in this area in the past (Gaglioti et al., 2020), although further studies are needed to assess the current state of the local *P. lividus* populations.

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 5 min, 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 psμ), in order to reflect the expected conditions at the study site once the local ecosystem was possibly restored.

2.3. Animal collection

Total 200 adult *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.

Paracentrotus 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).

2.4. Adult sea urchin rearing mesocosms

'Ad hoc' mesocosms comprising twelve independent, closed recirculating tanks (~50 L each), filled with natural sea water, were set up in the facility for the maintenance of marine organisms of Stazione Zoologica Anton Dohrn in Naples. 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 and physical variables of both sea water and sediments are reported by Ruocco et al. (2020).

2.5. Adult sea urchin experimental design

The bottom of eight tanks, randomly chosen out of the total 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; water depth: 3.8 m). This sediment came from the same sampling core and was uniformly mixed before being used. 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 the 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, while maintaining the same 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 for potential effects of hydrodynamics, independently of the presence of sediments and associated chemical and physical effects. A detailed illustration of the experimental setting and design was provided by Ruocco et al. (2020). To produce water turbulence, with the associated re-suspension of sediment, an electric pump (flow rate 4500L/h, power: 10 W) was placed in each experimental tank. The duration and frequency of experimental re-suspension events were established based on the records of natural storm events in the study area. Data on wind speed, wave height and period were collected daily from an oceanographic buoy within a monitoring program of Stazione Zoologica Anton Dohrn, as illustrated in detail 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 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 (Fig. 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*.

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 min 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 effects in response to the experimental 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 tank where adults were kept. 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

Fig. 3. *P. lividus* developmental stages. From left: arrested embryos, gastrulae, prism, early pluteus and echinopluteus.
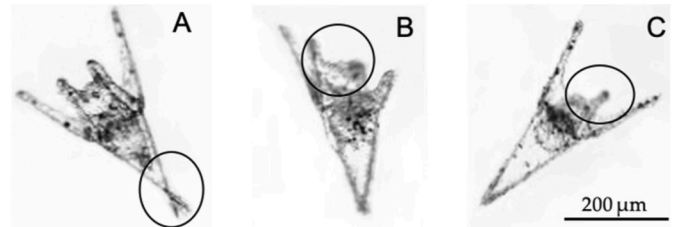

Fig. 4. Examples of anomalous 4-arm pluteus at 48 hpf. A: crossed spicules, B and C: asymmetrical or completely degenerated arms.
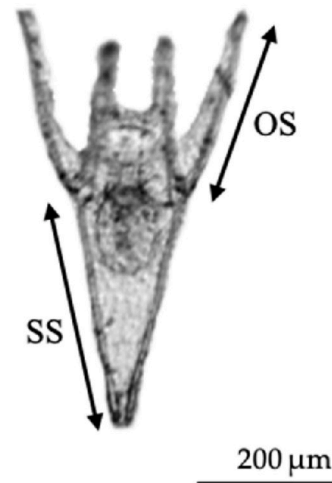

Fig. 5. Somatic (SS) and oral (OS) spicules at 48 hpf.

the potential to highlight: (a) specific effects of parental exposure on embryonic and larval development through comparing with larval populations reared in FSW; (b) selective pressure acting during gametogenesis and resulting into a better response to stress of offspring from adults reared in harsh conditions (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 at 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 h of
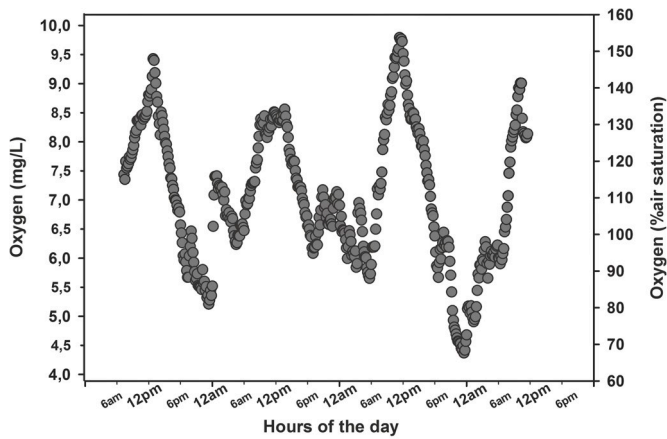
Fig. 6. Diel seawater dissolved oxygen fluctuations (5 min logging frequency).

exposure, and their frequencies were calculated. Five main classes were considered: 1) arrested, corresponding to undeveloped embryos; 2) gastrulae, distinguished by the onset of invagination; 3) prism, characterized by the early development of calcareous rods and the typical cuboid shape of the embryos; 4) early plutei, distinguished by the beginning development of the four appendixes; 5) echinoplutei, i.e., the 48 hpf stage characterized by the total development of oral and aboral rods (Fig. 3).

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

2.8. Statistical analyses

A non-parametric PERMutational multivariate ANalysis Of Variance, PERMANOVA (Anderson, 2001a) 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 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

Oxygen levels within the examined *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-h period) seawater oxygen fluctuations are shown in Fig. 6.

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 1). At 24 hpf, all the interactions between parental factors were significant (Table 1). Overall, the adults that had experienced a spaced pattern of

Table 1
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).

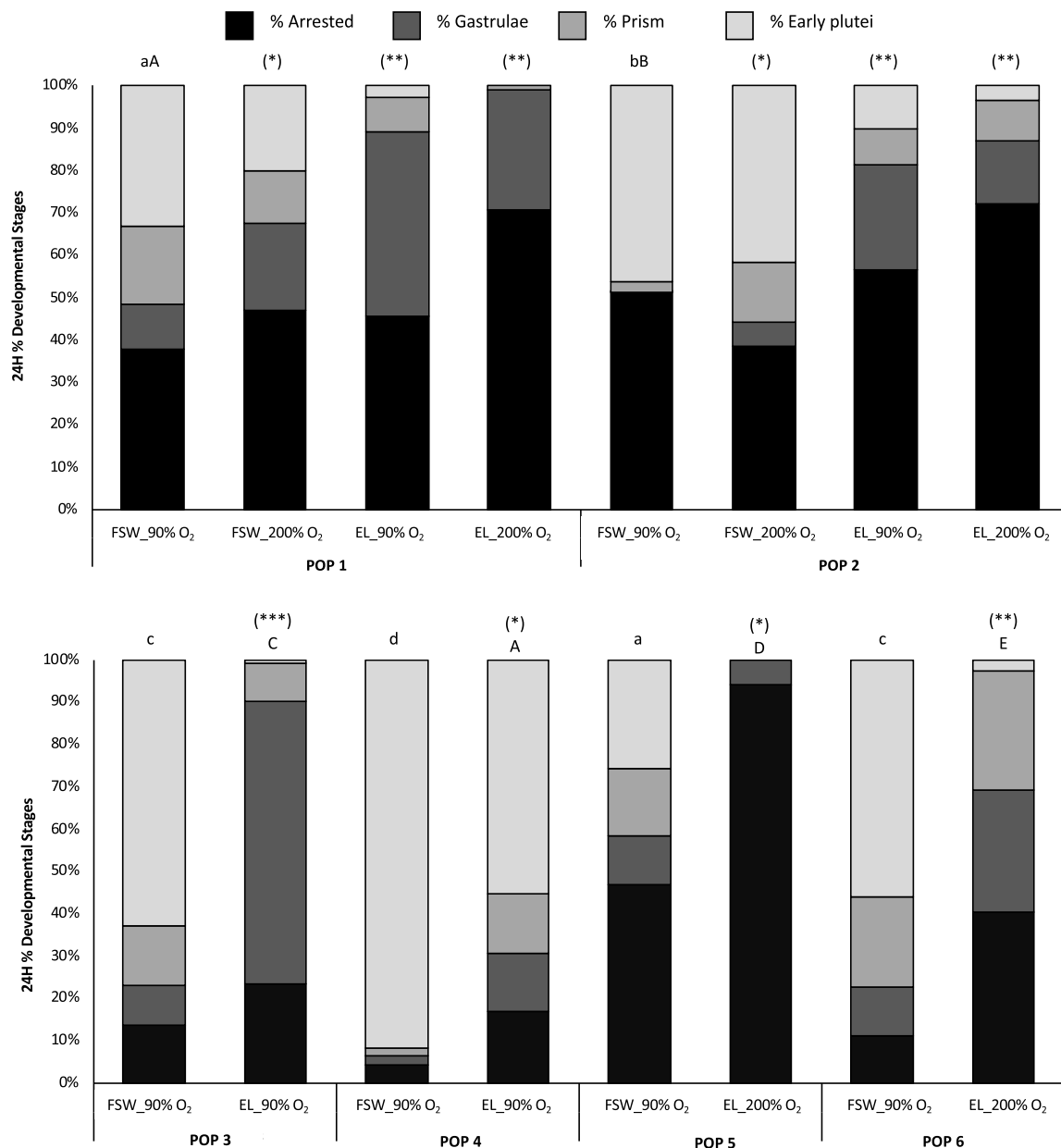|	Developmental stages 24h		Developmental stages 48h		Anomalies 48h		Somatic Spicules (SS) 48h		Oral Spicules (OS) 48h	
	FSW rearing	PC - rearing	FSW - rearing	PC - rearing	FSW - rearing	PC - rearing	FSW - rearing	PC - rearing	FSW - rearing	PC - rearing
Larval population	$F_{(5,17)} = 22.551$ $p_{(MC)}<0.001$	$F_{(5,17)} = 17.248$ $p_{(MC)}<0.001$	$F_{(5,17)} = 11.999$ $p_{(MC)}<0.001$	$F_{(5,17)} = 41.511$ $p_{(MC)}<0.001$	$F_{(5,17)} = 11.417$ $p_{(MC)}<0.001$	$F_{(5,17)} = 190.4$ $p_{(MC)}<0.001$	$F_{(5,17)} = 11.260$ $p_{(MC)}<0.001$	$F_{(5,17)} = 1043.8$ $p_{(MC)}<0.001$	$F_{(5,17)} = 33.995$ $p_{(MC)}<0.001$	$F_{(5,17)} = 246.920$ $p_{(MC)}<0.001$
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)	$F_{(1,17)} = 4.086$ $p_{(MC)} = 0.0271$	$F_{(1,17)} = 24.294$ $p_{(MC)}<0.001$	$F_{(1,17)} = 1.595$ $p_{(MC)} = 0.229$	$F_{(1,17)} = 8.8705$ $p_{(MC)} = 0.004$	$F_{(1,17)} = 0.170$ $p_{(MC)} = 0.749$	$F_{(1,17)} = 14.588$ $p_{(MC)} = 0.749$	$F_{(1,17)} = 1.321$ $p_{(MC)} = 0.272$	$F_{(1,17)} = 776.57$ $p_{(MC)}<0.001$	$F_{(1,17)} = 5.433$ $p_{(MC)} = 0.035$	$F_{(1,17)} = 205.34$ $p_{(MC)}<0.001$

Fig. 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).

turbulence gave birth to a higher percentage of early plutei than those having experienced an aggregated turbulence pattern. In general, an average 65% of early plutei from parents exposed to a spaced pattern of turbulence was recorded, while an average 41% of early plutei from parents exposed to an aggregated pattern was observed (Fig. 7).

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

At 48 hpf, the interaction between the pattern of turbulence and the presence of sediment were significant (Table 1). Overall, the averages of 4-arms echinoplutei from both parents exposed to the spaced and the aggregated pattern of turbulence were comparable (89% and 94%, respectively) (Fig. 8). At 24 hpf, the oxygen super-saturation had a negative effect on the average percentage of echinoplutei. In particular,

almost all (99%) larvae from POP 3 and POP 4 succeeded to develop up to the echinopluteus stage, while the average amount of echinoplutei between POP 5 and POP 6 was around 87% (Fig. 8). These results, however, take into account the total percentage of echinoplutei without distinguishing 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. Larvae grew better in FSW independently of the origin of their parents. For each experimental larval population, the percentage of developmental stages of larvae reared in FSW and of those reared in the presence of elutriates was always significantly different (Table 1). At 24 hpf, all the interaction between
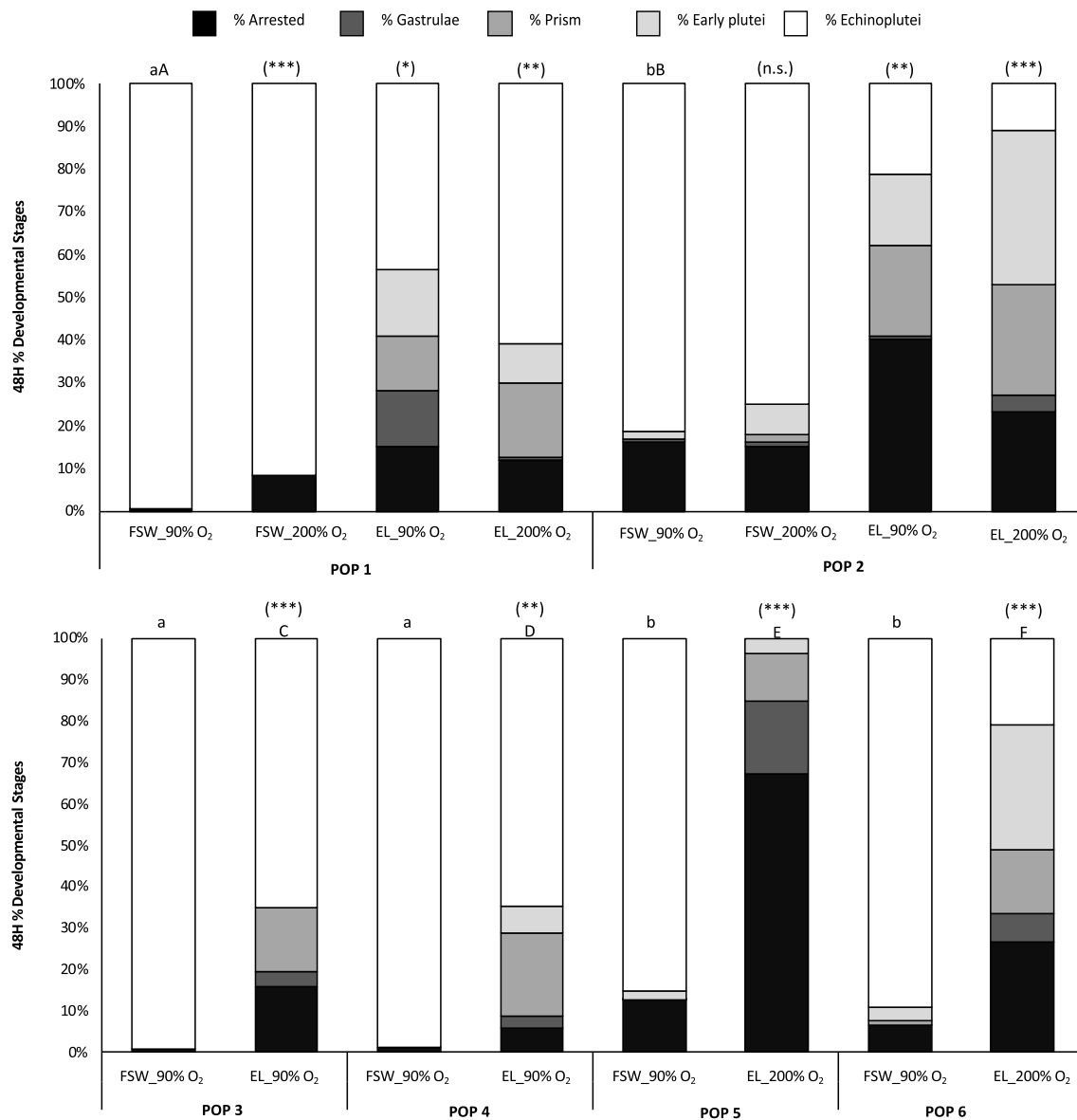
Fig. 8. Percentage of developmental stages at 48 hpf. On the X axis the parental populations (POP1-6) and larval exposure conditions are shown. Significant differences (p < 0.05) among larval populations reared in FSW for each experimental parental condition are represented with lower-case letters (a–b), 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).

parental factors were significant (Table 1). 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 (Fig. 7). Oxygen super-saturation had a negative effect on the average percentage of early plutei as well in all larval populations exposed to elutriates (Fig. 7).

At 48 hpf, all the interaction between parental factors were significant (Table 1). The same trend described for larvae at 24 hpf was recorded for the larval development at 48 hpf, i.e., a negative effect of adults' pre-exposure to both aggregated pattern of turbulence and oxygen super-saturation (Fig. 8).

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 1).

The interaction between turbulence pattern and the presence of sediment affected the normal formation of the calcareous structures in larvae maintained in FSW (Table 1). Overall, the percentage of normally developed echinoplutei from parents who experienced a spaced vs. an aggregated pattern of turbulence was comparable (77%) (Fig. 9). Oxygen super-saturation had a negative effect on the proportion of normally developed echinoplutei. In particular, an average 88% of normal echinoplutei between POP 3 and POP 4 was observed, while the average between POP 5 and POP 6 was around 65% (Fig. 9).

The length of both SS (Fig. 10 A) and OS (Fig. 10 B) was significantly affected by the parental population of origin in larvae reared in FSW (Table 1). In general, larvae reared in FSW had longer SS and OS compared to their siblings reared in elutriates (Fig. 10A–B), regardless of the parent of origin.
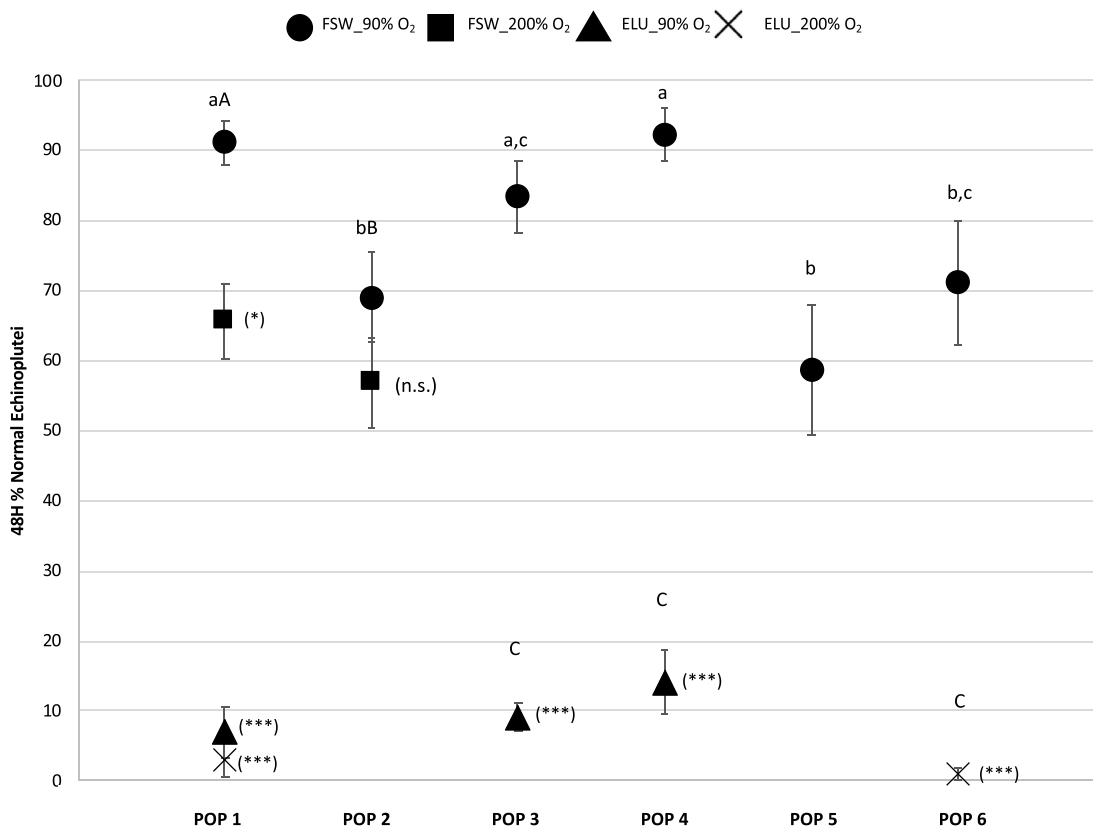
Fig. 9. Percentage ± SD (n = 3) of normal echinoplutei at 48 hpf. On the X axis parental populations (POP1-6) are shown. Larval exposure conditions are shown in the legend. Significant differences (p < 0.05) among larval populations reared in FSW for each experimental parental condition are represented 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).

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 1).

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

The length of both types of spicules was affected by parental exposure as well (Table 1). Larvae maintained in elutriates had shorter spicules compared to their siblings reared in FSW, regardless of the parent of origin, with a mean length reduction of 40% for both somatic and oral spicules (Fig. 10A–B).

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. A generally 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 on benthic organisms, those that experimentally examined the interaction between different types of physical, chemical and mechanical variables are scarce and often focused on a single life stage.

Furthermore, in natural habitats, increased water oxygenation due to the photosynthetic activity of algae and macrophytes can have a positive effect on the physiological performance of associated or nearby organisms. Giomi et al. (2019) found that 'super-saturated' (140 ± 3%) oxygen conditions increased the LT_{50} of six Red Sea species (one decapod, one echinoderm, one holothurian, one bivalve, two teleost fishes) by 2 °C compared to those maintained at normal oxygen saturation levels (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, although all larval populations were 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% O₂. These findings are in accordance with several studies that found the exposure to a mix of heavy metals present
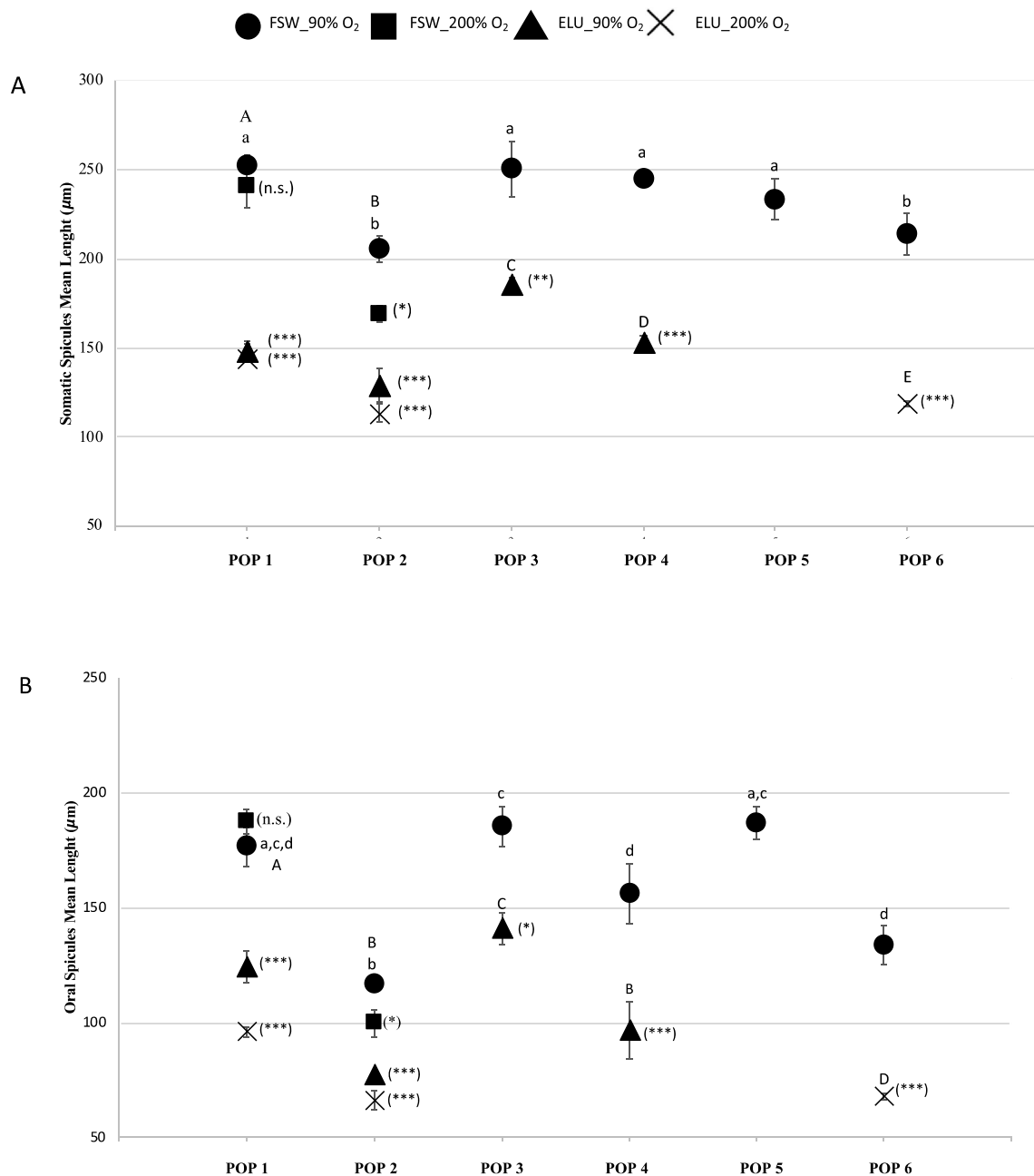
Fig. 10. A–B: Mean length ± SD (n = 3) of somatic (A) and oral (B) spicules at 48 hpf. On the X axis parental populations (POP1-6) are shown. Larval exposure conditions are shown in the legend. Significant differences (p < 0.05) among larval populations reared in FSW for each experimental parental condition 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.

in marine sediments to cause a negative effect on larval development in echinoderms, including the sea urchins *P. lividus*, *Psammechinus miliaris* and *Strongylocentrotus intermedius*, and the sea star *Asterias rubens* (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 heavy metals, such as Cd, Cu, Pb, Zn, had a negative synergistic effect on the larval development of *S. intermedius*. This can be explained with the presence of Cd,

which could have led to oxidative damage by causing the accumulation of reactive oxygen species (ROS) in larval tissues (Hassoun and Stohs, 1996), and the subsequent intensification of the sensitivity of cells to other co-occurring toxicants (Brzóska et al., 2008; Chowdhury et al., 1987).

Furthermore, abnormal larval development can 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 in sea urchin *Hemicentrotus pulcherrimus* larvae exposed to two PAHs, namely benz[a]anthracene (BaA) and 4-hydroxybenz[a]anthracene (4-OHBaA), for 53h post-fertilization (Suzuki et al., 2015). This was due to a reduction of the expression of several genes involved in the formation of spicules, which the authors attributed to the suppression of the transcription factor for spicule formation by 4-OHBaA. Furthermore, Pillai et al. (2003) suggested 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 (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 too short to trigger a transgenerational buffer effect against sea water pollution.

Furthermore, no positive effects of higher oxygen levels were observed, since larvae exposed to oxygen super-saturation showed an even higher percentage of abnormal larvae and echinoplutei compared to larval populations reared in FSW_90% O_2. Consistently, it was reported that 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 may be possible that increased oxygen availability fuels higher metabolic activity, resulting into 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 provide an important baseline for 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 the Bagnoli-Coroglio Bay.

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.

CRediT authorship contribution statement

Antonia Chiarore: Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Luigi Musco:** Conceptualization, Resources, Writing - original draft, Project administration, Funding acquisition. **Iacopo Bertocci:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Project administration. **Alessandra Gallo:** Conceptualization, Resources. **Antonio Cannavacciuolo:** Formal analysis, Data curation, Writing - original draft. **Mirko Mutalipassi:** Resources, Writing - original draft. **Davide Caramiello:** Resources. **Folco Giomi:** Resources, Writing - original draft. **Marco Fusi:** Resources, Writing - original draft. **Roberto Danovaro:** Writing - original draft, Funding acquisition. **Marco Munari:** Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2020.104967.

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