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Early-stage anomalies in the sea urchin (*Paracentrotus lividus*) as bioindicators of multiple stressors in the marine environment: Overview and future perspectives☆

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ABSTRACT

The morphological anomalies of the early development stages of the sea urchin *Paracentrotus lividus*, caused by exposure to environmental stressors, are used as biomarker in ecotoxicological and ecological investigations. Here, we reviewed the available literature and classified the embryo and larval anomalies identified so far, to highlight potential commonalities or differences related to the biological action of the different stressors and their ecological impact. Morphological anomalies are influenced by a) the developmental stage of exposure to stressors; b) the intensity of the stress; c) the intra- and inter-cellular mechanisms affected by the exposure to environmental agents. The classification and analysis of embryo and larvae anomalies, either observed by the authors of this review and reported in literature, indicate that sea urchin abnormalities, caused by exposure to different stressors, can be very similar among them and classified into 18 main types, which can occur individually or mixed. All anomalies can be used to calculate an Index of Contaminant Impact to assess the impact of multiple stressors and to identify relationships between morphological anomalies and compromised biological mechanisms. This approach could be useful for a first screening of the presence of potential stressors impairing the growth and development of the early life stages of marine organisms, thus providing a relevant advancement for in future monitoring activities devoted to assess the health status in coastal marine ecosystems.

1. Introduction

Biological models can integrate the toxicity effects of contaminants with those due to their detoxification/degradation products (Richardson et al., 2007), thus they are used in standard bioassays to assess the anthropogenic impact due to the presence of single or mixed contaminants or multiple stressors in natural ecosystems (Sarà, 2007). Sea urchins are amongst the most common model organisms for ecological and toxicological studies (Dinnel et al., 1988; Carballeira et al., 2012a, 2012b; Pagano et al., 2017a, b). Sea urchin fertilization and development are sensitive to environmental changes because they are subject to interactive processes between egg and sperm, among blastomeres and among all these and environment, in which pollutants may interfere. For this reason, embryonic and larval stages of sea urchin development have often been used as biomarkers of environmental alterations (Moulin et al., 2011; Carballeira et al., 2012a; Morroni et al., 2018; Chiarelli et al., 2019).

Sea urchin allows us to perceive risks to human health because it shares the mechanisms driving development and differentiation with higher organisms, including mammalians (Qiao et al., 2003). For this reason, it has been suggested as a model for the study of several human diseases (Buznikov et al., 2008). The transferability of results to mammalians is also supported by the "Sea Urchin Genome'' project (Sodergren et al., 2006), which revealed a high degree of homologies.

Paracentrotus lividus is a key species in shallow waters of the Mediterranean coasts, important in commercial terms, for food web

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functioning and for its ability to reshape marine habitats (Steneck, 2013). Among other echinoderms, *P. lividus* is emerging as a valid model for studies in marine ecotoxicology and ecology (Pagano et al., 2001; Losso et al., 2004; Volpi Ghirardini et al., 2005; Privitera et al., 2011; Morroni et al., 2016; Gambardella et al., 2020). It is considered one of the marine species to assess the toxicological risk of contaminated marine sediments by the Italian Ministerial Decree 173 (MD 173/2016), and an indicator species in European Directives (i.e. Habitat Directive 92/43/EEC, the Marine Strategy Framework Directive). In addition, it has been proposed as a promising alternative model (Aluigi et al., 2008; Falugi et al., 2008; Pinsino and Alijagic, 2019) to promote the Replacement, Reduction and Refinement of animals used in regulatory testing (Do Prado Duzanski et al., 2015). *P. lividus* can be used as an attractive proxy to human non-mammalian model for exploring the safety of several contaminants (e.g. nanoparticles (NPs), pharmaceuticals and personal-care products (PPCPs); Rosenfeld and Feng, 2011; Corinaldesi et al., 2017; Alijagic et al., 2020), which are defined "emerging" as their presence and environmental impact are under investigation since few years (Sauvé and Desrosiers, 2014).

Sea urchin toxicity bioassays included in national and international legislation are based on spermiotoxicity and embryotoxicity (ASTM, 1994, 1995, 2004; USEPA, 2002; MD 173/2016). Such bioassays based on gametes, zygotes, and embryos exposed to single or multiples stressors, are limited to provide the ratio of normal embryos or larvae/total number of exposed specimens (Warnau et al., 1996; Sartori et al., 2017), distinguished between normal developmental stages and deformed ones rather than the anomaly types.

In this review, we classified the main morphological anomalies caused by exposure to different contamininants (e.g. heavy metals-HMs-, organic compounds, nanoparticles -NPs, microplastics-MPs), as well as environmental stressors including climate change and other impacts. The aim of this review is to provide a guide of the main embryo and larvae morphological anomalies in relation to their developmental stage and contaminant/stressor types and concentrations. Embryo and larvae anomalies are shown by using original drawings and pictures owned by the authors of this review that have been elaborated with china ink to highlight the details of interest. Given the cause-effect relationship between contaminants and embryo and/or larval anomalies (Kobayashi and Okamura, 2004), the classification and analysis of the anomalies can be used to predict the type, the level of impairment of early developmental stage based on multiple stressors occurring in the environment.

2. Normal development of sea urchin

Sea urchin development has been described by several authors (Steinhardt and Epel, 1974; Giudice, 1986; Vacquier, 2011; Santella et al., 2012). *P. lividus* development occurs normally in optimal conditions of temperature: it proceeds synchronously up to pluteus (Shpigel et al., 2004; Falugi and Angelini, 2002), sustained by strong and straight skeletal rods (Fig. 1A–O). Skeletogenesis is fully driven by primary mesenchyme cells (PMCs), also responsible for organization of the embryo (McClay et al., 2000; Croce and McClay, 2006). At later stages, the skeletogenic PMCs are still present, scattered along the rods, and are responsible for larval plasticity maintenance (Fig. 1 N, O). Plutei up to 72 h can survive without feeding (Fenaux et al., 1985). Fed larval stages are seldom used for toxicity tests, as feeding may introduce confounding factors, according to the amount and quality of available food. Further details are present in Supplementary material.

3. Naturally occurring anomalies

Spontaneous anomalies may occur due to different factors, including: presence of confounding substances in seawater taken from the environment; fertilization of gametes of scarce quality; presence of a huge number of sperms; inappropriate temperature range; crowded population of developing embryos and larvae. The main spontaneous

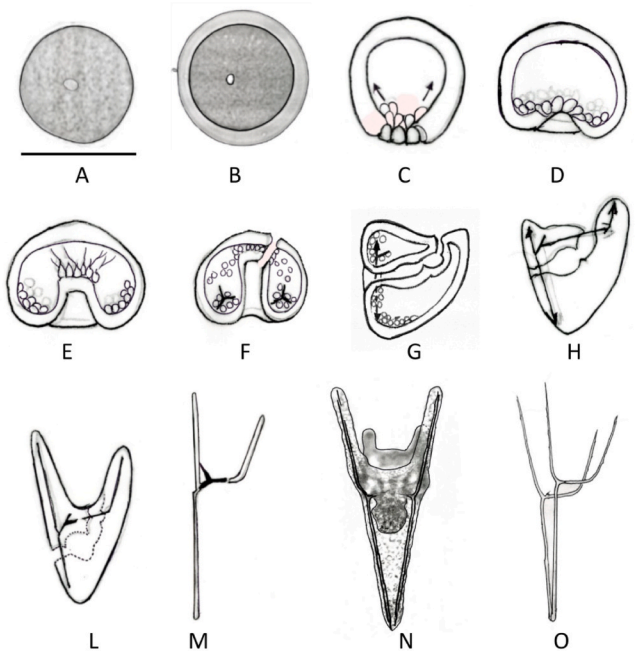

Fig. 1. *P. lividus* normal development A: unfertilized egg (gamete); B: fertilized egg before amphimixis (zygote); C: mesenchymal blastula stage-the first PMCs enter the coelom; D: beginning of gastrulation with invagination of the basal plate: ingress of the endoderm and of the secondary mesenchyme cells to reach the roof of gastrula; E, F: gastrula with the first skeletal rods (gastrula stage); G, H: prism stage and change of symmetry axes of the larva (larval stage); L: early pluteus; M–O: 24–48 h pluteus. Bars A-O = 100 μm.

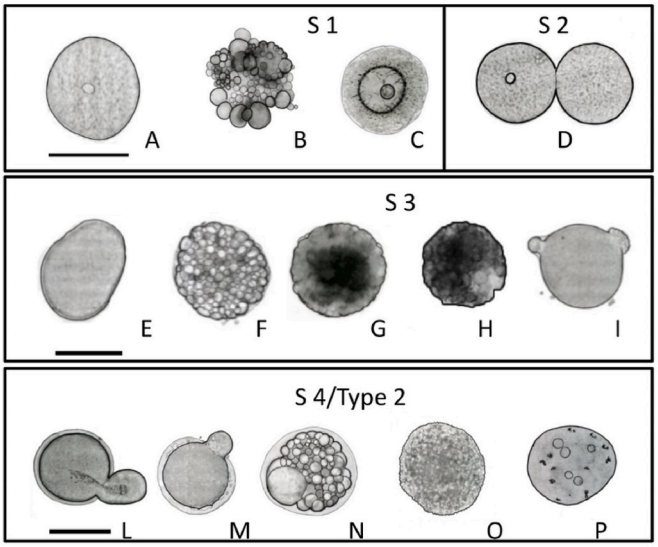

Fig. 2. Non-exposure-related anomalies of *P. lividus*: A: normal egg; B: piece of ovarian tissue released under stimulation; C: immature oocyte (B, C = S1 anomalies); D: membrane contact in eggs without jelly coat (S2 anomaly); E–I: aspects of damaged female gametes before fertilization (S3 anomalies); L–P: anomalous aspects of damaged eggs after fertilization (S4 anomalies). The same aspects are presented by eggs fertilized after acute exposure to pollutants (type 2 anomalies). Type 2 anomalies after exposure to sunscreen products. Bars = 100 μm.

anomalies are defined as Spontaneous (S) 1–4.

S 1: presence of immature oocytes. Sea urchin spawn fully ripe eggs, after the completion of meiosis. When spawning is forced at early

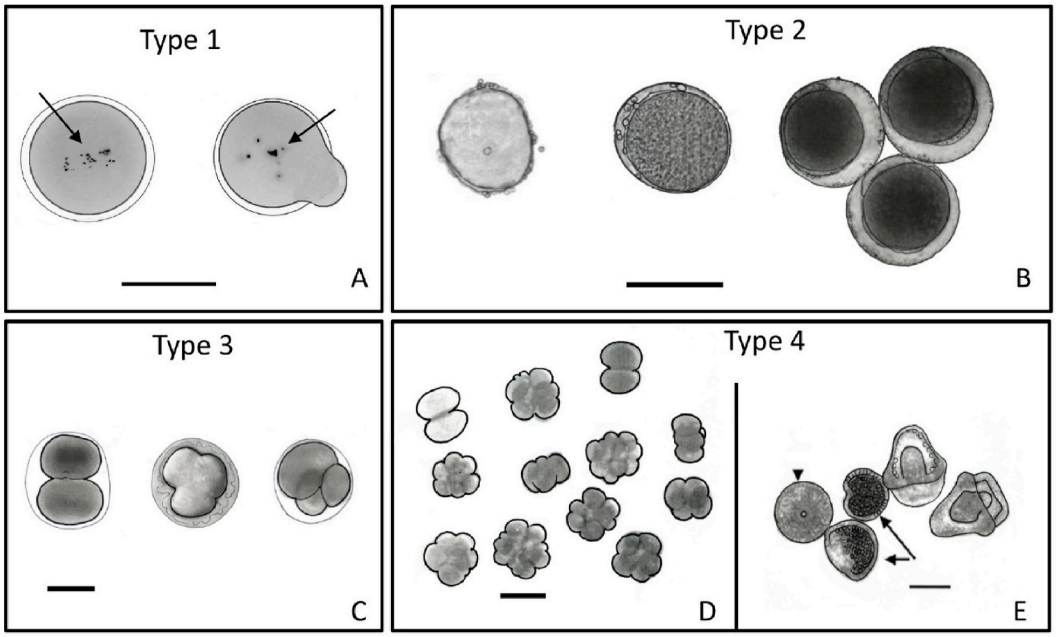

Fig. 3. *P. lividus* developmental anomalies due to early exposure (from gametes to zygote): A: polyspermic eggs 30–40 min after fertilization as a consequence of the alteration of the block of polyspermy (on the left polyspermic egg, and on the right polyspermic egg with destabilization of the fertilization membrane; the black arrows indicate sperm cells inside the eggs; see Figure S1); B: zygotes with blebs and dense material in the perivitelline space (type 2); C: anomalous cleavages from zygote to morula stage: the first on the left is reversible, the other two are irreversible and lethal (type 3 anomalies); D: asynchronous development at 3 h post fertilization; E: asynchronous development at 30 h after fertilization, with the presence of unfertilized eggs and anomalous gastrulae as indicated by the black arrows (type 4). Exposures to: A, C–E: pesticides; B: mercury. Bars = 100 μm.

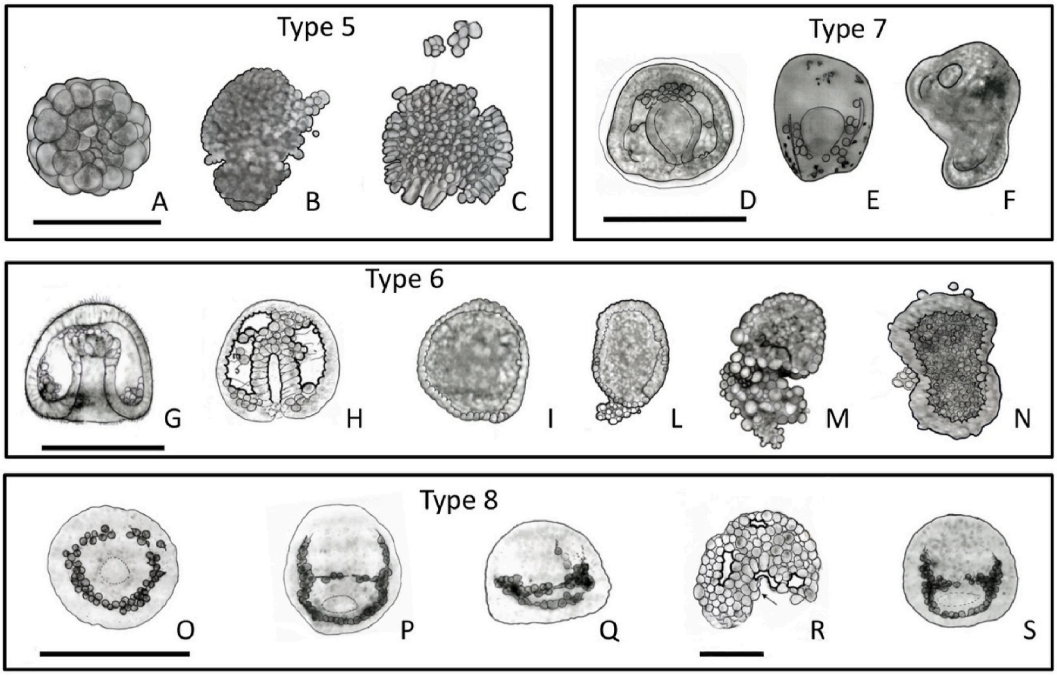

Fig. 4. *P. lividus* developmental anomalies due to early exposure to acute doses of pollutants: A: normal morula stage; B, C: disruption of cell adhesion in morula and early gastrula after exposure to mercury at zygote stage (type 5 anomalies); D: unhatched gastrula (type 7 anomalies); E, F: arrested gastrulae (type 7); G: normal gastrula, histochemical staining for AChE activity in PMCs; H: normal gastrula, semi-thin section showing wide blastocoel; I–N: exogastrulae (type 6 anomalies); O, P: normal migration of primary mesenchyme cells; Q, S: irregular migration of PMCs; R: resulting gastrula with narrow, if any, blastocoel (as indicated by the black arrow, type 8 anomalies). Staining of the PMCs by stain-coupled wheat germ agglutinin, WGA. Exposures to: B: nicotine; E, F: sunscreen products; I–N, Q–S: pesticides. Bars = 100 μm.

maturation stage, pieces of ovary or immature oocytes may be released (Fig. 2 B, C). Immature oocytes present small dimensions (diameter <70 μm) and huge nucleus containing a scarce cytoplasm (Fig. 2 C).

S 2: presence of eggs with damaged or lacking jelly coat: the eggs seem to stick together (Fig. 2 D).

S 3: eggs with irregular shape and size, frequently showing apoptotic-like appearance. This happens when ripe eggs are retained inside the ovaries or in the coelom due to climatic conditions or to health state of the adults (Fig. 2E–I).

S 4: immediately after fertilization, zygotes degenerate with apoptotic-like or swollen appearance, or blebbing (Fig. 2L–P).

S3 and S4 anomalies are like those caused by exposure to contaminants in the early sea urchin developmental stages.

4. Anomalies after exposure to environmental stressors

Environmental stressors are factors of natural or anthropogenic origin that perturb an ecosystem beyond its natural limits of variation (Crain et al., 2008; Hewitt et al., 2016). In marine ecosystems, these stressors induced by human activities (e.g. fishing and pollution) together with anthropogenically induced global climate change (e.g. ocean warming and acidification) are causing irreversible impacts on coastal marine ecosystems in a multitude of ways (Carrier-Belleau et al., 2021). Environmental stressors can affect ecosystems and biological processes in many organisms, including sea urchin development. The effect of these stressors on sea urchin development may differ according to 1- the exposure of the different stages (i.e. gametes, zygotes, early embryos, gastrula, larva); 2- pollutant mechanism of action, and 3- contaminants/mixtures concentrations to whom the samples are exposed (Sartori et al., 2017).

Morphological anomalies can be classified as reversible and irreversible. The first ones include anomalies due to natural causes that may be recovered once sea urchin larvae are brought again to optimal environmental conditions. Actually, the cause of the onset of these anomalies is not well understood although is frequently observed also in natural environment (Lister et al., 2017). For instance, in some cases spontaneous mutations may rise in maternal or paternal genomes, with devastating consequences on the entire pool of embryos (Hinegardner, 1975), in other cases changes in environmental factors (including diet and/or in water quality changes) may influence mathernal status, and produce embryo anomalies (Lister et al., 2017).

Irreversible anomalies are considered lethal, since they prevent further development and impair reproductive success. To assess the anomalies of gametes and zygotes, spermiotoxicity and embryotoxicity bioassays are carried out, respectively. In the first case the sperms are exposed before fertilizing the eggs; in the second case the eggs are exposed, or exposure occurs during fertilization or at zygote stage. The main anomalies are reported and described in Table 1.

The main anomalies of gametes and zygotes are:

- Type 1: alteration of the block of polyspermy, which produces a number of polyspermed eggs (Fig. 3 A). Lethal concentrations (LC) of the selected stressors can be measured.
- Type 2: the fertilization layer is elevated, with blebs or dense material in the perivitelline space (Fig. 3 B). Acute toxicity effects are represented by dead or degenerated zygotes (Fig. 2 L-P (S 4)).
- Type 3: odd blastomere cleavages or nuclear division not followed by complete cytoplasmic furrows (Fig. 3 C).
- Type 4: delay in the elevation of fertilization layer and loss of synchronicity in successive events (Fig. 3 D, E). This type of anomalies is partially reversible, provided that the optimal condition of water and temperature are restored.

When the effect is sub-lethal, the embryos may proceed in development, reaching the pluteus stage, and anomalies may appear lately, mainly represented by anomalous gastrulae or skeletal anomalies (Morale et al., 1998; Pesando et al., 2003).

The main anomalies that occur during cleavage and blastula stages are listed below:

- Type 5: exposure during the cleavage and blastula stage may cause the loss of adhesion among cells, followed by embryonic disaggregation (Fig. 4 B, C).

Table 1
Level of alteration of embryos and larvae of sea urchin, which is useful for calculating the index of contaminant impact (ICI).

Type of anomalies	Anomalies	Level of alteration
0	Healthy individual, with normal embryonic and larval development.	0
1	Loss of fertilizing ability of sperms and impaired block to polyspermy. In this case, the biomarker of toxicity is represented by the number of unfertilized eggs. Lethal concentrations (LC) of the selected stressors are measured in correspondence.	3
2	The fertilization layer is elevated, with blebs or dense material in the perivitelline space. Acute toxicity effects are represented by dead or degenerated zygotes (Fig. 2 L-P S 4).	3
3	Odd blastomere cleavages or nuclear division not followed by complete cytoplasmic furrows.	3
4	Delay in the elevation of fertilization layer and loss of synchronicity in successive events This type of anomalies is partially reversible, provided that the optimal condition of water and temperature are restored.	1
5	Exposure during the cleavage and blastula stage may cause the loss of adhesion among blastomeres, followed by embryonic disaggregation.	3
6	Anomalous of PMCs, which are unable to enter the coelom cavity and are extruded, forming exogastrulae.	3
7	Lack of hatching up to gastrula and prism stages. Arrested development at gastrula stage.	3
8	Altered aspects of PMCs migration, causing gastrulae lacking a coelom. The effect of exposure on PMC migration is also responsible for defective skeletogenesis at further stages (Type 9–12).	2
9	Small larvae, with swollen aspect, short and thin skeletal rods.	1
10	Short plutei, with little or no developed hind spines, so that the larva has a truncated appearance.	1
11	Larvae slightly smaller or equal to controls, with skeletal rods of the anterior arms fused, or with crossed tips. Sometimes both the aspects are present in the same larva.	1
12	Asymmetrical or bent larval body.	1
13	Presence of spines and supernumerary rods or entirely doubled skeleton.	2
14	Severe skeletal regression usually in the arms, or in both the parts of larval body.	3
15	Light skeletal regression, the arms are transparent or flabby.	1
16	Total or partial soft tissue retraction, so that the naked rods protrude out of the larval body.	2
17	Larvae with swollen and dilated intestine and anus.	2
18	Dead larvae or with lethal anomalies due to acute exposure to toxicants. These larvae may present a disrupted aspect, with vestigial skeleton and degenerating tissues. Although these anomalies are irreversible and deadly, often these larvae continue to swim on the bottom of the vessel or in the water column for some days.	3

- Type 6: collapsed ectodermal and/or endodermal cells, forming exogastrulae (Fig. 4I–N).
- Type 7: lack of hatching up to gastrula and prism stages (Fig. 4 D). Arrested development at gastrula stage (Fig. 4E and F).
- Type 8: Altered aspects of PMCs migration (Fig. 4 Q, S), causing gastrulae lacking a coelom (Fig. 4 R). The effect of exposure on PMC migration is also responsible for defective skeletogenesis at further stages (type 9–12).
- Type 9: Small larvae, with swollen aspect, short and thin skeletal rods (Fig. 5 A).
- Type 10: short plutei, with little or no developed hind spines, so that the larva has a truncated appearance (Fig. 5 B).
- Type 11: larvae slightly smaller or equal to controls, with skeletal rods of the anterior arms fused, or with crossed tips. Sometimes both the aspects are present in the same larva (Fig. 5 C).
- Type 12: asymmetrical or bent larval body (Fig. 5 D). These aspects were also described by Carballeira et al. (2012a) for larvae exposed to effluents from land-based turbot farms.
- Type 13: Presence of spines and supernumerary rods or entirely doubled skeleton (Fig. 5E–I).

In case of exposure at pluteus stages, the skeletal defects are secondary, because the skeletal rods and the body are already formed. Nevertheless, the following anomalies may be present:

- Type 14: Severe skeletal regression (Fig. 6A–E) usually in the arms, or in both the parts of larval body.
- Type 15: Light skeletal regression, the arms are transparent or flabby (Fig. 6 F, G)
- Type 16: Total or partial soft tissue retraction, so that the naked rods protrude out of the larval body (Fig. 6 B, Fig. 6 H, I).

These two types may also present clusters of pigment cells in the coelom and along the residual skeletal rods. The black and white figures show these clusters as dark spots (Fig. 6 B, C, D, H, I) (Ghilardi, 2016).

- Type 17: larvae with swollen and dilated intestine and anus (Fig. 6L–O).
- Type 18: Dead larvae or with lethal anomalies due to acute exposure to toxicants. These larvae may present a disrupted aspect, with vestigial skeleton and degenerating tissues (Fig. 6R–V). Although these anomalies are irreversible and deadly, often these larvae continue to swim on the bottom of the vessel or in the water column for some days.

Studies on the quantitative relevance of anomalies in early developmental stages of *P. lividus* developed indices for assessing the degree of toxicity and environmental impact of contaminants (Carballeira et al., 2012a; Corinaldesi et al., 2017). These indices could also be applied to all the different types of malformations identified in this work (Figs. 1–6). Corinaldesi et al. (2017) applied the Index of Sunscreen Impact to assess the environmental impact of sunscreen products. They grouped embryo and larvae anomalies according to their level of alteration (i.e. 0 = normal development; 1 = light anomalies, easily reversible; 2 = moderate anomalies; 3 = severe anomalies, leading to death and/or arrest of development; Table 1). By applying the formula described by Corinaldesi et al. (2017), on the frequency of anomalies for each degree of sea urchin embryo/larval alteration found in this review, an Index of Contaminant Impact (ICI) can be calculated, that summarizes the impact of pollutants, as follows:

ICI [= 0 x % level 0 + 1 x % level 1 + 2 x % level 2 + 3% level 3]/100

ICI index ranges from 0 (no impact) to 3 (high impact), also including the levels 1 (slight impact) and 2 (moderate impact).

The effects of exposure to different contaminants depend on the interference, which the contaminants may exert on the developmental events taking place during the exposure. Here, the anomalies and their severity related to the ICI.

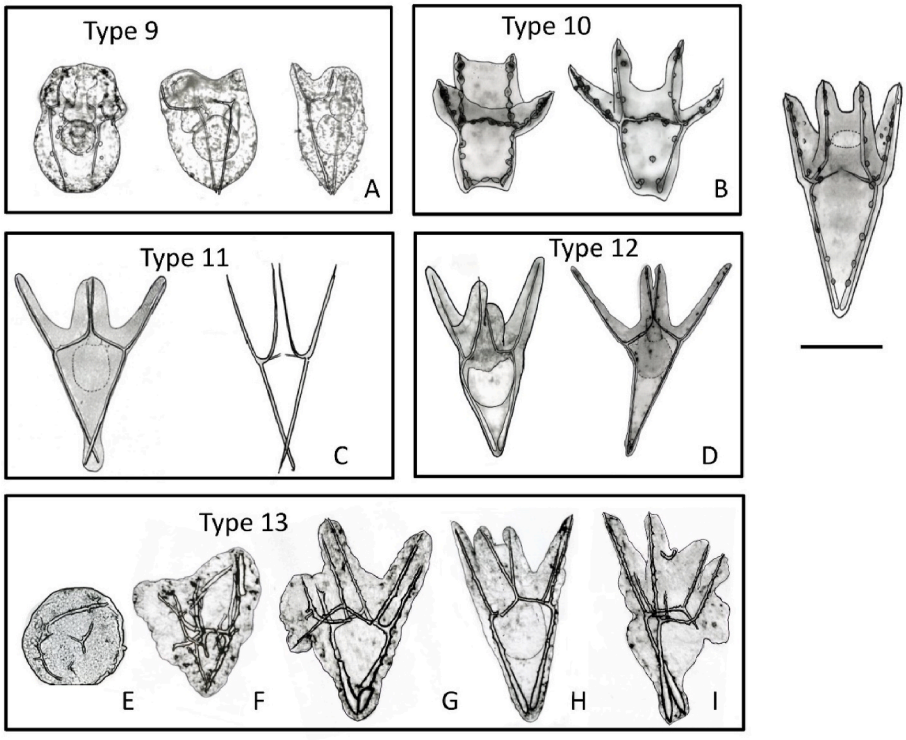

Fig. 5. *P. lividus* late developmental anomalies due to early exposure to sublethal doses of pollutants compared to the control (on the right of the figure): A: inefficient skeletogenesis, swollen vegetalized-like appearance (type 9); B: short larvae, not joined skeletal rods at the apex (type 10); C: crossed tips at the apex, partial fusion of the perioral arms (type 11); D: asymmetrical arms and general structure (type 12); E–I: different aspects of larvae, with supernumerary skeletal rods (type 13). Exposures to: A: antibiotics; B: nanoparticles and suntan products; C: nanoparticles; D: sunscreen products; E–I: SiO_2 nanoparticles. Bar = 100 μm.

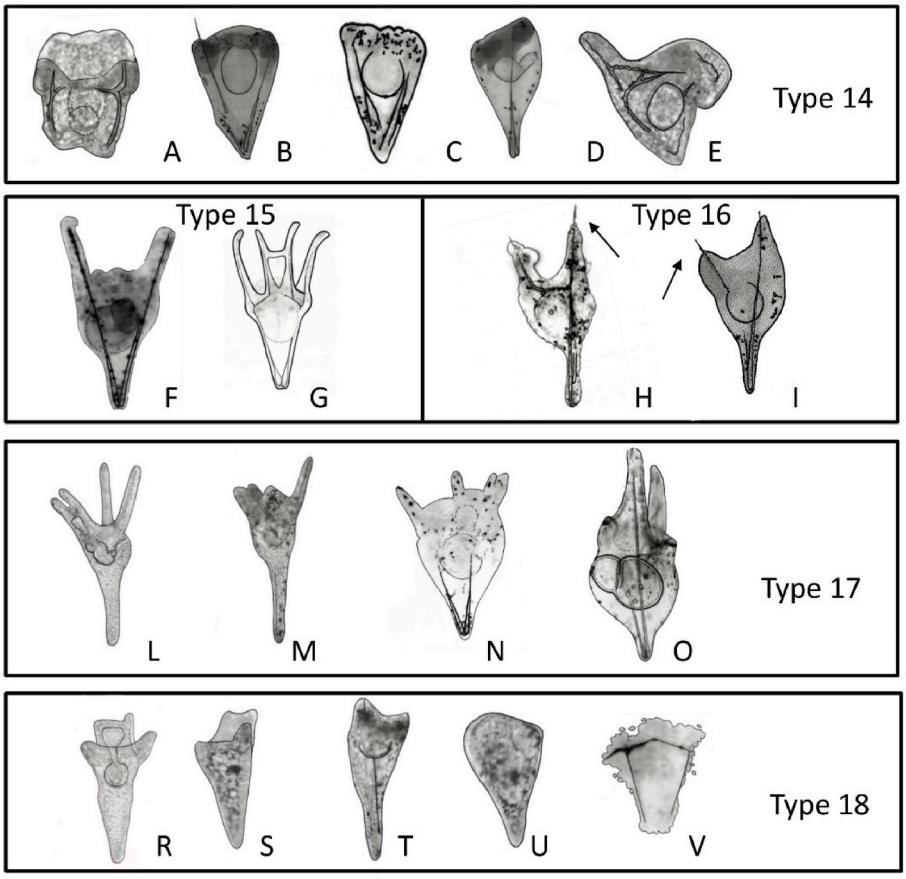

Fig. 6. *P. lividus* developmental anomalies due to late exposure (pluteus stage) to pollutants: A–E: 72 h plutei with resorbed skeletal rods and perioral arms (type 14); F, G: Plutei with partial resorption of spicules, and flabby arms (type 15); H, I: plutei with soft tissue retraction, leaving the skeletons naked (as indicated by the black arraows, type 16); L–O: various aspects of skeleton retraction; N, O: larvae with swollen intestine and anus (type 17); R–V: lethal forms of 72 h plutei (type 18). Exposure to sunscreen products.

5. Anomalies due to exposure to contaminants

5.1. Heavy metals

Anomalies of sea urchin fertilized eggs and first developmental stages have been reported after exposure to several heavy metals (HMs), including cadmium, manganese, copper, lead, chromium (Pagano et al., 1982a, b; 1983; Congiu et al., 1984; Warnau and Pagano, 1994; Russo et al., 2003; Kobayashi and Okamura, 2004, 2005; Filosto et al., 2008; Soualili et al., 2008; Pinsino et al., 2010; Migliaccio et al., 2014; Gharred et al., 2016). The main morphological anomalies of the embryos and larvae exposed to HMs are attributable to type 1 and type 7. The effects of HMs are exerted through interference in multiple mechanisms, including direct modification by reactive oxygen species, inflammation processes, alteration of neurotransmitter molecules (i.e. acetylcholine, ACh). As a result, cell communications between cells supported by ion exchange are altered, as well as the intracellular ion concentration, which explains the inhibition of sperm fertilizing ability (Runnström and Manelli, 1964).

Exposure to the inorganic salt $HgCl_2$ at high concentrations (10^{-4} - 10^{-8} M) impaired the block to polyspermy and caused block of development: the zygotes presented blebs at the cell surface (type 5, Fig. 4 B, C). At low concentrations (10^{-9} M), development could proceed to embryo stage, without reaching larval stages: the embryos showed anomalies like type 2 (Fig. 3B; Trielli et al., 1995; Berridge et al., 2000).

HMs exposure generally causes severe anomalies (levels of alteration 3). See further details in Supplementary information.

5.2. Persistent Organic Pollutants (POPs)

Early development of several sea urchin species can be sensitive to Persistent Organic Pollutants (POPs), such as PAHs (Bresch et al., 1972; Hose et al., 1983; Pillai et al., 2003; Suzuki et al., 2015) and PCB (Adams, 1983; Pagano et al., 1985; Anselmo et al., 2011). Exposure of benzo(a)pyrene and tobacco smoke condensate to gametes and zygotes of *P. lividus*, *Stronglycentrotus purpuratus* and *Psammechinus miliaris* did not induce anomalies in the early developmental stages (De Angelis and Giordano, 1974; Hose et al., 1983). Conversely, exposing zygotes of the sea urchin *Hemicentrotus pulcherrimus* and *P. lividus* to benz(a)anthracene, benzene and metabolites of PAHs caused skeletal anomalies, like type 10 (Fig. 5 B; Pagano et al., 1988; Suzuki et al., 2015). Exposure of *Lytechinus anemesis* embryos to PAHs, caused exogastrulae with evaginated archentera (Pillai et al., 2003) or retarded the cleavage with a loss of adhesion among blastomeres (Hose et al., 1983), ascribable to type 5 and 6 (Fig. 4, B–C, I–N). PCB caused anomalies in the early development mainly represented by the presence of short or abnormal arms (Pagano et al., 1985; Anselmo et al., 2011), attributable to the types 9 and 10 (Fig. 5 A, B). All these anomalies can be classified as low and severe anomalies (levels of alteration 1 and 3). Further information is reported in the Supplementary Material.

5.3. Endocrine disrupters

Environmental endocrine disrupting compounds (EDCs) are exogenous substances or mixtures that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub) populations (Damstra et al., 2002) (IPCS, 2002). They include organic synthetic compounds such as Bisphenol-A (BPA), hormones, acetylsalicylic acid and some pesticides, PPCPs and surfactants (described in the next paragraphs). These EDCs have been shown affect the development of sea urchins (Roepke et al., 2005; Özlem and Hatice, 2008; Da Silva and de Souza Abessa, 2019).

Roepke et al. (2005) exposed developing sea urchin embryos (*Strongylocentrotus purpuratus*, *L. anamesus*) to natural and environmentally relevant synthetic EDCs, including BPA. Exposure from fertilization to 96 h development, caused morphological anomalies at all the investigated stages, according to other studies on the sea urchins *P. lividus* and *E. locunter* testing the effects of BPA, 17α-ethinylestradiol and acetylsalicylic acid (Özlem and Hatice, 2008; Da Silva and de Souza Abessa, 2019). The mechanism of action of EDCs may be due to a deregulation of calcium at cellular level, that may explain the skeletal anomalies in sea urchin larvae, similar to type 10, 14 and 18 (Fig. 5 B; Fig. 6 A-E; Fig. 6 T-V; Roepke et al., 2005).

PCB exposure of zygotes and embryos mainly caused short or abnormal arms (Pagano et al., 1985; Anselmo et al., 2011), similar to *P. lividus* anomalies type 9 and 10 (Fig. 5 A, B).

EDCs-related defects can be classified as low and severe anomalies (levels of alteration 1 and 3).

5.4. Pesticides

Exposure of sea urchin gametes and zygotes to neurotoxic pesticides (i.e. organophosphates, carbamates) causes type 1, 3, 4 and 6 anomalies (Morale et al., 1998; Falugi and Angelini, 2002; Aluigi et al., 2010). Exposure before gastrulation causes gastrulae without a well-defined blastocoel (type 8, Fig. 4 R), for impaired PMCs migration (Fig. 4 Q, S; Pesando et al., 2003). The exposure to lower concentrations of neurotoxic pesticides at early stages causes type 10, 11, 14 anomalies (Fig. 5 B, C), while high doses of pesticides (i.e. 10^{-3} M diazinon) causes arrested development or death (type 18 anomaly; Ohta et al., 2009).

Based on the levels of alteration of sea urchin embryos and larvae, pesticides can cause levels of alterations ranging from 1 to 3. Additional information on pesticides effects on sea urchin are reported in the Supplementary Material.

5.5. Pharmaceuticals and personal care products (PPCPs)

Environmental contamination by PPCPs is increasing worldwide, since they are not regulated and their concentrations in the environment are lower than those of traditional contaminants (Arpin-Pont et al., 2016). Among PPCPs, sea urchin development is affected by 17α-ethynylestradiol (EE2), acetylsalicylic acid (ASA), antibacterial agents and sunscreens. The exposure of EE2 and ASA to several sea urchin species reduced fertilization success and impaired the development, causing anomalies similar to types 6 (Figs. 4 I), 8 (Fig. 4 P), 13 (Figs. 5 E) and 14 (Fig. 6, E; Roepke et al., 2005; Capolupo et al., 2018; Da Silva and de Souza Abessa, 2019).

Exposure of sea urchin fertilization to different concentrations of effluent waters containing antibiotics caused dose-dependent anomalies in embryos of P. *lividus* and *Arbacia lixula*, including S3–S4 types abnormalities, gastrulation abnormalities similar to 7 (Fig. 4 E, F) and 8 (Fig. 4 Q, R) types. Exposure to minor concentrations allowed development up to pluteus larva, with anomalies attributable to types 9, 10, 11, 12 (Fig. 5A–D) and 17 (Fig. 6 L, M).

Among antibacterial agents, triclosan (TCS) is one of the most used in Europe. Low TCS concentration does not affect *S. nudus* and *P. lividus* development, differently from high doses (Macedo et al., 2017). Indeed, the development in some embryos was delayed or tended to be arrested, without reaching the pluteus stage (type 7 anomaly Fig. 4 E; Hwang et al., 2014).

Sunscreen products generally contain active ingredients to protect human skin from UV radiation, such as organic UV filters (e.g. benzophenones) and/or inorganic filters (e.g. TiO_2 and ZnO), which prevent or limit UV penetration. Other ingredients normally found in almost all commercial products include preservatives, adjuvants, moisturizers, and antioxidants.

Sunscreen products and UV filters can affect embryo-larval development of sea urchins (Au et al., 2002; Catalano et al., 2020; Corinaldesi et al., 2017), causing development block (type 7, Fig. 4 E, F), signs of necrosis (type 2, Fig. 3 B) or exogastrulae formation due to anomalous PMCs migration (type 6, Fig. 4H–N). The effects of sunscreen products were even more evident on the sea urchin larval development, showing death or lethal anomalies (types 17, 18, Fig. 6L–V). Different types of larval anomalies (type 10–16, Figs. 5 and 6) were observed when *P. lividus* was exposed to different brands of sunscreen products (Corinaldesi et al., 2017). Further information about PPCPs effects on early stages of sea urchin is reported in the Supplementary Material. By classifying the anomalies, it can be observed that PPCPs cause a wide range of anomalies (levels of alteration 1, 2, 3). Further information is reported in the Supplementary Material.

5.6. Surfactants

Surfactants are the chemicals which react with lipid and lipoprotein of the cell membrane, disturbing the membrane structure (Bont et al., 1969). Effects on several surfactants (i.e. sodium lauryl sulfate (SLS), sodium deoxycholate (DOC), cetyltrimethyl ammonium bromide (CTAB), Tween 20, Tween 80, linear alkylbenzene sulphonat) have been demonstrated in sea urchin cleavage and further development of the sea urchins *Hemicentrotus pulcherrimus*, *Temnopleurus toreumaticus, Pseudocentrotus depressus* and *P. lividus* (Tanaka, 1976, 1979; Bresch and Ockenfels, 1977; Bressan et al., 1989, 1991). Normal development has been observed after exposure to low surfactant concentrations, while high doses caused an altered PMC migration (type 8, Fig. 4 Q) responsible for exogastrulae formation (type 6, Fig. 4 M, N) and abnormal embryos characterized by few cells in their blastocoel (type 4, Fig. 3 E). The main anomalies found at larval stage were exerted on skeletal development (type 10, 11, Fig. 5 B, C) that was completely inhibited at high doses (Bressan et al., 1989). Overall, surfactant mechanism of action is maximum at the end of gastrulation, when Ca^{2+} uptake is high, presumably related to the beginning of the skeletal growth, that may affect calcium availability for morphogenesis (Bressan, 1991).

Based on the levels of alterations, surfactants determined a wide range of alteration levels (1–3: from light to severe anomalies).

5.7. Microplastics (MPs)

MPs are derived from large plastic degradation (Andrady, 2011). The exposure of the sea urchin *Tripneustes gratilla* showed scarce sensitivity, and no developmental anomalies after ingestion of polyethylene MPs (Kaposi et al., 2014). On the contrary, *P. lividus* larvae showed aspects like the type 18 (Fig. 6 T, U, V) after exposure to high doses of polystyrene MPs (Della Torre et al., 2014; Messinetti et al., 2018), MP leachates (Oliviero et al., 2019) and pellet (Rendell-Bhatti et al., 2020), while sublethal MP concentrations caused type 7 (Fig. 4 E, F), 10 (Figs. 5 B) and 14 anomalies (Fig. 6 A-E; Pinsino et al., 2017). Similar anomalies were observed in the sea urchin *L. variegatus* exposed to virgin and beach-stranded plastic pellets (Nobre et al., 2015) and in *Sphaerechinus granularis* exposed to MPs (Trifuoggi et al., 2019).

MPs caused anomalies can be classified as low and high anomalies, respectively (levels of alteration 1 and 3). Further information is reported in the Supplementary Material.

5.8. Nanoparticles (NPs)

NPs are tiny materials having size ranges from 1 to 100 nm. They can be classified into different groups based on their properties, shapes or sizes and have different applications. NPs including TiO_2, CeO_2, Ag, SnO_2, Co and Fe_3O4, generally exert an inflammation and oxidative stress in cells and tissues (Jeevanandam et al., 2018). Inflammation is generally related to alteration in the cholinergic system, causing an overexpression of AcetylCholine (Ach) (Wessler and Kirkpatrick, 2001). In *P. lividus* larvae, inflammatory process is demonstrated by the enhancement of ACh expression; thus, NP exposure causes anomalies

like types 10, 11, 13, including delayed development, bodily asymmetry and shortened or irregular arms (Gambardella et al., 2013, 2014; Šiller et al., 2013).

Ingested metal oxide NPs (SnO_2, CeO_2, Fe_3O_4) can enter and accumulate into the immunity cells, embryos, and larvae of *P. lividus,* causing developmental anomalies like type 10, 11, 13 (Falugi et al., 2012; Gambardella et al., 2015; Burič et al., 2015; Mesarič et al., 2015).

These anomalies can be classified from low to moderate anomalies (levels of alteration 1 and 2). Additional information on nanoparticles effects on sea urchin are reported in the Supplementary Material.

6. Other anthropogenic impacts and multiple stressors driven by climate change

Besides traditional and emerging contaminants, other stressors have been reported to impact early developmental stages of sea urchin. Among them, electromagnetic fields (EM) radiations caused an impairment of sea urchin development after exposure of fertilized eggs, due to damage of the membrane structures (Koldayev and Shchepin, 1997) and anomalies attributable to the type 3 (Fig. 3 C; Ravera et al., 2006). Conversely, larvae exposed to EM pulses did not cause developmental anomalies (Falugi et al., 1987).

The environmental exposure to radioactivity of adult specimens caused damage to DNA in the spawned gametes and block of development at blastula stage, with a high percentage of anomalies, mainly due to impaired cleavages and loss of symmetry (like type S4/types 2 and 3) (Gabel et al., 1979). Shoulkamy at al. (2018) observed that exposure to X-rays caused anomalies according to the developmental stage of exposure. Exposure of early stages and before gastrulation caused a scarce number of migrating PMCs (type 8, Fig. 4 Q), while exposure after gastrulation caused skeletal anomalies, like types 10, 11, 12.

Sea urchins are subjected to multiple impacts which also include climate change (e.g., ocean warming, acidifying, and increasing in pCO_2). These impacts can act simultaneously affecting their fertilization processes (Byrne et al., 2009, 2010). High temperatures (>30 °C) affect fertilization success (Mejía-Gutiérrez et al., 2019), showing alteration in the development, such as asymmetrical cleavage (type 3, Fig. 3 C). UV-radiations can also affect the cleavage stage embryo, besides the skeleton growth (Bonaventura et al., 2005, 2006), inhibiting development and caused exogastrulae formation in two sea urchin species (like type 6, Fig. 4 L, M; Akimoto et al., 1983; Lesser et al., 2003; Ding et al., 2019).

According to Byrne et al. (2010), ocean warming might negatively affect sea urchin fertilization, especially sperm activity. Conversely, acidification did not affect fertilization and embryogenesis (Martin et al., 2011). Contrasting information about the impact of acidification was reported in other studies conducted on *T. gratilla* (Sheppard Brennand et al., 2010). Increased acidity/pCO_2 and decreased carbonate mineral saturation significantly reduced larval growth resulting in decreased skeletal length (attributable to types 14, Fig. 6), such as shortened perioral arms (Fig. 6, type E).

Some categories of natural compounds produced by diatoms have been reported to cause stress and malformations in early developmental stages of *P. lividus* (Romano et al., 2010). In particular, natural oxylipins such as polyunsaturated aldehydes (PUAs) and hydroxyacids (HEPEs) have been demonstrated to exert a teratogenic effect on *P. lividus* embyos (Marrone et al., 2012; Varrella et al., 2016) and a strong developmental delay with increasing decadienal concentrations (from 1.32 μM up to 5.36 μM, Romano et al., 2010). Further studies demonstrated that PUAs induced malformations in *P. lividus* larvae of type 10, 11, 12, 17 and 18 (Varrella et al., 2014). HEPEs also caused anomalies of type 11 at lower concentrations (6–10 μM) and anomalies of type 7 at higher concentrations (15–30 μM; Varrella et al., 2016).

The anomalies produced by stressors described in this paragraph can cause a wide range of anomalies at all the level of alterations (i.e. 1, 2, 3). Further information is reported in the Supplementary Material.

7. Main remarks and future perspectives

Morphological anomalies due to the exposure to different contaminants and multiple stressors are attributable to similar dose-dependent mechanisms of interference in cell-to-cell communication driven by intracellular or intercellular ion alteration. The acute exposure to contaminants causes almost immediately lethal or irreversible anomalies, while the effect of subacute exposure is observed over long times (ca. 10 days) and not at the first developmental stages. As more diluted are the toxic compounds, as later the effects appear visible (Marchi et al., 1996; Carballeira et al., 2012a), potentially because the embryonic development is a multiphasic event. Thus, effects which at early development stages may appear negligible, are generally amplified during development, and become evident and often lethal at later stages.

A common effect of the exposure of the early developmental stages of sea urchin to different stressors including pesticides (Sultatos, 1994), metallic NPs (Gambardella et al., 2013), EDCs (Sarkar et al., 2006) and sunscreen products (Corinaldesi et al., 2017) is the alteration of acetylcholinesterase (AChE) activity. This activity is one of the main biomarkers for several pollutants, and is present during morphogenetic events, including PMC and lateral laminae of mammalians (Drews, 1975). Data summarized in this review suggest that the effects of AChE alteration may be due to the impairing of the regulation of calcium-related events since the earliest stages (Harrison et al., 2002; Jennings et al., 2008). Besides AChE, other biochemical and molecular mechanisms can be altered in sea urchin development due to contaminant exposure, therefore they need to be further considered. The different kinds of anomalies here classified may depend on time/-concentration/stressor type, impinging on the mechanism of positional information occurring during the exposure (Table 2). Therefore, the anomalies themselves can be considered specific biomarkers of the presence and degree of impact of the responsible stressors. However, further investigations are needed to demonstrate the cause-effect relationships between sea urchin development and stressors, also by using molecular and biochemical approaches to better understand the responses of the organisms (Buznikov et al., 2001; Pinsino and Matranga, 2012; Gambardella et al., 2013; Corinaldesi et al., 2017).

In this light, the identified types could be used as specific biosensors for the different pollutants in monitoring plans and help us to make: i) a first screening of the potential contaminants/stressors in marine environment, which should be identified with specific analyses, and ii) a prediction of the degree of environmental impact (Table 1) to deepen investigations.

The rationalization of the information collected in this review further highlight the utility of sea urchin as a model to assess the effects of multiple stressors and contaminants in marine environment. However, although several experimental studies and ecotoxicological bioassays have been already conducted using sea urchins, this review underlines the importance to increase information on the analyses of embryo and larvae morphological anomalies and mechanisms behind these biological responses aiming at upgrade and expand the categories of malformations related to the different contaminants and stressors. In the light of the current and future scenarios of climate change, we claim to urgently investigate the effects of multiple anthropogenic impacts on early developmental stages of sea urchin to forecast the effects of climate change and define specific management strategies and conservation in marine environment.

Table 2
Types of anomalies related to the stage of exposure and concentration of pollutants in early developmental stages (gametes, embryos, larvae) of *Paracentrotus lividus*. Also reported are impaired events, altered mechanisms and timing at which all factors can be observed (according to the exposure time). High dose = concentration ≥ EC50; low dose = between NOEC and EC50.

EXPOSURE STAGE	EXPOSURE	ANOMALIES	IMPAIRED EVENTS	ALTERED MECHANISMS	TIMING
Early exposures (gametes and fertilization)	High dose	Unsuccessful fertilization Sperm inhibition (type 1)	Sperm motility	General toxicity, cell death Altered membrane permeability, block to ATP production Altered intracellular [Ca^{2+}] needed for flagellar motility	Few minutes
			Acrosome reaction	Altered release of calcium ions, which are involved in microtubules assemblage to form the acrosome process Altered release and function of lytic enzymes from the acrosome	
		Egg inhibition (type 2, Figure 2 O-P)	Cortical reaction, (no fertilization layer visible)	Altered Influx of Na^{+} molecules through electrochemical receptors which ease membrane fusion of gametes Insufficient release of calcium ions from the internal stores	20-40 min
		Zygote damage	a) Cell death b) Egg fragmentation, cortical blebbing (type 2, Figure 2 L-N)	a) Altered membrane permeability b) Excessive [Ca^{2+}] shock causing sudden contraction of cortical web cytoskeleton (actin and microfilaments)	
		Anomalous cleavages (type 3)	a) Multiple spindles b) Odd number of blastomeres	a) Altered block to polyspermy (either fast or slow) caused by altered calcium release from the internal stores b) Anomalous direction of the spindles (intracellular anomalous distribution of ions	40 min from the exposure stage From 70 min up to 18 h
	Low dose	Asynchronous development (types 3, 4)	Compromised clock mechanisms	a) Different speed in sperm motility	From 20 min to 1 h
				b) Different speed in cell cleavages (unknown mechanisms)	From 70 min up to 18 h
Zygote- gastrula	High dose	a) Block of development b) anomalous gastrulae (types 6-8)	a) Total or partial inhibition of PMCs ingression b) Wrong PMCs direction inside the coelomic space	a) Malfunction of cadherins release due to altered [Ca^{2+}] b) Lack of collagen and fibronectin deposition inside the coelomic wall (Huggins and Lennarz, 2001)	18-24 h 18-22 h
	Low dose	Skeletal anomalies at larva stage (types 9-13)	Anomalous migration of PMCs along anomalous fibronectin patterns		40-48 h up to 72 h
Larval stages	Low dose	a) Resorption of larval structures (types 14-16) b) Swollen gut, red pigmented cells (type 17)	a) Decalcification of skeletal rods (types 14-16) b) Inability of feeding due to slow, asynchronous, or arrested ciliary movements (type 17)	a) Acidification of the whole organisms b) Anomalous coordination of ciliary movements due to neural-like ionic intra-cellular dynamics	40-48 h up to 72 h
	High dose	Arrest of development/ death (type 18)			From few hours up to 24 h

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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