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MINI REVIEW

Cadmium stress effects indicating marine pollution in different species of sea urchin employed as environmental bioindicators

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Abstract

In recent years, researches about the defense strategies induced by cadmium stress have greatly increased, invading several fields of scientific research. Mechanisms of cadmium-induced toxicity continue to be of interest for researchers given its ubiquitous nature and environmental distribution, where it often plays the role of pollutant for numerous organisms. The presence in the environment of this heavy metal has been constantly increasing because of its large employment in several industrial and agricultural activities. Cadmium does not have any biological role and, since it cannot be degraded by living organisms, it is irreversibly accumulated into cells, interacting with cellular components and molecular targets. Cadmium is one of the most studied heavy metal inductors of stress and a potent modulator of several processes such as apoptosis, autophagy, reactive oxygen species, protein kinase and phosphatase, mitochondrial function, metallothioneins, and heat-shock proteins. Sea urchins (adults, gametes, embryos, and larvae) offer an optimal opportunity to investigate the possible adaptive response of cells exposed to cadmium, since these cells are known to accumulate contaminants. In this review, we will examine several responses to stress induced by cadmium in different sea urchin species, with a focus on *Paracentrotus lividus* embryos. The sea urchin embryo represents a suitable system, as it is not subjected to legislation on animal welfare and can be easily used for toxicological studies and as a bioindicator of environmental pollution. Recently, it has been included into the guidelines for the use and interpretation of assays to monitor autophagy.

Keywords Cadmium-stress · Pollution · Defense strategies · Autophagy · Apoptosis · Sea urchin

Introduction

A wide range of anthropogenic contaminants finally ends up in the marine environment, which constantly receives several chemicals originating from terrestrial sources (Islam and Tanaka 2004; Meena et al. 2018). Monitoring and understanding the toxic effects of chemical pollution in the marine ecosystems is becoming more and more urgent for environmental management (European Marine Board 2013). In the sea, surface waters often have low discharge or renewal rates, hence pollutant contamination from industries have a high negative impact on the physico-chemical and biological quality of water. The elements that primarily threaten marine organisms are biological (bacteria, viruses, fungi, protozoa), chemical (metals, metalloids, organic compounds), and physical contaminants (X- and UV rays) (Elliott 2003; Chiarelli and Roccheri 2012; Matranga et al. 2013).

Contaminants are often biopersistent pollutants that accumulate at the top of the food chain, entering into living organisms via food, drinking water, and air (Chiarelli and Roccheri 2014, 2015). Biomonitoring is essential for the assessment of the health of marine ecosystems and for evaluating possible risks for human health. Many studies have been carried out to assess the status of chemical pollutants in marine ecosystems using embryos or adult organisms as bioindicators.

Among chemical contaminants, the seawater quality is especially affected by those pollutants deriving from industrial discharges, and among these, the most dangerous are heavy

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metals: cadmium, mercury, chromium, lead, arsenic, and thallium (Viarengo 1989; Chiarelli and Roccheri 2014, 2015; Yılmaz et al. 2017; Meena et al. 2018). Heavy metals are non-biodegradable and persist in the environment for long periods. Cadmium (Cd), a non-essential metal, is one of the most powerful poisons, able to cause different types of damage, including cell death (Hamada et al. 1997; Templeton and Liu 2010). The main cause of Cd-toxicity comes from persistence and accumulation in biota and in aquatic environments (Waisberg et al. 2003; Chora et al. 2009).

Cd is commonly detected in aquatic and terrestrial environments, released from natural sources (leaching of Cd-rich soils or volcanic activities), as well as from anthropogenic activities (production of plastics stabilizers and nickel-cadmium batteries, mining, electroplating, smelting, production of pigments) (Bargagli 2000). Several reports show evidence of Cd pollution in the marine environment. Polluted areas were revealed in the Mediterranean sea around Sicily island, with levels comprising between 0.56×10^{-3} and 80.4×10^{-3} μg/L (Censi et al. 2002); in the Northern Atlantic Ocean with about 4×10^{-3} μg/L (Kremling and Streu 2001); in the Baltic Sea, with levels comprising between 12 and 16×10^{-3} μg/L (Pohl and Hennings 2005). along the Galitian coasts Cd concentration in the seawater ranged from 13.4×10^{-3} to 1.49 μg/L in very polluted sites (Beiras et al. 2003).

The presence of this metal is highly dangerous since it easily penetrates into cells via transport mechanism normally used for other purposes, where it is then slowly eliminated. Sublethal effects of Cd on population growth rate, gametogenesis, and embryogenesis have been described in various species of aquatic invertebrates (Schröder et al. 2005; Roccheri and Matranga 2010). Therefore, the effects of Cd on these organisms have been studied examining its accumulation in adult tissues, as well as in embryos where it perturbs embryonic development and triggers stress protein synthesis, expression of detoxification genes, apoptosis, autophagy, and the related pathways involved (Roccheri et al. 2004; Agnello et al. 2007; Chiarelli et al. 2011; Ragusa et al. 2013; Migliaccio et al. 2015; Chiarelli et al. 2016).

Marine invertebrates inhabit a key position as intermediate consumers in the pelagic as well as in the benthonic food chains, making them suitable model systems for ecotoxicological studies.

Here, we will analyze Cd as a stressor for the induction of defense strategies, purely as a toxic insult. The doses used for this purpose are usually above the environmental concentrations. On the other hand, previous reports showed that long-lasting exposure to Cd concentrations, similar to those found in moderately or highly polluted seawaters, causes severe developmental delays and abnormalities during sea urchin embryo development. This demonstrated that even very small amounts of Cd, if accumulated in cells, can produce significant cytotoxic effects (Filosto et al. 2008).

In this review, our analysis will focus on Cd stress in several sea urchin species at different levels: gametes, embryos, and adults. Then, we will analyze the role of the protective system against the consequences of Cd stress, discussing its implications in the survival mechanisms that cells adopt as defense strategies or, in extreme cases, by death processes.

As stated above, scientific literature covering the link between Cd and sea urchins is very rich, and we will here focus only on the studies analyzing a stress response and, in particular, the studies regarding the stress responses at the morphological, cellular, and molecular levels. The latter, in fact, represents an optimal and innovative approach as, at present, the specific pathways activated by stressors have been scarcely investigated in marine organisms. The goal of this discussion is to highlight specific markers of Cd stress that better reflect marine pollution.

Sea urchins as sentinel organism to test the marine environment

Among the benthonic organisms, echinoderms represent a simple, though significant, model system to test how specific stress can simultaneously provoke dangerous effects on growth and vitality of organisms. Different sea urchin species are sensitive to several aquatic contaminants, often showing different sensitivities and molecular effects (Burić et al. 2015; Martino et al. 2018). Sea urchins provide an attractive and exceptional model for investigating environmental pollution. Although these aquatic invertebrates are known to accumulate high levels of heavy metals in their tissues, nevertheless they are able to survive in polluted environments (Dallinger and Rainbow 1992). Metals often penetrate into their cells via transport mechanisms normally used for the physiological uptake of nutrients and are irreversibly accumulated in cells where they interact with cellular components and molecular targets. Sea urchins are also ideal organisms for in vivo toxicity tests, because of their ability to modulate different defense strategies, depending on the nature of the physical and/or chemical stress (Hamdoun and Epel 2007; Matranga et al. 2013).

To study the toxic effects induced by chemical and physical agents, many researchers use different typologies of samples from sea urchin: adult organisms, ovarian, eggs, sperms, coelomocytes, embryos, and larvae. The sea urchin embryo has proved to be a versatile model system for different research areas. Advantages include the large number of gametes that can be easily obtained, external fertilization, rapid development and growth, transparency and easy manipulation, and rapid cell divisions during cleavage stages (Walker et al. 2013; Chiarelli and Roccheri 2014). It has long been used in developmental biology, as it provides an attractive model for exploring the mechanisms of successful development. In the sea urchin embryo, the cell fate of territories at the appropriate time and space is controlled by gene regulatory networks (GRNs). Currently, among the GRNs

available from other organisms, the sea urchin embryo endomesoderm GRN is the most nearly validated and completed (Peter et al. 2012). Having potent cellular mechanisms protecting them against changes in the external environment, the sea urchin embryo represents an excellent model system to study the regulatory pathways that alter their development in response to the adverse environmental conditions encountered, providing them protection, robustness, and resistance (Hamdoun and Epel 2007; Martino et al. 2017b, 2018). In addition, having echinoderms an extensive endoskeleton composed of magnesium calcite, they represent a suitable model system to study biomineralization and material properties of biominerals (Matranga et al. 2001). This is becoming more and more important as changes in the seawater chemistry due to human impact can potentially modify the mechanical properties of marine organism skeleton (Smith et al. 2016; Martino et al. 2019).

The most important sea urchin species used as a model system to study the defense strategies induced by Cd stress are *Paracentrotus lividus*, *Anthocidaris crassispina*, *Arbacia punctulata*, *Psammechinus microtuberculatus*, *Sphaerechinus granularis*, *Strongylocentrotus intermedius*, and *Strongylocentrotus nodus*. These species were used to report the effects of this environmental stressor at several levels, such as morphological anomalies (eggs, embryos, and larvae), skeletal injuries, archenteron malformations and abnormal development (embryos and larvae), alteration of spermatic parameters (curvilinear velocity, average path velocity, straight line velocity, germ plasm substance of spermatogonia), fertilization rate, reversibility of metal exposure effects, synthesis of cytoprotective proteins (HSPs and MTs), oxidative stress, DNA damage, apoptosis, and autophagy.

Effects induced by Cd stress in Paracentrotus lividus: adults, gametes, embryos, and larvae

Most of the studies investigating the effects of Cd stress were conducted on *Paracentrotus lividus*, a sea urchin species belonging to the Parechinidae family and commonly known as the purple sea urchin (Fig. 1). It is the type species of the genus and occurs in the Mediterranean Sea and eastern Atlantic Ocean (World Register of Marine Species). The sea urchin embryo *P. lividus* is one of the most important marine invertebrate used as a bioindicator of metal/heavy metal pollution and an important model organism in developmental biology (Chiarelli and Roccheri 2012, 2013, 2014, 2015). It has been recognized as a suitable model for ecotoxicological and environmental studies aimed at determining the effects of chemical pollutants, both in the field and in laboratory experiments (Russo et al. 2003), as it continuously faces environmental, chemical, physical, and biological stressors (Matranga et al. 2010, 2013).

Reproduction and development

Experiments to test the effects induced by Cd were conducted on *P. lividus* since 1982. Several treatments have been made on different stages (gametes, blastula, gastrula, and pluteus) showing that concentrations of $CdCl_2$ of 10^{-8}–10^{-3} M are able to induce skeletal injuries, producing abnormal embryos and larvae (Pagano et al. 1982). Treatments of sperms of *P. lividus* with Cd at the concentration of 10, 100, 200, and 400 μg/L provoked alterations in the egg fertilization rate and abnormal embryogenesis (alterations on length of spicules, crossed spicules, separated spicules, atrophy of arms, fractured ectoderm and fused arms and blockage of development at different stages: fertilization, segmentation, gastrulation, and prepluteus stage) (Gharred et al. 2016). Retarded, malformed larvae and developmental arrest were observed in embryos treated since the pluteus stage with 1000–2500 μg/L of Cd (larvae affected in skeletal or gut differentiation, larvae with normal shape and symmetry, but with reduced size) (Manzo et al. 2010). More recently, Morroni and colleagues observed the reversibility of metal exposure effects as an additional ecological value to the sea urchin bioassay, in order to expand the potentiality of the recently developed integrative toxicity index (ITI). The classical toxicity criteria are based on distinguishing between normal and abnormal embryos at pluteus stage but in this case embryos were observed after different periods of exposure and recovery to metals. The onset and reversibility of effects by trace metals were more efficiently discriminated by the use of the ITI, which recognized and weighted the delay and degree of various abnormalities: lower toxicity values are given to delayed embryos (embryos with delay in development and absence of malformations) and higher scores are attributed to abnormal embryos (embryos with delay in development and malformations) with no chance to recover development (Morroni et al. 2018).

Molecular response

It was shown that exposure to different toxics causes the synthesis of heat-shock proteins (HSPs), providing a protective role during stress condition. For example, coelomocytes of adult *P. lividus*, treated with 10^{-5}–10^{-3} M of $CdCl_2$ show increased level of HSP70 (Matranga et al. 2002). The synthesis of a specific set of HSPs was demonstrated in *P. lividus* embryos continually exposed to 1 mM of $CdCl_2$ at the blastula (15 h) and gastrula (24 h) stages (Roccheri et al. 2004). In addition, a low increase of HSC70, associated with development without gut and degeneration, was reported after exposure of embryos to 1 mM of $CdSO_4$ for 20 h (Geraci et al. 2004).

Taken together, these data suggest that the first response to the Cd insult is an alteration of normal development, probably inducing alternative pathway of growth as demonstrated from

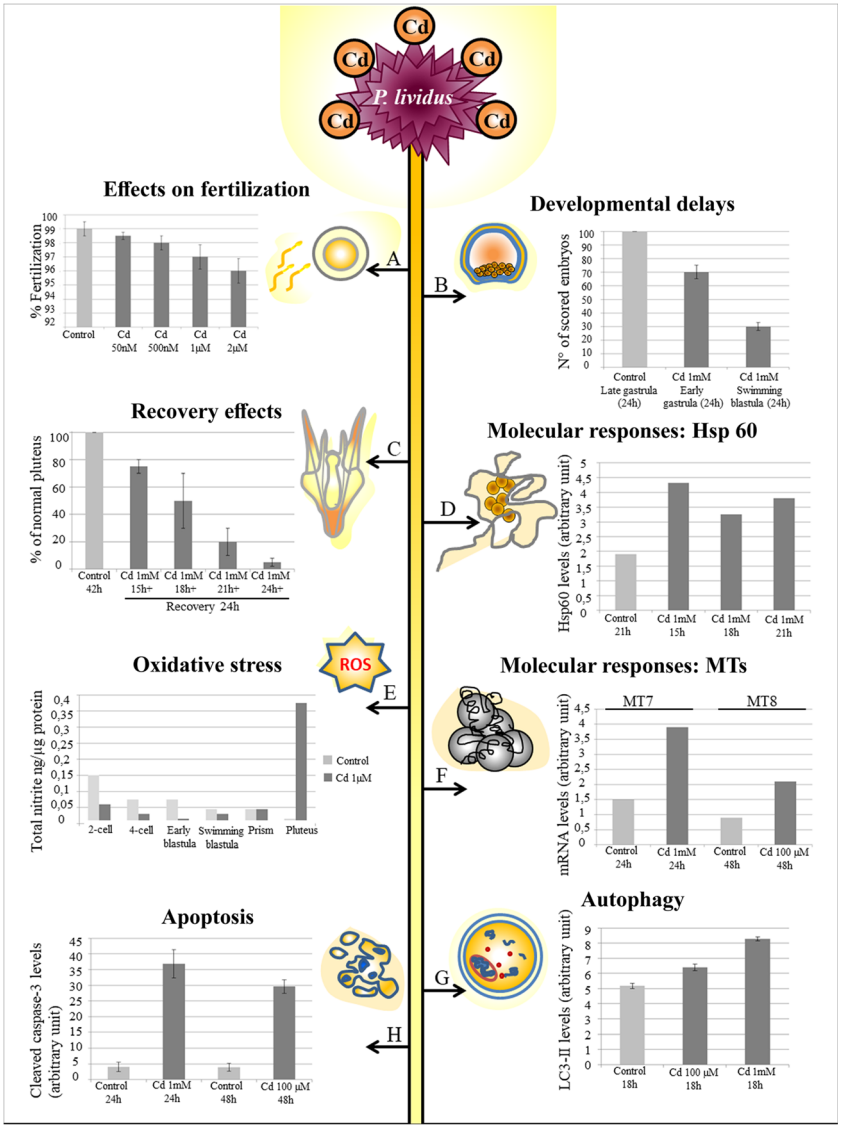

Fig. 1 Overview of effects induced by Cd stress in *P. lividus* embryos and larvae. A) Effect on fertilization (Gharred et al. 2016). B) Developmental delays (Roccheri et al. 2004). C) Recovery effects (Russo et al. 2003). D) Molecular responses: Hsp60 (Roccheri et al. 2004). E) Oxidative stress (Migliaccio et al. 2014). F) Molecular responses: MTs (Ragusa et al. 2013). G) Autophagy (Chiarelli et al. 2011). H) Apoptosis (Chiarelli et al. 2014). For each effect a quantitative data of the most representative responses has been processed

the presence of several typologies of embryo morphology. Contextually, embryos activate molecular mechanisms of defense and one of the first is the induction of HSPs, producing a detoxifying and antioxidant effect in order to block the action of the toxic metal. Another molecular mechanism is the induction of metallothioneins (MTs), a type of metal-binding proteins. MT expression (Pl-MT gene) was reported at different stages of development and treatment with 1 mM of $CdCl_2$: morula (6 h), hatching blastula (12 h), gastrula (24 h), and pluteus (48 h), indicating that Cd-exposed embryos increase the level of these low–molecular weight proteins in order to activate a detoxification process (Russo et al. 2003). Other experiments related to the expression of the MT were carried out for PlMT4, PlMT5, PlMT6, PlMT7, and PlMT8 genes in gastrula (24 h) and prisma (30 h) exposed to 1–100 μM of $CdCl_2$. These data suggest a hierarchical and orchestrated response of the *P. lividus* embryo to overcome differential environmental stressors that could interfere with normal development (Ragusa et al. 2013).

Oxidative stress

The metal has been associated with blockage of oxidative phosphorylation, glutathione depletion and antioxidant enzymatic activity inhibition, production of ROS, DNA damage, and inhibition of relative repair mechanisms (Kim et al. 2014; Tamás et al. 2014).

Although extensive research has been undertaken to elucidate the signal pathways in Cd-induced stress, at present, oxidative stress has been considered an important possible mechanism of Cd toxicity. In Cd-exposed sea urchin embryos, nitric oxide (NO) production was reported after treatment with 0.5–30 μM of $CdCl_2$ at different stages of development (2-cell, 8-cell, early blastula, swimming blastula, prisma, pluteus) and

this data, together with HSPs and MTs, open new perspectives on the role of NO as a sensor of different stress agents in sea urchin developing embryos (Migliaccio et al. 2014, 2015).

Apoptosis

Sea urchin embryos are able to induce apoptosis as a survival mechanism in response to different chemical and physical stressors: emetine, etoposide, ultraviolet radiation, hydrogen peroxide, staurosporine, camptothecin, and Cd. Moreover, treatment with TPA (12-O-tetrade-canoylphorbol-12-acetate) or high concentrations of 2-trans-4-trans-decadienal, a polyunsaturated aldehyde, caused apoptosis in *P. lividus* embryos (Roccheri et al. 1997; Voronina and Wessel 2001).

Results obtained by TUNEL assay, performed on whole mount embryos, showed most nuclei with DNA fragmentation and the activation of caspase-3, one of the key molecules of apoptosis (Agnello et al. 2006). During Cd exposure, an increase of the cleavage of caspase-3 substrates as a-fodrin and lamin A was also observed (Agnello et al. 2007).

Other studies on the apoptotic processes activated in *P. lividus* were conducted after a long-lasting exposure to low Cd concentrations, similar to those found in moderately or highly polluted seawaters. These exposures caused severe developmental delays and abnormalities in the larvae, suggesting that even very small amounts of Cd, if accumulated in cells, can produce significant cytotoxic effects and apoptosis (Filosto et al. 2008). Finally, we can assume that in sea urchin embryos/larvae, apoptosis can be considered part of a defense strategy that, by sacrificing a few cells, can safeguard the whole organism and the developmental program, provided that the exposure to Cd is not excessively prolonged or too intense.

Autophagy

Another molecular process studied in sea urchin embryo is autophagy (Chiarelli et al. 2011). Autophagy is a mechanism of self-eating described as an important intracellular pathway responsible for degradation and recycling of long-term proteins and cytoplasmic organelles. In some circumstances, autophagy functions as a non-apoptotic form of cell death. On the other hand, during periods of nutrient shortage, autophagy provides the constituents required to maintain the metabolism essential for survival (Codogno and Meijer 2005).

Although it is a normal mechanism of clearance since oogenesis and early development (Agnello et al. 2016), it seems to be critical during stress because, in some extreme cases, it offers high plasticity to allow the survival of embryonic cells. Autophagy has been recently observed in eggs and embryos, in response to stress induced by Cd and other stressors (Agnello et al. 2016; Chiarelli et al. 2011; Martino et al. 2017b). In particular, it was shown that autophagy is triggered as a survival program in unfertilized eggs destined to die by apoptosis after inactivation of MAPK1/3 (ERK2/1). However, eggs can use autophagy as a survival program when the cell cycle is blocked (Houel-Renault et al. 2013). Most stress-induced autophagic studies have been carried out using Cd as a stress agent. Results of these studies revealed a higher level of autophagosomes in embryos exposed to Cd for 18 h. After 24 h of exposure, embryos show a lower level of autophagosomes, because in this condition apoptosis becomes massive (Agnello et al. 2007; Agnello and Roccheri 2010). Autophagy may represent a key evolutionarily conserved response to toxic metals/metalloids and other stressors. The relationship between autophagy and apoptosis and their molecular regulation need to be explored in connection with exposure to specific toxicants.

Many experiments have been conducted on sea urchin embryos exposed to Cd to study the relationship between autophagy and apoptosis. Results suggest that autophagy may have a key role in energy supply necessary for apoptosis, providing ATP molecules by recycling damaged cellular components (Chiarelli et al. 2014).

Cd is known to induce stress at various levels in *P. lividus* embryos, causing damage that affects organelles and proteins. The autophagic process would seem able to restore equilibrium in the cell, removing damaged structures that, accumulating massively inside the cell, would otherwise induce a deterioration of the normal vital functions.

Recent studies suggest that autophagy is important for the clearance of protein aggregates that are formed in cells following stress, and in this process, the p62/SQSTM1 protein (sequestosome 1) appears to play a key role (Bjørkøy et al. 2006). p62/SQSTM1 is a multifunctional, multidomain adaptator protein which resides at the autophagosome membranes (Komatsu 2011). It is an autophagosome cargo protein that targets other proteins that bind to it for selective autophagy. This protein is conserved across metazoans, but not in plant and fungi (Katsuragi et al. 2015). However, as seen from the alignment of p62/SQSTM1 sequences from mammals, opossum, chicken, frogs, fishes, sea urchin, and honeybee, it shows specific conserved motif (Pankiv et al. 2007).

To highlight any protein aggregates concomitantly with the peak of autophagic vacuolation, control *P. lividus* embryos and embryos exposed to 1 mM $CdCl_2$ for 18 and 24 h were submitted to the immunofluorescence/confocal laser scan microscopy protocol and qualitative/quantitative analysis (Chiarelli et al. 2014), using the anti-p62/SQSTM1 heterologous antibody (Fig. 2).

Qualitative analysis showed a diffuse globular signal that is often referred to as Ibs (inclusion bodies), suggesting that the presence of polyubiquitinated protein aggregates was intended for autophagic degradation; the quantitative analysis, carried out by means of densitometric analysis of the p62/SQSTM1 signal, after grouping in stacks of the embryonic optical sections, showed a parallel trend to the signal

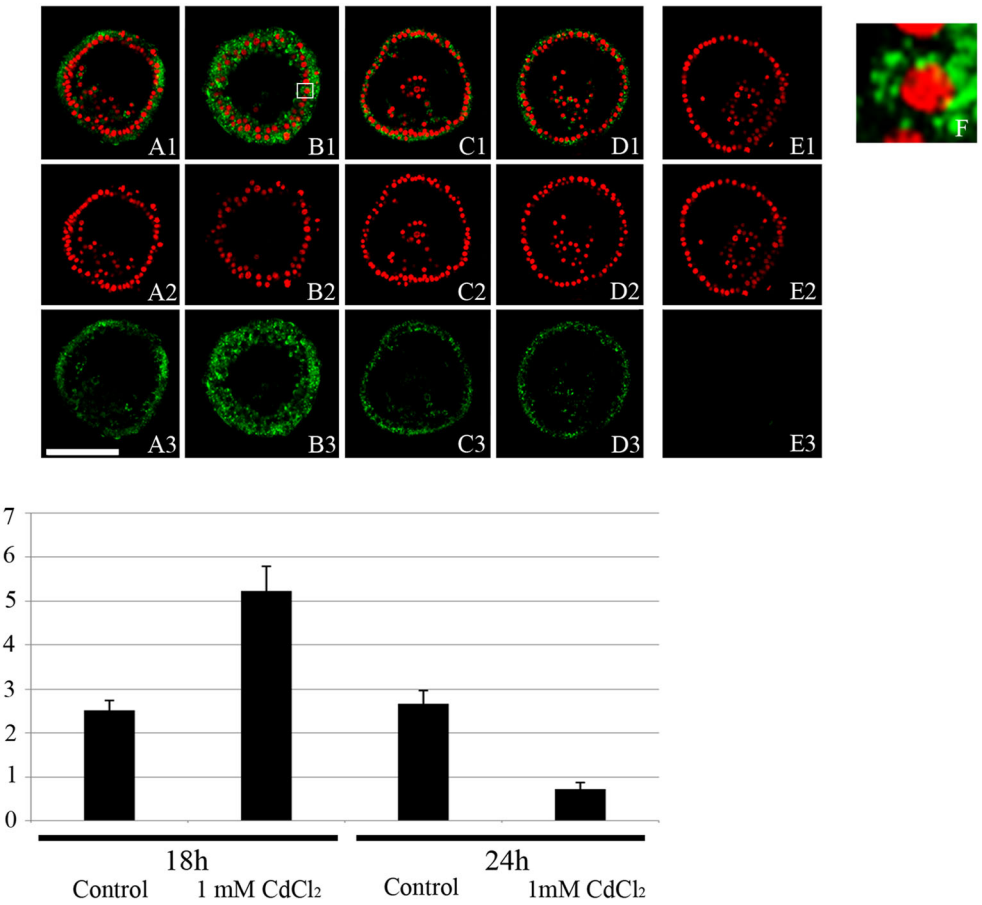

Fig. 2 Detection by immunofluorescence of p62/SQSTM1 protein on whole-mount embryos (p62/SQSTM1 antibody dilution 1:500, Sigma-Aldrich). The images of representative embryos at 18 h and 24 h of development show equatorial optical sections observed under CLSM. Merge of signals from p62/SQSTM1 protein detection and nuclei stained with propidium iodide (A1–E1). Nuclei stained with propidium iodide (A2–E2). p62/SQSTM1 protein detection (A3–E3). Control embryo after 18 h of development (A1–A3). Cd-treated embryo for 18 h (B1–B3). Control embryo after 24 h of development (C1–C3). Cd-treated embryo for 24 h (D1–D3). Negative control embryo (E1–E3). Enlargements (F) of a section of Cd-treated embryo for 18 h (B1). Bar = 50 μm. Data in the histograms report the quantification of p62/SQSTM1 signals, and are presented as the mean of triplicate experiments

related to the LC3-II protein (Chiarelli et al. 2011). A peak of the signal was recorded after 18 h of treatment with $CdCl_2$ 1 mM, while after 24 h of treatment, a decrease in the p62/SQSTM1 signal was observed. A basal signal was found in control embryos at 18 h and 24 h of development (histogram in the Fig. 2). Taken together, these data indicate that in conjunction with the peak of autophagic vacuolation, there is an intense formation of protein aggregates that would be sent to degradation mediated by the autophagic process. After 24 h of 1 mM $CdCl_2$ treatment, however, there is a reduction in the signal since, under the same treatment conditions, there is a phase in which many autophagosomes become autophagolysosomes, in which the autophagic degradation starts for damaged proteins and for the same p62/SQSTM1 protein.

Reported data about the defense mechanisms induced by Cd in *P. lividus* suggest a hierarchical choice of defense strategies; strikingly, the temporal choice of activation of different mechanisms depends on the fact that the embryo tries to face the stress conditions using, initially, defense strategies that are less deleterious to preserve the developmental program. If these processes are not sufficient to offset the damage caused by stress, the autophagic and apoptotic mechanisms are activated (Chiarelli et al. 2016; Klionsky et al. 2016) (Table 1).

Effects induced by Cd stress on other species of sea urchin

Other species of sea urchin have been used in order to detect the Cd-induced stress response; however, few data have been identified in each studied species. Further studies are needed to build a complete profile of the defense strategies activated in each species, as was done on *P. lividus*. It is now understood that this species is well equipped with defense systems to cope with stress agents. These studies would help to identify the most sensitive species to Cd stress and the most appropriate markers of marine pollution.

The short-spine sea urchin *Anthocidaris crassispina* (Echinometridae family) lives in tropical and subtropical coastal waters. This species has long been used as a bioindicator for environmental pollution.

Adults were exposed to 1.5 and 10 ppm Cd^{2+} and subsequently processed to study different seminal parameters. The analysis of several parameters, such as sperm VCL (curvilinear velocity), sperm VSL (straight line velocity), and sperm VAP (average path velocity) highlighted a good relationship between sperm motility and the exposure concentration/time (Au et al. 2000).

Table 1 Effects induced by Cd stress in sea urchin *Paracentrotus lividus*: adults, gametes, embryos, and larvae

Tipology of biological sample	Cadmium concentration	Studied effects	References
Gametes, blastula, gastrula, and pluteus	10^{-8}–10^{-3} M of $CdCl_2$	Alterations in embryo and larva development and skeletogenesis	Pagano et al. (1982)
Sperms	10, 100, 200, and 400 μg/L of $CdCl_2$	Reduction of egg fertilization rate and embryogenesis	Gharred et al. (2016)
Pluteus (48 h)	0,1–10 mg/L of $CdCl_2$	Alterations in embryo and larva development	Manzo et al. (2010)
Gastrula (24 h)	1000–2500 μg/L of $CdCl_2$	Reversibility of metal exposure effects using the delay and degree of various abnormalities	Morroni et al. (2018)
Adults (coelomocytes)	10^{-5}–10^{-3} M of $CdCl_2$	Increase of HSP70 levels	Matranga et al. (2002)
Blastula stage	1 mM of $CdSO_4$	Alterations in embryo development and increase of HSC70 levels	Geraci et al. (2004)
Blastula (15 h), gastrula (24 h)	1 mM of $CdCl_2$	Increase of the synthesis of a specific set of HSPs	Roccheri et al. (2004)
Morula (6 h), hatching blastula (12 h), gastrula (24 h), pluteus (48 h)	1 mM of $CdCl_2$	Increase of the metallothionein expression (Pl-MT gene)	Russo et al. (2003)
Gastrula (24 h); prisma (30 h)	0.1 μM–100 μM of $CdCl_2$	Increase of the metallothionein expression (PlMT4, PlMT5, PlMT6 PlMT7 and PlMT8 genes)	Ragusa et al. (2013)
2-cell, 8-cell, early blastula, swimming blastula, prisma, pluteus	0.5 μM–30 μM of $CdCl_2$	Oxidative stress (increase of nitric oxide production)	Migliaccio et al. (2014)
Adult female	10^{-6} M of $CdCl_2$	Reduction of fertilization process, increase of NO production, alteration in embryo development, increase of the expression of several genes involved in stress response, alteration in skeletogenesis, detoxification, multidrug efflux processes	Migliaccio et al. (2015)
Blastula (15 h), gastrula (21 h and 24 h)	1 mM of $CdCl_2$	Apoptosis (by TUNEL assay and by caspase-3, α-fodrin, lamin-A immunodetection)	Agnello et al. (2007)
Pluteus (38 h)	100 μM of $CdCl_2$	Apoptosis (by TUNEL assay and caspase-3 immunodetection)	Agnello et al. (2006)
Larva (10–30 days)	1 μM,1 nM,1 pM of $CdCl_2$	Apoptosis (by TUNEL assay)	Filosto et al. (2008)
Hatching blastula (12 h), blastula (15 h), gastrula (18 h, 21 h and 24 h)	1 mM of $CdCl_2$	Autophagy (by acridine orange (AO) and neutral red staining, LC3-II immunodetection)	Chiarelli et al. (2011)
Gastrula (18 h, 24 h)	1 mM of $CdCl_2$	Autophagy (by LC3-II immunodetection)	Chiarelli and Roccheri (2012)
Pluteus (38 h)	100 μM of $CdCl_2$	Autophagy (by AO staining)	Chiarelli and Roccheri (2014)
Gastrula (24 h)	100 μM of $CdCl_2$	Autophagy (by AO staining)	Chiarelli et al. (2016)
Gastrula (24 h)	1 mM of $CdCl_2$	Apoptosis-autophagy interplay (by TUNEL, caspase-3 and LC3-II immunodetection, AO staining)	Chiarelli et al. (2014)

Reproductive impairment and gamete quality were studied after exposure of adults to 0.01 and 0.1 mg l^{-1} of Cd^{2+}, such as sperm motility, egg morphology, fertilization rate, and the dynamics of first cleavage (Au et al. 2001a). These studies also allowed us to obtain information on sperm development; the exposure of adults to Cd induced cytological alterations of sperm cells and alterations in morphology of "nuage" in spermatogonia. The discrete, granular bodies of nuage were substituted by a large electron-dense body.

Results showed an increased number of spermatozoa with short, incomplete, and "broken" tails or tails of spermatids/spermatozoa with extraordinary electron density in gonads exposed to both levels of Cd. Mitochondrial cristae deformation was observed at all stages of development for sperm cells. The sperm plasma membrane was showed to be more convoluted, but the acrosome was intact. The observed cytological alterations of sperm tails and mitochondria/midpiece may explain the decline in motility and poor resolution in sperm produced by sea urchins exposed to Cd, causing a lower percent fertilization and cleavage rate, implying that male sea urchins were more sensitive than females to chronic Cd exposure (Au et al. 2001b).

Further studies by Vaschenko et al. (1999) were carried out by exposing spermatozoa and eggs to different concentrations of Cd to study the effects on the fertilizing capability of spermatozoa, dynamics of the first cleavage, and pluteus formation. The exposure of sperms to Cd concentrations of 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, and 10.0 mg l^{-1} for 30 min caused a significant reduction of the fertilizing capability of spermatozoa at all doses. Cd exposure of eggs to concentrations of 0, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, and 5.6 mg l^{-1} for 30 min, as well as embryo exposure, caused: reduction of percentage of divided zygotes in dose/time-dependent manner, abnormal and retarded plutei, pre-plutei, dead embryos/larvae (small malformed plutei, oval embryos with rudimentary skeleton, poorly differentiated intestines, and no mouth, pre-pluteus stage). Results indicated that sperms of *A. crassispina* are more sensitive to Cd if compared with other sea urchin species. In addition, analysis of the dynamics of the first cleavage may be used as a very sensitive and useful bioassay in the studies of embryotoxicity triggered by chemical agents (Vaschenko et al. 1999).

Gametes and larvae of the Atlantic purple sea urchin *Arbacia punctulata* (Arbaciidae family) have largely been used in embryological studies and toxicity bioassay testing.

Table 2 Effects induced by Cd stress in different species of sea urchin: adults, gametes, embryos, and larvae

Tipology of biological sample	Cadmium concentration	Studied effects	References
Adult *Anthocidaris crassispina*	1.5 and 10 ppm Cd^{2+}	Reduction of three velocity parameters: sperm VCL (curvilinear velocity); sperm VSL (straight line velocity); sperm VAP (average path velocity)	Au et al. (2000)
Adult *Anthocidaris crassispina*	0.01 and 0.1 mg $l^{-1}$of Cd^{2+}	Dose-dependent reduction of sperm motility, fertilization rate and dynamics of first cleavage Presence of large eggs at the highest Cd concentration	Au et al. (2001a)
Adult *Anthocidaris crassispina*	0.01 and 1 ppm Cd^{2+}	Sperm development: deposition of electron-dense materials and cytological alterations	Au et al. (2001b)
Sperms *Anthocidaris crassispina*	0.32, 0.56, 1.0, 1.8, 3.2, 5.6, and 10.0 mg l^{-1}	Reduction of the fertilizing capability of spermatozoa in all Cd concentrations	Vaschenko et al. (1999)
Eggs and embryos *Anthocidaris crassispina*	0.18, 0.32, 0.56, 1.0, 1.8, 3.2, and 5.6 mg l^{-1}	First cleavage kinetics assay: reduction of percentage of divided zygotes in dose/time-dependent manner Abnormal and retarded plutei, preplutei, dead embryos/larvae (small malformed plutei, oval embryos with rudimentary skeleton, poorly differentiated intestines and no mouth, pre-pluteus stage)	Vaschenko et al. (1999)
Adult *Arbacia punctulata*	8.9×10^{-7} M (100 ppb) Cd	Reduction of viability of eggs and sperms, reduction of the fertilization rate, alterations in embryonic development and larvae	Bowen and Engel (1996)
Gametes, blastula, gastrula, and pluteus *Psammechinus microtuberculatus*	10^{-8}–10^{-3} M of $CdCl_2$	Increase of abnormal embryos-larva and skeletal injuries in dose/time-dependent manner	Pagano et al. (1982)
Gametes, blastula, gastrula, and pluteus *Sphaerechinus granularis*	10^{-8}–10^{-3} M of $CdCl_2$	Increase of abnormal embryos-larva and skeletal injuries in dose/time-dependent manner	Pagano et al. (1982)
Ovaries *Strongylocentrotus intermedius*	1.50 and 100 μg/L of $CdCl_2$	Reduction of the number and size of oogonia, reduction of the activity of acid and alkaline phosphatases at the highest Cd concentration	Lipina et al. (1987)
Adult *Strongylocentrotus intermedius*	0.001, 0.05, 0.1, and 1 mg/L of $CdCl_2$	Embryo development: delayed archenteron formation; decrease in the size of larvae	Vashchenko et al. (1988)
Adult *Strongylocentrotus nodus*	0.001, 0.05, 0.1, and 1 mg/L of $CdCl_2$	Embryo development: delayed archenteron formation; decrease in the size of larvae	Vashchenko et al. (1988)
Adult *Strongylocentrotus nodus*	0.01 mg/L of $CdCl_2$	Destruction of germ plasm substance of spermatogonia	Reunov et al. (2005)

Bowen and Engel's experiments were aimed at understanding if an extended sublethal exposure of sexually mature sea urchins to Cd would affect the viability of eggs and sperms, and subsequently fertilization, embryonic development and, ultimately, the larvae. Results supported the hypothesis that Cd exposure affected spermatogenesis and oogenesis (Bowen and Engel 1996).

Psammechinus microtuberculatus (Parechinidae family), the green sea urchin, is a species of sea urchin found in the western and eastern Atlantic Ocean, in the Adriatic and Aegean Sea, and throughout the entire Mediterranean Sea, despite it being very rare. This species of sea urchin is used as a bioindicator of marine environmental pollution related to several toxicants. Several treatments at concentrations ranging from 10^{-8} to 10^{-3} M of $CdCl_2$ have been made on gametes, blastula, gastrula, and pluteus and caused skeletal injuries and the development of abnormal embryos and larvae, in a dose/time dependent manner (Pagano et al. 1982).

Sphaerechinus granularis (Toxopneustidae family), known as the purple sea urchin, lives in the Mediterranean Sea and the eastern Atlantic Ocean. This species was used to test several toxicants, such as heavy rare earth elements. In particular, *S. granularis* showed a significantly higher sensitivity to rare earth elements, if compared with *A. lixula* and to *P. lividus* (Trifuoggi et al. 2017). Early life stages of *S. granularis* were used to investigate the impact of relevant levels of UV-B radiation (Nahon et al. 2009).

Cd-exposed gametes and embryos from the blastula to the pluteus stage showed skeletal injuries and abnormal development (Pagano et al. 1982).

The species *Strongylocentrotus intermedius* (Strongylocentrotidae family) was used to investigate the differences among several populations for their conservation, sustainable fishery production, and aquaculture through ecological and genetic studies. This species has also been tested for Cd exposure: treatment of ovarian to 1.50 and 100 μg/L of Cd had significant effects on the number and size of oogonia and activity of acid and alkaline phosphatases (Lipina et al. 1987). Treating adults of *S. intermedius* with 0.001, 0.05, 0.1, and 1 mg/L of Cd alterations of development were visible at the gastrula stage: embryos displayed aberrations in archenteron formation (Vashchenko et al. 1988).

The effect of Cd exposure during archenteron formation was also analyzed in the sea urchin *Strongylocentrotus nodus* (Strongylocentrotidae family), a dominant sea urchin species in the northwest Pacific found on intertidal and subtidal rocky sea bottoms. (Vashchenko et al. 1988). *S. nodus* was used to study the processes of germ plasm destruction in spermatogonia exposed to Cd. Sea urchins at the stage of active gametogenesis were exposed to the toxicant and spermatogonia were subsequently analyzed through ultrastructural study. These spermatogonia showed the appearance of vesicles, probably lysosomes produced by the Golgi complex and located in the germ plasm area. This allowed us to hypothesize that an autophagic process was involved in the destruction of the germinal substance (Reunov et al. 2005) (Table 2).

Conclusions

The marine environment has become the basin for a range of anthropogenic contaminants, mainly originating from terrestrial sources (Islam and Tanaka 2004). Monitoring chemical pollution in the marine ecosystems has become an essential goal for the scientific community, to understand their toxic effects for environmental management (European Marine Board 2013). The sea urchin embryo has long been largely employed for this purpose, in an attempt to investigate its developmental, cellular, and molecular response to environmental stressors such as metals and other emerging contaminants. Numerous studies have investigated the impact of toxicants, including metals (e.g., Cd, Mn, Pb, and Zn) and other stressors (e.g., UV and X-rays) on sea urchin development, focusing on gene expression and using the skeleton as a sensitive trait for ecotoxicology (Russo et al. 2003, 2010; Bonaventura et al. 2005; Matranga et al. 2010; Pinsino et al. 2010; Matranga et al. 2013, Bonaventura and Matranga 2017). Recent studies focused on the impact of pharmaceuticals, medical agents that are increasingly of global concern (Brausch et al. 2012; Brooks and Huggett 2012; Tiwari et al. 2017) as they are manufactured to be biologically active compounds. Among pharmaceuticals, Gadolinium, a medical agent recognized as an emerging environmental pollutant (Telgmann et al. 2013), was showed to cause severe morphological malformations in different sea urchin species at environmentally relevant doses, to induce autophagy and to alter gene expression (Martino et al. 2017a, b, 2018). Coexposure to Cd and sulfamethoxazole, an antibiotic commonly introduced into the marine environment after utilization in aquaculture, caused impaired development and was able to attenuate transcriptional response in *P. lividus* sea urchin embryos (Ragusa et al. 2017). Exposure of *P. lividus* and *Arbacia lixula* sea urchins to heavy rare earth elements affected embryogenesis, fertilization, cytogenetic, and redox endpoints (Oral et al. 2017). Other studies focused on the fate, behavior, and effects of nanomaterials (NMs) in the marine environment, showing their toxicity for sea urchin embryos at different larval stages (Fairbairn et al. 2011; Burić et al. 2015; Gambardella et al. 2016; Kanold et al. 2016; Torres-Duarte et al. 2016).

In the last years, industrial activities caused an increase of carbon dioxide and other greenhouse gases. Subsequently, oceans have warmed and showed a reduction of pH. These two events, warming and acidification, are predicted to continue and increase during the twenty-first century (Rodolfo-Metalpa et al. 2011; IPCC 2013; Hoegh-Guldberg et al. 2014), posing an alarming treat for marine biodiversity and hence representing a high priority for science, policy, and

management (Byrne and Przeslawski 2013). It has been reported in several marine invertebrates that acidification, global warming, and excessive evaporation can lead to an increase of the concentration of chemical substances, such as heavy metals, inside the cells of aquatic organisms after exposure (Shi et al. 2016; Nardi et al. 2017, 2018). In particular, pH may influence the solubility and speciation of metals in seawater, increasing the release from polluted sediments. On the other hand, temperature was shown to modulate the uptake and toxicity of metals through accelerated metabolic rates, oxidative stress, impairment of mitochondrial function, damages to lysosomal system and DNA, and accumulation of lipid peroxidation products (Sokolova 2004; Baines et al. 2005; Cherkasov et al. 2006, 2007; Mubiana and Blust 2007; Ivanina et al. 2008; Sokolova and Lannig 2008; Guinot et al. 2012; Izagirre et al. 2014; Múgica et al. 2015).

Reciprocal interactions between temperature/acidification and Cd have been recently described (Benedetti et al. 2016). Although the possibilities for interactions of global change on toxicological responses to environmental pollutants have been addressed (Byrne 2012), specific pathways of activation by multiple stressors have been scarcely investigated in marine organisms.

Considering the importance of stress response to Cd at different levels (organisms, tissues, cells, and molecules), it is relevant to observe that sea urchins of different species activate specific defense mechanisms to safeguard their survival. Many studies have been conducted on the embryonic stages, since they represent the most sensitive system to the environmental pollution.

The most primitive response to stress induced by Cd in embryos and larvae is abnormal development and morphological anomalies. In order to survive, the cells of organisms have evolved the capability to adapt, constantly and quickly, to environmental changes inducing molecular and cellular mechanisms. In general, the first response to Cd-exposure is an increased level of cytoprotective proteins (HSPs and MTs). Contextually, we see the activation of molecular processes such as autophagy and apoptosis. Autophagy is involved in the turnover of cellular components, including proteins and organelles, and in the maintenance of tissue homeostasis of adult organisms and developing embryos; this process must undoubtedly have a crucial role in organisms exposed to stress conditions. In both invertebrate and vertebrate taxa, autophagy operates as a protective mechanism, playing an essential role in the response to stress, sometimes in concert with the apoptotic machinery (Chiarelli and Roccheri 2012).

This review, although not comprehensive in its reporting, is aimed at providing an overview of the current knowledge on the role of mechanisms triggered after stress induced by Cd in different species of sea urchin: *Paracentrotus lividus*, *Anthocidaris crassispina*, *Arbacia punctulata*, *Psammechinus microtuberculatus*, *Sphaerechinus granularis*, *Strongylocentrotus intermedius*, and *Strongylocentrotus nodus*.

As it is a suitable bioindicator of marine pollution, a lot of data are available about the effects on *P. lividus*, considering that different markers have been studied in this organism to understand the dangerous effects induced by chemical substances and physical stress. However, more work is still needed to expand this research field in order to discover the most suitable biomarkers reflecting the effects of Cd, and the development of new technologies may be very useful. For example, the impact of next-generation sequencing technologies (NGS) may allow us to obtain new and valuable information about gene regulation through RNA sequencing and subsequent expression analyses of genes participating in the defense reactions. The application of NGS can be considered a suitable screening tool for the assessment of environmental risks and water quality, including the development of bioindicators of marine pollution (Tan et al. 2015).

Scientific research, while it has long highlighted the problem of marine pollutants, needs to be further developed to obtain a complete profile of the effects of pollutants on living beings. It is critical to analyze those aspects that could threaten the quality of the marine environment in order to determine a toxicological profile of each pollutant, analyzing the different effects on gametes, embryos, larvae, and adult organisms, with the final aim to discover specific marker that better reflect marine pollution.

Molecular and cellular aspects, since they are governed by specific pathways, may have a key role in the determination of Cd pollution, representing specific markers that better reflect the health of the marine environment from the insults of this heavy metal.

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