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Review

Life under Climate Change Scenarios: Sea Urchins' Cellular Mechanisms for Reproductive Success

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Abstract: Ocean Acidification (OA) represents a major field of research and increased efforts are being made to elucidate its repercussions on biota. Species survival is ensured by successful reproduction, which may be threatened under detrimental environmental conditions, such as OA acting in synergy with other climate change related stressors. Achieving successful gametogenesis, fertilization, and the development of larvae into healthy juveniles and adults is crucial for the perpetuation of species and, thus, ecosystems' functionality. The considerable vulnerability of the abovementioned developmental stages to the adverse conditions that future OA may impose has been shown in many species, including sea urchins which are commonly used due to the feasibility of their maintenance in captivity and the great amount of gametes that a mature adult is able to produce. In the present review, the latest knowledge about the impact of OA on various stages of the life cycle of sea urchins is summarized with remarks on the possible impact of other stressors. The cellular physiology of the gametes before, at fertilization and, at early development, is extensively described with a focus on the complex enzymatic machinery and the intracellular pH (pH_i) and Ca^{2+} homeostasis for their vulnerability when facing adverse conditions such as acidification, temperature variations, or hypoxia.

Keywords: fertilization; cytoskeleton; cellular division; sea urchin; Ocean Acidification

1. Threats to the Life Cycle Of Echinoids under Ocean Acidification

Ocean Acidification (OA) represents a threat to the survival of marine organisms and its impact has been demonstrated in a wide range of species, both calcifiers and non-calcifiers [1–7]. In 2007, modelers published alarming future climate change scenarios [8] which have formed the basis of a growing awareness on presumed OA effects. Further studies have assessed the potential reversibility of the effects of emissions forecasted for the present century, the influence of OA on the primary productivity, on the export of particulate organic carbon, on the future ocean carbon uptake, and on the sensitivity of pelagic organisms [9–12]. These studies highlight the climate change impact on relatively small timescales. The Arctic Ocean, as well as Southern Ocean regions, will be severely affected by aragonite undersaturation, large pH changes, and synergic warming influence [13,14].

Under these conditions it is expected that many processes in living organisms such as physiological performance and reproduction will be affected (Table 1). While physiological performance strongly influences the adaption feasibility of a population, reproduction is a critical phase for the survival of species and populations within an ecosystem and is highly sensitive to any environmental perturbation. Reproduction is a very complex process involving different life stages of an organism and comprising highly-regulated mechanisms during gametogenesis, fertilization, development of larvae into adults and adult performance. Echinoderms, such as sea urchins, are expected to suffer from the detrimental effects of reduced seawater pH in the oceans along their life cycle [15,16], thus affecting all of those

mechanisms. The present review summarizes the impact of OA on the reproduction of sea urchins with emphasis on the processes of fertilization and further development.

Gametogenesis, Spawning and Fertilization of Sea Urchins

For echinoids, six gametogenic stages are described: recovery after spawning, growing, premature, mature, partially-spawned, and spent [17]. The gametogenic stages are characterized by the accumulation of nutrients, the proliferation of gonial cells, and their differentiation into gametes, as well as a reproductive quiescent period after spawning, in which the residual gametes are absorbed [17].

Table 1. Processes under the influence of changing climatic conditions. Environmental variables: pH (in black), Hypoxia/Anoxia (in blue), Temperature (in red). ⇑ = increase; ⇓ = decrease; ⇔ = no effect. Detailed descriptions in the text.

Stage	Studied Effect [Reference]	Observations
Gametogenesis	- ⇑ HCO_3^- compensation mechanisms and dissolution of carbonate structures [18] - ⇓ Food intake and gonadal growth [19] - ⇓⇓ Ability to spawn [20] - Delayed maturation and spawning at low pH [21]	Temperature and photoperiod regulate the gametogenesis cycle; future studies could focus on the effects of climate change variables on the hormone system behind gametogenesis regulation in different taxa.
Fertilization rate	- ⇓⇓ Proportion of fertilized eggs [22,23] - ⇓ Fecundity (reversible effect) [24] - ⇓ Fertilization success at longer exposure periods before fertilization [25] - ⇑ Polyspermy rates [26]	Depending on species origin and methods used, there are inconsistent results indicating the need of a standard protocol.
Gamete's viability	- ⇑ Life spam under laboratory conditions [27–30]	Stated under laboratory conditions; more studies are required including other environmental variables. Temperatures out of the range of the habitat conditions reduce the viability of the gametes but within that range an increase may contribute to gametes' contact.
Sperm cells	- ⇓⇔ Sperm motility (due to pH dependent activation of dynein ATPase) [31–33] - ⇓⇓ Sperm swimming speed [34,35] - ⇓ Mitochondrial activity [36]	Responses depend on species. Some species show no effect or even positive impact of decreasing pH. That could indicate acclimation or adaption patterns. More studies are required to demonstrate the effects of pH, T, and oxygen depletion on sperm motility, swimming speed, mitochondrial activity, ATP generation for motility, and ionic mechanism involved on sperm activation, capacitation, and acrosomal reaction.
Sperm-Egg recognition and contact	- ⇓ Egg jelly induction of acrosomal reaction at low pH [37]	The pH effect on the induction of the acrosomal reaction was elegantly studied in *S. purpuratus*. The authors demonstrated the resilience of this process until pH_e = 7 but pH changes were not achieved with CO_2 manipulations. Studies are required for T and H/A effects.
Egg cells and early zygotes	- ⇓ pH_i due to exposure to acidified seawater matrices before and after fertilization [22] (with still unstudied impact on Ca_i^{2+} sequestration and cytosolic buffer affinity for Ca^{2+}) - ⇓ Proportion of perfect FE and HL formation [22] - ⇓ Actin polymerization in the cortex region [38] - Cleavage retardation/inhibition at low pH leading to delayed larval formation [38,39] - ⇑ Alterations of the division plane [40,41] - ⇑ Embryo radialization [42–44]	There are few studies dealing with T and oxygen depletion impacts on eggs before and immediately after fertilization. The strong dependence of all stages after fertilization on the redox state of the zygote, the pH_i and Ca_i^{2+} levels and the sensitivity of the molecular machinery to pH (and probably T and H/A too), make of these stages a great-unexplored field requiring further attention. Markers ease to be studied at these stages: cortical reaction, CGSP1 activity which decreases with acidic pH, exocytosis and endocytosis cycles, ROS formation/elimination, enzymatic activity, cytoskeleton, and mitochondrial activity.
Larval development	- ⇑⇑ Larval malformations (e.g., exogastrulation, defect spiculogenesis and arms' formation *etc.*) [45–48] - ⇓ Development efficiency and larval settlement [49]	Many studies use the larval stages for the demonstration of environmental effects but few of them studied the molecular mechanisms behind the processes supporting the larval development. Studies are required to elucidate the impact of oxygen depletion.

Sea urchin gametogenesis occurs within the gonads triggered by specific environmental cues e.g., photoperiod and temperature [17], depending on the health status of the adult organisms. The reproductive health can be determined by gonadal structure, gonadal development, and fertilization success [50]. During a gametogenic cycle, the gonads of *Strongylocentrotus droebachiensis* change in mass and depend predominately on anaerobic metabolism (lactate pathway) to circumvent the hypoxic conditions resulting from the weak perfusion of the circulatory system in these organs [51]. Bookbinder and Shick [51] studied the change in perivisceral coelomic fluid in relation to the gametogenic cycle and detected a perivisceral decrease in pH before spawning and an increase after spawning. Moreover, they found an increase in protein concentrations, thereby increasing the buffering capacity of the coelomic fluid during the periods of gonadal development. Under OA exposure *S. droebachiensis* undergoes a shift in resource allocation and increases its bicarbonate (HCO_3^-) concentration in the perivisceral region as a compensation mechanism, mostly mediated by the coordinated regulation of the intestinal and peritoneal epithelia [18]. The intestinal epithelium actively imports HCO_3^- and prevents its loss while the peritoneal epithelium contributes to the generation of a buffer capacity via the dissolution of carbonates from osiccles and spines [18].

The quality and quantity of gametes shed by adult organisms exposed to detrimental environmental conditions during active gametogenesis may decrease [52]. Adult individuals of *S. droebachiensis* exposed to high concentrations of carbon dioxide (CO_2) showed a significantly reduced food intake which was reflected in reduced gonadal growth [19]. Similarly, adults of *Hemicentrotus pulcherrimus* delayed their gonad maturation and spawning under OA exposure in addition to their suppressed food intake [21]. Interestingly, the negative synergic impact of temperature and pH on gonadal development of *Echinometra* sp. differs between females and males, being stronger in males [53]. These authors found that low pH leads to differential spawning when comparing control and treatment conditions, and degeneration/reabsorption of the eggs in females unable to support the generation of new gamete cohorts, as it is the case in species with a long spawning season. In males there was a decline in gonad conditions in addition to a reduced ability to spawn [20,53]. Thus, compensation and gametogenesis are concurrent processes in terms of energy budget and adults might need to expend larger resources in order to cope with the stressful conditions imposed by acidification, instead of investing in gonadal development. The impact of additional stressors might exacerbate this situation.

Many organisms, besides the broadcast spawning echinoids, release their gametes for external fertilization in the water column. Fertilization must be performed under synchronic spawning events of the same species in order to ensure that male and female gametes meet. As spawning of different species may occur at the same time under natural conditions, gametes are equipped with mechanisms for species-specific recognition to avoid hybridization and cross-fertilization [54–56]. Released eggs and sperm cells are highly exposed and sensitive to adverse environmental conditions and, hence, are at high risk due to a variety of factors, ranging from natural physical conditions to artificially-created environmental modifications including chemical pollution, hypoxia, temperature variations, and OA. The time period during which the gametes may be exposed to adverse environmental conditions depends on the population distribution and spawning behavior [57–60].

The spawning behavior of sea urchin species is variable and mass spawning events are rarely observed in nature [27]. Aggregation in sea urchin populations may be influenced by food availability or by reproductive behavior, both factors determining the fertilization rates and the outcome zygote production [59]. Thus, variable population dynamics and behavior at spawning may lead to different exposure periods of the gametes. For instance, the spawning densities of *Diadema antillarum* are relatively low, inducing in this manner asynchronous spawning events [60]. Similarly, only a small proportion of *S. droebachiensis* population has been seen taking part of natural spawning [59]. Himmelman, *et al.* [57] documented the spawning behaviour of this species in its natural habitat and stated that spawning lasts longer than one hour, with numerous males spawning first and eggs remaining on the aboral surface of the females before slowly advecting and dispersing into

the water column to meet the sperm for fertilization. Under this behavior, gametes might become exposed to increased CO_2 levels for long periods before they are able to accomplish fertilization. During this period the gametes might be also exposed to variable environmental conditions in terms of temperature, oxygen availability and pressure and this might influence their performance once fertilization takes place.

The viability of sea urchin gametes is variable and little is known about their survival under natural conditions. Experimental studies showed that oocytes of *S. droebachiensis* are viable for 8 h in sterilized sea water [28], 24 h in filtered sea water [27], 2–3 days under laboratory conditions [29], and up to 1–2 weeks under anoxic storage conditions [30]. The fertilization during natural sperm release events may last as much as 48 h with temporal patterns that allow a success window for fertilization of 24 h under natural sperm release [29]. Similarly, Lera and Pellegrini [61] reported an egg viability of 2–3 days to up to a week for *Paracentrotus lividus*.

A reduction of fertilization rates and development efficiency has been referred to occur in species such as *Strongylocentrotus franciscanus* [16,26], *H. pulcherrimus*, and *Echinometra mathaei* [45,62]. Echinoids from shallow intertidal waters, commonly used in ecotoxicological and OA studies, are considered to be robust and to have higher stress tolerance to OA effects [63]. Under OA conditions there is a decrease in fecundity and larval settlement by long-term pre-exposure of *S. droebachiensis* adults, which can be ameliorated in cases where the outcome larvae of the following generations are further reared under control conditions [24]. This reveals the putative reversibility of physiological conditions induced by acidification and the genetic plasticity supporting robustness and adaption mechanisms of echinoids.

To avoid equivocal conclusions based on fertilization success experiments using intertidal species, the phenotypic plasticity and/or the adaption to natural daily changing conditions in their habitat has to be taken into account [26,53,63]. Significant impact of multistressors like OA and ocean warming on intertidal species indicates that the physiological tolerance limits are outstripped and that even relatively resilient species may be push to lethal levels under the expected climatic conditions for the end of the century [64]. Some stages in the life cycle of echinoids species, e.g., larval stages, are more sensitive regardless of their regional origin [65] and are prone to become the bottleneck for species success in a changing ocean. The sensitivity of physiological processes also vary and it is, therefore, crucial to identify vulnerable stages and physiological markers, taking into account species-specific and sexual differences in order to use them as endpoints and reference for damage in climate change research.

Once spawning takes place, the eggs must be fertilized by only one sperm, as polyspermy typically results in the zygote's death. Death is usually due to disruptions to the cleavage furrow formation by the presence of multiple inherited paternal centrioles guiding the process [66]. Nevertheless, Hiramoto [67] obtained zygotes that divided regularly regardless of polyspermic fertilization and suggested as a possible explanation that the second sperm does not participate in the mitotic process. Moreover, Runnström and Manelli [68] showed that polyspermic zygotes are also able to develop into larvae, however, with an injured endomesoderm-mesenchyme. To avoid polyspermic events, the fertilized eggs develop a fast and a slow polyspermy block involving a rapid change in membrane potential and the formation of the fertilization envelope (FE) [69–74]. Simultaneously to the formation of the FE, the eggs' metabolism becomes activated [75]. This activation is related to the redox status of the eggs [76], the intracellular levels of calcium (Ca_i^{2+}) and pH_i [77–79], the further activation of synthesis [80,81], and the cellular reorganization needed for posterior cytokinesis [82–85]. All of these events offer a broad spectrum of markers for OA research and are described in detail in the following sections.

2. Synergy between pH_i and Ca^{2+} Homeostasis at Fertilization

Fertilization is the fusion of gametes leading to the formation of a zygote from which a new organism develops. What seems to be the simple gathering of gametes is actually a very complex

and precisely scheduled cascade of enzymatic reactions that allows life to arise. Hence, fertilization represents a vulnerable step in the life cycle of an organism confronted with environmental stressors because the complex enzymatic machinery behind it might become affected by pH and temperature changes beyond their optimum window for physiological performance. Enzymes' structure and activity require precise control of the pH_i and this is reflected in its rigorous regulation in different cell types and within organelles [86]. Sea urchins are one of the preferred model organisms for developmental biology studies. Fertilization and larval development of sea urchins have, therefore, been extensively studied. In the present section the published knowledge, relevant in the context of OA and climate change research, are reviewed.

2.1. Sperm Activation, Chemoattraction, and Acrosomal Reaction

Information about the impact of climate change scenarios on the physiology of the sperm cells of sea urchins is, generally, scarce. There are only a few pioneering studies in the frame of OA research tackling the impact of pH on sperm swimming speed, sperm motility, and the physiological performance of sperm's mitochondria, and from these results it can be concluded that only few taxa might be resilient to acidification (Table 2). The sperm activation, the process of chemoattraction, and the acrosomal reaction are crucial for a successful fertilization and are prone to be affected by changing climatic conditions.

Table 2. Effects of pH/temperature changes on sperm cells performance.

Phylum, Species	pH/T Levels Tested	Results	Ref.
Echinodermata			
Centrostephanus rodgersii	pH = 8.10 pH = 7.80–7.60 (−0.30 until −0.5 pH units)	- Reduced mitochondrial membrane potential - Slight decrease in sperm swimming behaviour - Significant inter-individual variability	[36]
Heliocidaris erythrogramma	pH = 8.12 pH = 7.80–7.60 (−0.32 until −0.52 pH units)	- No effect on sperm swimming speed - Reduced proportion of motile sperm - Significant inter-individual variability	[87]
Heliocidaris erythrogramma	pH = 8.1 pH = 7.7 (−0.4 pH units)	- Reduced sperm swimming speed - Reduced percent sperm motility	[34]
Psammechinus miliaris	pH = 8.06 pH = 7.95–7.67 (−0.11 until −0.39 pH units) T = 14 °C; 17 °C; 20 °C	- Improvement of swimming speed and percent motility at reduced pH - Temperature affects swimming speed with no impact on percent motility	[35]
Holothuria spp.	pH = 8.03 pH = 7.77–6.55 (−0.26 until −1.48 pH units)	- Reduced sperm flagellar motility	[31]
Mollusca			
Mytilus galloprovincialis	pH = 8.0 pH = 7.6 (−0.4 pH units)	- Reduced proportion of motile sperm - Reduced sperm swimming speed	[88]
Crassostrea gigas	pH = 8.21–7.81 (−0.35 pH units)	- No effect on sperm swimming speed and sperm motility	[32]
Annelida			
Galeolaria caespitosa	pH = 8.10 pH = 7.80–7.60 (−0.30 until −0.50 pH units)	- Reduced swimming speed and percent motility	[33]
Cnidaria			
Acropora digitifera	pH = 8.17 pH = 8.05–7.74 (−0.12 until −0.43 pH units)	- Reduced sperm flagellar motility	[89]
Acropora digitifera	pH = 8.03 pH = 7.77–6.55 (−0.26 until −1.48 pH units)	- Reduced sperm flagellar motility	[31]
Chordata			
Gadus morhua	pH = 8.08 pH = 7.55 (−0.53 pH units)	- No effect on sperm swimming speed and sperm motility	[90]
Salmo salar	pH = 6.8 pH = 6.0–3.4 * (−0.8 until −3.4 pH units)	- Reduced sperm motility	[91]

* adjusted with H_2SO_4

The activation and capacitation of the sea urchin spermatozoan result from the dissolution into seawater which produces a drastic environmental change for the sperm cells: from K^+-rich (>30 mM), low-oxygen tension and acidic conditions in the gonads, to lower K^+ concentrations, oxygen-rich and basic pH of around 8.2 in seawater [92,93]. The conditions within the gonads inhibit the hydrolysis of ATP from which the sperm motility depends and keep the sperms in a quiescent state with repressed motility [92–94]. Under OA scenarios models suggest that seawater might arise pH levels far below the required value for activation [8], thus, distressing the intracellular regulation of pH during capacitation. OA may affect this process because once in water the sperm begins a Na^+-dependent acid extrusion due to the coalescent activities of the Na^+-H^+ exchangers (NHE) and the Na^+-K^+-ATPase in the flagellar plasma membrane (Figure 1) together with an active oxygen consumption for respiratory ATP production and motility [95–97], which might be additionally limited under hypoxic conditions. Only when pH_i increases, the axonemal dynein ATPase becomes active and the ATP production of the mitochondria via fatty acid oxidation starts [92,96].

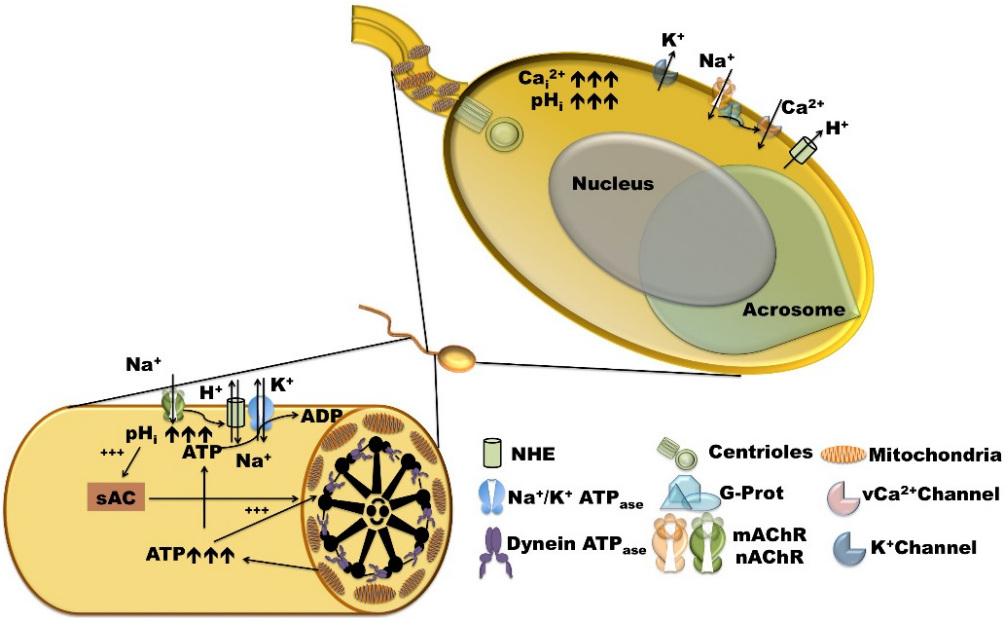

Figure 1. Main mechanisms involved in sperm capacitation and motility activation. The regulation of pH_i and Ca_i^{2+} levels within the sperm depends on the activity of NHE, voltage activated Ca^{2+} channels, Na^+/K^+-ATPase and the ATP availability regulated by oxygen tension in the media. NHE: Na^+-H^+ exchanger; AChR: nicotine acetylcholine receptors; sAC: soluble adenyl cyclase. Detailed description included in the text.

Acetylcholinesterase activity has been detected in the sperm flagellum and this enzyme is thought to play an active role at fertilization by restoring the excitability of nicotine acetylcholine receptors (AChR) that gate the NHEs and actively participate in the sperm propulsion [95]. The signal sensor for pH_i is the soluble adenyl cyclase (sAC) which: (i) is stimulated by HCO_3^- in the presence of Mg^{2+}; (ii) has a steep sensitivity to pH between 7 and 7.5; (iii) can be inhibited by millimolar Ca^{2+} concentrations; and (iv) is closely in contact with plasma membrane proteins like dynein, NHE, creatine kinase, guanylyl cyclase, cGMP-specific phosphodiesterases, the receptor for speract, and α- and β-tubulins [98,99]. The sAC elicits the cAMP-dependent phosphorylation of proteins in the flagellum and triggers the initiation of motility [98].

Motility and respiration are positively influenced during chemoattraction by peptides (sperm activating substances e.g., speract, resact, and sperm-activating peptide-I isolated from *Strongylocentrotus purpuratus*, *Arbacia punctulata*, and *H. pulcherrimus*, respectively) mainly present in the eggs' jelly coats that guide the movements of the sperm towards the egg [93,100,101]. It is known that K^+

and Ca^{2+} channels are activated by speract and that through chemotaxis the muscarinic AChR are activated and associated with G-proteins for triggering the Ca^{2+} cascade necessary for the acrosomal reaction [95]. The signal of chemotaxis is processed by the formation of cGMP and ion fluxes, via the membrane-bound isoforms of the sperm guanylyl cyclase acting as a cell-surface receptor in a pH dependent manner, and by the transient/fluctuating cGMP-mediated Ca_i^{2+} increase via voltage-gated Ca^{2+} channel [102–106]. All together, these processes affect the flagellar motion and determine the direction of sperm movement.

After the sperm has encountered the egg, the acrosomal reaction takes place. The species-specificity of the acrosomal reaction is guaranteed by diverse sulfaction patterns of the fucose-sulphated polysaccharide (FSP) of the egg jelly coat of each species [55,56]. The FSP glycoconjugate activates receptors in the sperm plasma membrane that trigger the exocytosis of the acrosomal vesicle via activation of ionic channels, with a net influx of Ca^{2+} and Na^+ and an efflux of H^+ and K^+ [93,107]. The extracellular pH of the media plays in this stage a fundamental role and may influence the pH_i and Ca_i^{2+} dynamic of the sperm required for the acrosomal reaction [37]. During this event, the sperm exocytoses the acrosomal vesicle from the anterior region of the head [96,108]. Simultaneously, the sperm releases lytic enzymes (a chymotrypsin-like protease) for the digestion of the egg jelly coat and the vitelline layer followed by the elongation, via polymerization of actin, of the acrosomal process whose surface is covered by the sperm-egg binding protein "bindin" [54,95,107]. Actin polymerization depends on pH [109], though under acidified conditions the formation of the acrosomal process might be repressed.

"Bindin" mediates the attachment and adhesion of the sperm to the plasma membrane [96]. This protein has a high affinity for FSP, typical of the egg receptor in the egg's surface, and their binding sites are later digested by the trypsine-like protease released during cortical granules exocytosis [110]. A second receptor, named Egg Bindin Receptor 1, unrelated to FSP, exists in the jelly coat of *S. franciscanus* providing a second "fail-safe" adhesion system for gamete interactions [110]. Additionally, the mature egg owns molecules in the membrane that are immunologically related to choline acetyltransferases, which can autonomously synthesize acetylcholine and may activate the AChRs that trigger the acrosome reaction of the sperm [95].

2.2. Egg Fertilization, Cortical Reaction, and Metabolic Activation

Sea urchin fertilization success has been extensively studied in recent years under OA and ocean warming scenarios but there are not many studies looking for the mechanism behind the climate change stressors at physiological, cytological, or genetic levels [18,111–114]. In this section the main physiological processes of the fertilized egg that might be impacted by environmental change are reviewed.

The mature eggs of sea urchins are in a dormant state within the gonads: the cell cycle is paused at G1 of first mitosis (first phase within interphase), pH_i is between 6.50 and 7.38, depending on the species, and the respiration levels are low until the contact with the sperm restarts the eggs' cell cycle events and metabolism via an increase in cytosolic free Ca^{2+} [78,115–120]. The eggs are kept in interphase by suppression of cyclin synthesis under the acidic pH_i prevalent in the unfertilized eggs [121].

In any case, the consequence of the gametes' contact is the development of an initial low and step-like depolarization (also called fast block to polyspermy) that allows the fusion of the gametes' membranes followed by a latent period of about 13 s and a longer fertilization potential that triggers the Ca^{2+} response and alkalinization of the cytosol within the eggs [95,122]. The initial depolarization is mainly generated by Na^+ and Ca^{2+} entry from external sources reinforced, via nicotinic acid adenine dinucleotide phosphate (NAADP), by the opening of voltage-sensitive T-type Ca^{2+} channels in the plasma membrane, and Ca^{2+} channels of lysosome-like acidic organelles which initiate the Ca^{2+} cortical flash responsible for the cortical granules' (CG) exocytosis (cortical reaction) [95,121,123,124]. The T-type Ca^{2+}-channels are dependent on depolarization and facilitate the actin-mediated sperm

incorporation into the fertilization cone [121]. As the sperm acrosomal process has the ability to synthesize acetylcholine after the acrosomal reaction, it might be possible that the initial influx of Na^+ into the egg is directly triggered by the sperm acting on nicotine AChR in the eggs' membrane [95].

Parrington, *et al.* [123] found that the NAADP-induced Ca^{2+} release is biphasic and after an initial surge of Ca^{2+} within the cortex, of about 2 s after sperm interaction, newly synthesized NAADP, targeting Ca^{2+} channels located in the membranes of acidic organelles, supports the following global Ca_i^{2+} increase that extends throughout the egg in a wave-like fashion. In addition, Morgan and Galione [125] found that a NAADP-mediated vesicle alkalinization (independent of the Ca^{2+} wave, of pH_i, of vesicular membrane potential and of the activity of the H^+-ATPase) takes place and potentially influences the activity of key enzymes contained in eggs' vesicles like the CGs. This alkalinization is not only restricted to the CGs but comprises additional deeper exocytotic cortical vesicle types, like the basal laminar and the apical vesicles involved in the formation of the hyaline layer (HL) or other acidic pigmented vesicles that have been observed near the cortex and other regions of the cytoplasm [126].

The global Ca_i^{2+} increase response is mainly due to the activation of the eggs' phospholipase C (PLCγ) by a tyrosine kinase (TyrK) of the Src family and/or GTPase activity: PLCγ, after its activation by the TyrK-G protein receptor complex, acts in a Ca^{2+}-dependent manner and hydrolyzes large amounts of polyphosphatidylinositol 4,5-biphosphate (PIP_2), present in the eggs' membranes, into inositol triphosphate (IP_3) and diacylglicerol (DAG), both known as second messengers in the Ca^{2+} signaling pathway [96,115,121–123,127]. The following Ca_i^{2+} response is due mainly to the release of this ion from intracellular sources [122,123,128]. The IP_3 mobilizes Ca^{2+} from the endoplasmic reticulum (ER) via IP_3-Receptors (IP_3R) while DAG, acting throughout the activation of protein kinase C (PKC), activates the Ca^{2+} release via nitric oxide synthase-cGMP-ADPrybosyl-cyclase activation of ryanodine receptors (RyR) and NAADP-dependent Ca^{2+} channels in the ER [123,124].

Berridge, *et al.* [124] referred that the ADPrybosyl cyclase has synthase and hydrolase activity and it is inhibited by ATP and nicotinamide adenine dinucleotide phosphate (NADP); thus, it may act in a dual manner in the formation and degradation of cyclic ADP Ribose (cADPR) and NAADP, as well as a sensor for the cellular metabolism state. Accordingly, the Ca^{2+} signal, dependent on cADPR and NAADP, gives a reinforcing boost and prolongs the main IP_3-R-mediated Ca_i^{2+} signal [121]. Additional modulation of the IP_3-R (through kinases like Ca^{2+}/calmodulin kinase (Ca^{2+}-CaMK), PKC, cGMP-dependent protein kinase and cAMP-dependent protein kinase) and the fine-tuned function of cytosolic buffers allow the generation of frequency modulated Ca^{2+} shifts of different amplitude, duration, intracellular location, and recovery time that specifically regulate the metabolic activation and the differential gene transcription after egg activation [124,129].

The generated Ca^{2+} wave is intricately related to different processes such as: (i) β-NAD kinase-mediated control of the redox state; (ii) Ca^{2+}-mediated cortical granules exocytosis; (iii) pronuclear migration; (iv) activation of protein and DNA synthesis; (v) amino acid transport; and (vi) mitosis and further cleavage cycles that lead to the progression of larval development [76,130–136]. The Ca^{2+}-dependent activation of NHE accounts for the alkalinisation of the cytoplasm [80,120,121]. The NHE, localized in the plasma membrane and cortical granules, requires for its activity basal levels of F-actin within the cortex region of the egg and the regulatory action of Rho/Rho-kinase proteins linked to the modulation of the actin cytoskeleton [137]. Rangel–Mata, *et al.* [137] proposed that the exchangers in the plasma membrane control the resting pH_i of unfertilized eggs while, once the cortical reaction takes place, new exchangers proceeding from the CGs become inserted into the plasma membrane and actively participate in the fast alkalinization of the cytoplasm. Moreover, the exchanger activity is affected by ATP depletion while the levels of PIP_2 are directly affected by the amount of ATP, as well as the functionality of kinases and phosphatases [127]. Therefore, it has been suggested that the ATP dependence of the exchanger results from its direct association to the PIP_2 dynamics [127]. In order to ensure adequate levels of ATP, a rapid activation of the pentose phosphate pathway (PPP) accounts for considerable levels of anaerobic metabolism in newly fertilized eggs [80]. Swezey and Epel [133] found that the main enzyme of the PPP, the G-6-PDH in unfertilized eggs, is inactive because

it is bound to particulate element of the cell and is essentially released after alkaline conditions are achieved. Together with NAD-kinase, which catalyses the formation of NADP and is activated by calmodulin during the Ca^{2+} rise [76,138], these enzymes guarantee the redox balance required for DNA synthesis (for which increased levels of reduced nucleotides (NADP/NADPH) are necessary) [80] and for the protection of the egg from the effects of reactive oxygen species (ROS) generated during the formation and hardening of the FE [129].

The Ca^{2+}-dependent exocytosis of the CGs leads to the formation of the FE, which swells over the course of 40 s after the entry of the sperm due to the associated rapid intake of seawater [139]. This process involves the combined action of enzymes released from the CGs which modify the vitelline layer (VL) and the extracellular matrix (ECM) to form a protective coat of around 80 nm thick [140]. Among the released enzymes, the Cortical Granule Serine Protease 1 (CGSP1), whose activity is pH-dependent, and the ovoperoxidase (OVOP) become autoactivated by the alkaline conditions of the seawater [141,142]. CGSP1 destroys via proteolysis the transmembrane integrin-anchoring between the microvilli and the VL leading to the autoassembly of additionally-released proteins with the VL, while the OVOP catalyses the formation of di-tyrosine bonds between adjacent proteins giving the FE its rigidity [141,143]. The OVOP achieves its maximal activity at pH 8 and has an unusual pH activity behavior called hysteretic response being inhibited at excessive hydrogen peroxide (H_2O_2) concentrations or at acidic conditions [144–146]. Hysteresis has been proposed as a valid mechanism for the control of the OVOP activity: when activated within the cell, the transient acidification of the egg's surroundings may rapidly convert this enzyme to its inactive form, which slowly regains its activity at the sea water pH of 8 [145]. Therefore, the pH_i transitions experienced by the OVOP during fertilization may be enough to provide the minimal conditions for hysteresis, thereby affording a functional timing mechanism for controlling its activity [144]. αBβC integrin subunits are released with the content of the CGs and cross-link together with the VL by the action of the OVOP and it has been suggested that integrins participate in the assembly and activation of cell-surface signaling complexes mediated by Src-like kinases during the activation of the egg via PLC [143].

Immediately after fertilization and during the FE lifting-off there is an elevated oxygen uptake directly linked to the formation of H_2O_2 [138,147]. The H_2O_2, needed for the hardening reaction, belongs to the ROS and is produced by the Ca^{2+}-activated Udx1 (a NADPH-dependent urchin dual oxidase anchored to the membrane of the CGs) supported by the activation of the PPP [141,147]. ROS are extremely reactive and toxic for proteins and DNA, representing an oxidative risk for the cell: ROS may induce cellular damages, inhibition of respiration, and activation of the apoptotic pathway in mitochondria [129,148]. The egg circumvents the negative effects of the ROS via the oxidation of an intracellular amino acid, ovothiol, to ovothiol disulphide [149] and its recycling through reduction by the glutathione reductase pathway [150]. Alternatively, before it damages the embryo, the Udx1 extracellular catalase-like peroxidase domain may neutralize the H_2O_2 produced by the NADPH oxidase domain of the same enzyme [141].

After completion of the FE formation the egg proceeds with further exocytotic events leading to the formation of the HL [139]. The HL has been extensively studied [151–155]. It has been demonstrated that it is necessary in order to hold cleaving blastomeres together, to maintain the shape of the embryo and the orientation of the cells during cellular division, and to participate in the process of mesenchyme cells invagination at the moment of archenteron formation [156,157]. The main protein of the HL is hyalin, an acidic filamentous protein that is extruded with the content of the CGs during the cortical reaction [151]. Hyalin aggregates with core proteins in a Ca^{2+} dependent manner and provides the backbone for the further addition of other proteins belonging to the ECM [151,152]. The CGs contain enough hyalin to supply embryogenesis until gastrulation [151]. Nevertheless, *de novo* formation of hyalin in embryos in the mesenchyme blastula stage and in reaggregation experiments of early embryos have been also described [152,158]. During development, amino acids are actively incorporated into the HL and when this structure is digested or weakened, e.g., by acidic media, it becomes difficult to detect [154,158] indicating its probable destruction. A time-sequenced exocytotic process achieves

the extrusion of other proteins contained into basal laminar vesicles, apical vesicles (which load the backbone of hyalin with glycoproteins), echinonectin vesicles (importing echinonectin for the union of the HL to the plasma membrane), and vesicles containing maternal cadherin, all required for the formation of the lamellar structure of the HL [139].

It has been demonstrated that the levels of Ca_i^{2+} and pH_i, dependent on external Na^+, Ca^{2+}, and HCO_3^- concentrations, are crucial in order to ensure the accurate levels of second messengers involved in the abovementioned signalling pathways and the progression through the mitotic cycles following fertilization and metabolism activation [112]. The physiological performance of both gametes will be potentially affected. A summarized overview of the vulnerable mechanisms at fertilization and metabolism activation is shown in Figure 2. More studies are necessary to determine, precisely, the impact at the cellular level.

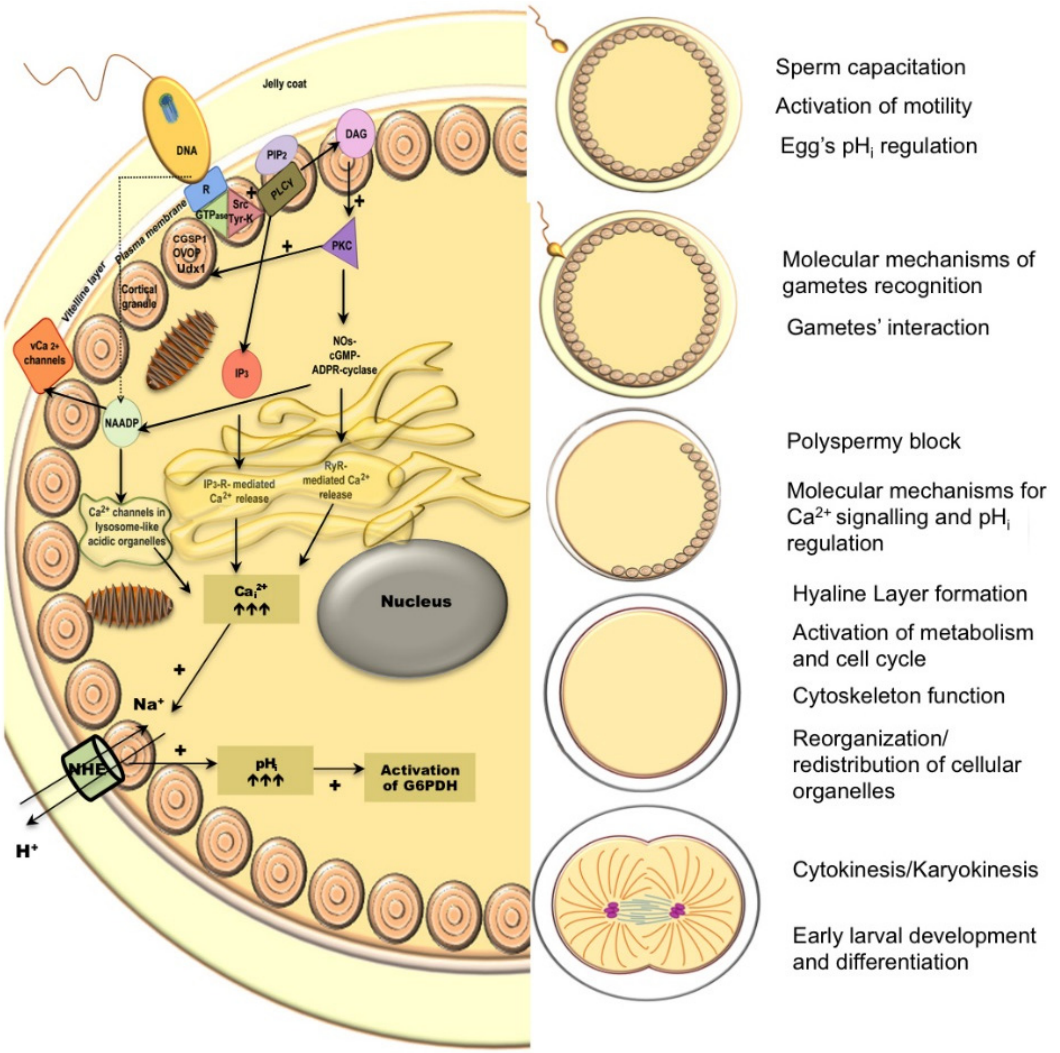

Figure 2. Overview of the main mechanisms at fertilization prone to be susceptible under climate change scenarios. AChR: nicotine acetylcholine receptors; ADPRcyclase: ADPribosylcyclase; CGSP1: cortical granule serine protease 1; DAG: diacylglycerol; GTPase: G protein; G6PDH: glucose-6-phosphate dehydrogenase; IP_3: inositol triphosphate; IP_3R: IP_3-receptors; NAADP: nicotinic acid adenine dinucleotide phosphate; NHE: Na^+-H^+ exchanger; NOs: nitric oxide synthase; OVOP: ovoperoxidase; PIP_2: polyphosphatidylinositol 4,5-biphosphate; PKC: protein kinase C; PLCγ: phospholipase C; RyR: ryanodine receptors; TyrK: tyrosine kinase of the Src family. More details of the mechanisms are explained in the text (adapted from the concepts published on Santella, *et al.* [122], Morgan and Galione [125], Whitaker [121], Runft, *et al.* [115], and Swezey and Epel [133]).

2.3. Cytokinesis

The pH_i and Ca_i^{2+} increases are also detected before and during the cellular division and are essential for the resumption of the cell cycle mediated by an increase in the synthesis of cyclins and the phosphorylation of other key cell cycle control proteins [159]. The Ca^{2+} and CaMKII are required for the assembly of the mitotic spindle, for the control of centrosome duplication/separation and for the activation of cell cycle kinases-cyclins complexes involved in the progression of meiosis to the final cytokinesis [129,131,160]. Slightly acidic pH_i conditions have been seen to retard or inhibit cleavage [39]. The separation of sister chromatids is supplemented with a brief, sharp and small Ca^{2+} transient of about 1–2 min before spindle elongation occurs [121]. Furthermore, Ca^{2+}, in a still alkaline cytosol, mediates actomyosin-based translocation of vesicles to the plasma membrane and their exocytosis for the furrow formation is coincident with the reorganization of the ER at fertilization [121].

Under the pH conditions of the unfertilized eggs, microtubule-associated proteins (MAPs), which are essential for tubulin polymerization and tubulin-actin interactions, are in a phosphorylated state that strongly inhibits their interaction with actin and also prevents microtubule assembly [161]. Nishida, *et al.* [161] found that the maximum interaction of actin filaments with unphosphorylated MAPs is achieved at pH between 6.8 and 7.4, which could be linked to the increased pH_i and kinase activity observed at fertilization. Direct effects of the pH_i shift (by the manipulation of the pH of the media) on actin dynamics have been already demonstrated [109,162,163]. Santella and co-workers found that, after fertilization, there is a rapid migration of subplasmalemmal actin fibers towards the inner cytoplasm and that disruptions in the actin structure of the cortical region lead to larger proportions of polyspermy, failures in the lifting-off of the FE, and to abnormal fertilization cones failing to engulf the sperm, mainly due to actin filaments alterations [164]. Thus, increases in pH_i, as well as Ca_i^{2+}, at fertilization control the performance of the main cytoskeleton elements involved in the fertilization, itself, and in the following cellular division of the eggs. When the elevation of pH_i is suppressed, the inhibition of microfilaments assembly accounts for an impaired formation of the fertilization cone affecting the incorporation of the sperm into the egg cytoplasm, there is an inhibition of microtubule assembly leading to the prevention of pronuclear migration and nuclear movements and this comes along with chromatin decondensation of the sperm nucleus [165]. Moreover, as an increase in Ca_i^{2+} and pH_i ensures the disassembly of microtubules on maternal centers, leading to the coordinated nucleation solely by sperm centrioles under a pH-mediated regulation of the mitotic organizing centers [165], altered pH_i and Ca_i^{2+} conditions within the zygote might lead to irregularities during the establishment of the division plane.

Furthermore, during the CG exocytosis there is an increase in the surface of the egg's plasma membrane, which is initially controlled by active elongation of the microvilli and subsequently reduced by Ca^{2+} mediated endocytosis [135,163]. The endocytosis begins 15–30 s after the completion of the cortical reaction and internalizes the membrane covering the microvilli and originated from the CGs [135]. This process proceeds in a biphasic manner independently of cytoplasmic alkalinisation: an initial endocytotic burst which last between 3 and 5 min and a basal level of endocytosis that follows after the first cleavage [135]. The endocytotic membrane retrieval takes place throughout clathrin-coated vesicles that initially appear after the microvilli have elongated and before the actin bundles within the microvilli become rigid, coincident with the first signs of HL formation. Near the end of the endocytotic burst large vesicles which migrate toward the cortex, together with pigment granules, evidencing high-regulated active and independent transport systems for endocytosis and exocytosis processes at fertilization [135]. The cytoskeleton elements play an essential role at this stage.

The pH_i plays also an important role during cellular division. The GTP_{ase} Rho, which controls the activity of the NHE, has been identified as the signaling link between the distribution of cytoskeleton elements in the inner cytoplasm (e.g., microtubules of the central spindle) and elements of the contractile ring that forms in the cortex and allows the correct positioning of the furrow and a successful cytokinesis [40,41].

As a result of the complex regulation of pH_i the progression to larval development ensues without a misreading of the Ca^{2+} signals, which are quite similar to those observed during apoptosis [129]. Mitochondria may act as sentinels of ER-mediated apoptotic signals via the Ca^{2+}-conducting voltage-dependent anion channel that is located in the outer mitochondrial membrane and is able to detect Ca^{2+} released from ER [129]. PIP_2, PLC, cADP-rybosyl cyclase, as well as IP_3-R and RyR have been found in the nucleus and may modulate gene expression and translocation of transcription factors from the cytosol to the nucleus during early larval development [166]. The performance of lysosome-like organelles as Ca^{2+} sources depends on the Ca^{2+}-H^+ antiporter in their membranes, which is driven by low internal pH in the lumen of these organelles [121].

Moreover, the pH changes may influence Ca^{2+} signalling by altering the intracellular affinity of cytosolic buffers for Ca^{2+}. The pH optimum for Ca^{2+} sequestration is about 7.4 with a minimum at pH 6.8 [165]. F-actin may also act as a Ca^{2+} reservoir, as Ca^{2+} bound to F-actin becomes inaccessible to Mg^{2+} for exchange and actin assembly can be directly linked to or controlled by the pH_i [164]. Thus, during fertilization and the first developmental stages, the influence of pH on mitochondria, ER, nucleus, and other organelles' functions might support and coordinate the regulation of the Ca^{2+} signals within the zygotes. For all these reasons it is clear why larval development is among the most compromised stages under climate change scenarios.

3. Main Mechanisms of pH_i Regulation in Sea Urchins

To maintain adequate levels of pH_i, the eggs require mechanisms for extrusion of H^+ to resist acidification and mechanisms that counteract this process. Casey, *et al.* [86] showed that while cellular compartments are protected from small localized shifts in pH_i by their inherent buffering capacity, these are not sufficient to neutralize sustained pH_i shifts which could be promoted by the tendency of the cytosol to become acidified throughout acid equivalents generated during metabolic reactions. Thus, pH_i homeostasis requires an active ATP-mediated extrusion mechanism and/or the activity of exchangers and co-transport mechanisms to counteract the natural cytosolic acidification [86].

The NHE is one of the most important mechanisms responsible for pH_i homeostasis at fertilization [167]. NHE has a pH sensing regulatory site (H^+-sensor) that acts by minimizing the dissipation of transmembrane Na^+ gradient in situations of undue cytosolic alkalinization, while sharply increasing its activity when the cytosol becomes acidified [86]. Na^+-HCO_3^- co-transporters (NBC) have been found in sea urchin primary mesenchyme cell transcriptomes [49]; thus, the sea urchin eggs might also rely on this system for acid extrusion and control of pH_i. The functioning of the NBCs depends on the Na^+ gradient across membranes and the transmembrane potential which, once it is outstripped, inverts the mechanism of the NBC, meaning that HCO_3^- is extruded instead of being incorporated into the cell, leading in this manner to acidification of the cytosol [86].

At the onset of fertilization a membrane depolarization escorts the initial Ca_i^{2+} and pH_i increases [73]. Consequently, it can be speculated that this mechanism counteracts the hyperpolarizing effect of the electrogenic isoforms of the NBCs supporting the alkalinisation effect of the NHE with an additional admission of HCO_3^-. In line with this, Ciapa and Philippe [112] suggested that NBCs and Na^+-Ca^{2+} exchangers must work together with NHEs in the early embryos of *P. lividus* in order to maintain the alkaline pH_i after fertilization. Nevertheless, the eggs must also control the alkalinization of the cytosol to avoid shifts far beyond necessary levels for metabolism activation because alkaline-dependent apoptosis has been proposed to occur in mouse ganglion neurons, opposed to the common acidosis-dependent origin suggested for apoptosis [112].

The Cl^-–HCO_3^- exchangers are also likely to be involved in pH_i regulation as they are found in direct relation to the activation of sperm motility [168,169]. The function of these exchangers is based on the driving force of Cl^- gradients across membranes for the extrusion of HCO_3^- and it is self-controlled by acidification of the cytosol: in acidic surroundings this activity sharply decreases by about 80%–90% [86]. The combined action of this mechanism and the NHE exchanger in *Xenopus* oocytes results in the maintenance of the pH_i near 7.2 [170].

The Ca^{2+} wave is also mediated by Ca_i^{2+} release from sources other than the ER, e.g., acidic vesicles and reserve granules [171]. The accumulation of Ca_i^{2+} within these organelles is mediated by vacuolar-type-H^+-translocating ATP hydrolases, Ca^{2+}-H^+ exchangers, and Ca^{2+} pumps, for which pH homeostasis is of major concern [86]. Morgan and Galione [125] examined the spatio-temporal variation of organellar pH at fertilization and found that vesicle alkalinization is coincident with NAADP-mediated Ca_i^{2+} release and probably directly exerted by NAADP on the acidic vesicles in the cortex area of the eggs. The role of this alkalinization is still to be elucidated, but is potentially related to the fast activation of the OVOP in a controlled environment reducing the probability of ROS formation within the cytosol, close to the region where pronuclear fusion must take place [149,150,172].

All these mechanisms might be negatively influenced by other environmental stressors than pH changes: temperature variations could impose suboptimal conditions for the function of the required enzymes and, as most of these mechanisms require a link to the energy machinery of the eggs, oxygen availability is also crucial, and life in increasing hypoxic environments will force organisms to their limits and will negatively influence their cellular performance.

4. Evidence of Subcellular Alterations Due to Ocean Acidification Exposure

OA affects the reproduction of sea urchins in many aspects. It has been reported that, under acidified conditions, besides the morphological alterations detected at fertilization, a delay in larval formation occurs, the larvae may suffer deformations, e.g., reduced calcification and budding, which are malformations resembling the process of exogastrulation [46–48,173]. Exogastrulation can be caused by exposure to heavy water, lithium, and antibodies for the hatching enzyme, by the administration of estrogens, by calcium deficiency, and due to chilling [174–179]. Exogastrulation is due to the incorrect accumulation of β-catenin in all blastula cells (by the treatment for instance with lithium chloride), instead of being only in the cells disposed to become endoderm and mesoderm, which leads to the transformation of presumptive ectoderm cells into endoderm driving the evagination of the archenteron [66]. The β-catenin signalling pathway is a primitive regulator of endomesoderm specification and is redox regulated [42]. The larval budding report of Chan, *et al.* [47] might point toward disruptions in translocation mechanisms of transcription factors like β-catenin probably also involving disorders in cytoskeleton elements and the functioning of mitochondria. Thus, temperature and pH changes as environmental stressors should be carefully analyzed in relation to this findings.

Mitochondria play a key role in the control of Ca_i^{2+} within the eggs and zygotes supporting the regulation of the redox state, which is closely related to pH_i. About 10% of the respiratory energy between 50 and 90 s after insemination is used for the synthesis of H_2O_2, NADP, and NADPH, and in this initial respiratory burst after fertilization, the electron transport energy is also directly coupled to the entry of Ca_i^{2+} into the mitochondria [180]. Mitochondria are known to interact with the ER, to function as Ca^{2+} buffers and their Ca_i^{2+} uptake regulates its metabolic activity and the production of ROS acting as second messengers in further enzymatic cascades [42]. Moreover, mitochondria are asymmetrically distributed in the unfertilized egg, being differently inherited by the blastomeres after cleavage and contributing in this manner to the specification of the oral ectoderm [43].

Under hypoxic conditions, enforcing a relative reducing redox state, there is a suppression of the specification of the oral ectoderm along which the ciliated band ectoderm, the mouth morphogenesis, and other mesodermal cell types differentiate [43,181]. At blastula stage the ectodermal cell fate depends on the localized expression of "nodal" in the forthcoming zone of oral ectoderm and its expression is redox regulated via mitochondrial H_2O_2-activation of a stress-activated protein kinase p38, required for this process [181]. Under hypoxic conditions the suppression of the oral ectoderm specification, even when embryos seem to be normal at blastula stage, leads after hatching to the formation of radialized embryos presenting flattened stomatodeum and ciliated band, with a tendency to form retarded and non-localized spiculogenesis, and having a radially-symmetric archenteron instead of being displaced as in normal development [43].

Coffman and Davidson [44] suggested that cytoskeletal activity may trigger subtle respiratory asymmetry in the early zygote promoting a persistent signal throughout cleavage for oral-aboral axis specification and that the mechanisms regulating the cytoskeleton function are, therefore, highly dependent on the correct pH environment. In terms of regulation, the alkalinization of the cytosol appears to be more than just a complementary event at fertilization.

Ciapa and Philippe [112] investigated the effects of buffer mediated acidification on the control of mitosis in early larval stages of the sea urchin *P. lividus*. They found that Ca_i^{2+}/pH_i levels and Extracellular Regulated Kinase (ERK)-mitotic promoting factor activities vary together regulating mitosis and survival of the larvae. ROS, such as H_2O_2, produced by mitochondria or in the cytosol, stimulate the Mitogen Activated Protein Kinase and the ERK pathways involved in cell proliferation and differentiation [42]. The results of Ciapa and Philippe [112] show that sea urchin embryos are actually equipped with mechanisms for the close regulation of their pH_i in response to fluctuations of pH in the media. Nevertheless, in Ciapa and Philippe's experiments the fertilization was performed under normal (control conditions) and after 15–20 min the fertilized eggs were transferred to incubation medium having altered ionic composition and pH, which could explain why these authors did not find alterations during the transformations of the ECM structures (e.g., FE, HL). Moreover, the acidification levels of the incubation media in their experiments were not obtained by CO_2 enrichment, thus, perhaps introducing other mechanisms than those present under OA scenarios. For instance, Kurihara and Shirayama [62] detected a more severe effect of CO_2-enriched seawater on the fertilization rate of the sea urchins species *H. pulcherrimus* and *E. mathaei* than of HCl-acidified seawater had. Interestingly, Ciapa and Philippe [112] found eggs showing cellular constrictions and nuclear envelope breakdown, probably related to alterations in the Ca_i^{2+} regulation under cytosolic acidosis caused by the Na^+ deprivation outside the cell, followed by compensation throughout additional removal of HCO_3^- from the media. Constricted eggs which failed to progress into the cell cycle were also observed by Bögner, *et al.* [22].

For the accomplishment of cytokinesis a highly-regulated sequence of events concerning the nucleus and the cytosol must occur and the communication between these compartments is essential. The cortex region of the zygote is in close contact with tubular membrane networks originated from the reorganization of the ER at fertilization [82,182]. The double membrane structure of the nuclear envelope that separates the nucleoplasm from the cytoplasm is actively involved in Ca_i^{2+} balance within the nucleus and the inner cytosol and it communicates with the lumen of the ER which mediates the Ca_i^{2+} signaling by means of sequestering or extruding Ca_i^{2+} from its interior [166]. The abundant nuclear pore complexes of the nuclear envelope represent only a weak barrier for the diffusion of H^+ and consequently the pH of the nucleus and the ER may be similar to the pH_i [86]. Moreover, the presence of IP_3R in the inner and outer nuclear envelope membrane enabling the release of Ca_i^{2+} directly into the nucleoplasm has been proved [166]. During mitosis, the onset of anaphase is stimulated at metaphase by increasing concentrations of free Ca_i^{2+} ions contributing to the initiation of chromosome separation [183]. Thus, a link between the cortical pH_i increase and the Ca_i^{2+} signals may lead to nuclear transcription events and the membranous structures derived from the ER may mediate the amplification of the signals in the cortex to inner regions of the cell for the coordination of cellular division. These events are supported by cytoskeleton elements. As actin polymerization is affected under acidified conditions while the microtubule's assembly is enhanced at slightly acidic pH_i (with a stabilization of the spindle leading to an enlargement of the mitotic apparatus and to cleavage retardation) [39,109,163,184], it might be speculated that cellular constrictions are the result of the direct effect of extracellular pH on the Ca_i^{2+} and pH_i homeostasis of the zygote. This is supported by the fact that the position of the mitotic apparatus triggers spatial cues governing the localization of the cortical actomyosing contractile ring needed for the furrow formation at the beginning of telophase [40,185].

Changes in pH_i may also affect the completion of cytokinesis by disrupting the centrosomes. Centrosomes are composed of a mother and a daughter centriole that are duplicated before mitosis, the absence of which causes defects in completing cytokinesis and further cell cycle progression [186].

Schweitzer and D'Souza–Schorey [186] reported that the depletion of "centrolin", found in association with the mother centriole and required for the successful completion of cytokinesis, leads to daughter cells connected by intercellular bridges that may undergo apoptosis or enter a following cell division cycle. Similar effects were observed in fertilization experiments using *Psammechinus miliaris* under acidification exposure [187]. Climate change related stressors might, therefore, adversely influence most of the signalling pathways activated at and after fertilization.

5. Conclusions and Future Perspectives for Research

Under CO_2-mediated acidification, hypoxic conditions, and temperature variations, the gametes of many echinoids are prone to suffer at almost every event before, at, and after fertilization takes place. Processes, such as the pH_i and Ca_i^{2+} homeostasis merit closer insight and should be addressed in future research. The threatened events before fertilization include the capacitation of the sperm, the acrosomal reaction and the basal maintenance of pH_i in sperm and egg cells. There are few references to disruptions in sperm motility and longevity under acidified conditions, and studies focusing on sperm mechanisms for pH_i regulation in the context of OA research are still missing. During fertilization, events such as the gametes' interaction, the engulfment of the sperm by the egg, the cortical reaction, the protective response against ROS in the zygote, the activation of the PPP, the pH_i and Ca_i^{2+} increase response, the formation of the ECM structures and the mitotic spindle, the correct establishment of the furrow plane for cellular division, and the function of important organelles like ER and mitochondria may all be affected under climate change scenarios. The evaluation of the consequences of the cortical pH_i shifts for the main organelles within the cytosol of the fertilizing gametes, as well as whether their potential functional disruption may affect the process of zygote's development, require further research. The molecular background of findings, like the larval budding, are also very interesting subjects that require still-additional evaluation to determine whether there is, in fact, a negative impact of OA on signaling pathways during larval development or an adaptive response to the acidified conditions through which small larvae might develop asexually from the buds. Intersexual variability, transgenerational reversibility, adaption, and resilience should be also evaluated when analysing cellular/physiological performance.

Studies at cellular level in the context of OA research remain scarce. Echinoid gametes are very suitable for this kind of research and many tools and protocols e.g., fluorescence staining, histochemical detection of enzyme or mitochondrial activity are available and have been well established in this model organism. This kind of study may offer a wider understanding on the multi-stressor impact, on the underlying mechanism, and on the chances that broadcast spawning organisms may have to cope with this problem.

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Abbreviations

The following abbreviations are used in this manuscript:

AChR	acetylcholine receptor
Ca_i^{2+}	intracellular calcium
Ca^{2+}-CaMK	Ca^{2+}/calmodulin kinase
cADPR	cyclic ADP ribose
CG	cortical granules
CGSP1	cortical granule serine protease 1
CO_2	carbon dioxide
DAG	diacylglicerol

ECM	extracellular matrix
ER	endoplasmic reticulum
ERK	extracellular regulated kinase
FE	fertilization envelope
FSP	fucose sulphated polysaccharide
H_2O_2	hydrogen peroxide
HCO_3^-	bicarbonate
HL	hyaline layer
IP_3	inositol triphosphate
IP_3R	IP_3-receptors
MAPs	microtubule-associated proteins
NAADP	nicotinic acid adenine dinucleotide phosphate
NADP	nicotinamide adenine dinucleotide phosphate
NBC	Na^+-HCO_3^-co-transporter
NHE	Na^+-H^+ exchanger
OA	ocean acidification
OVOP	ovoperoxidase
pH_i	intracellular pH
PIP_2	polyphosphatidylinositol 4,5-biphosphate
PKC	protein kinase C
PLCγ	phospholipase C
PPP	pentose phosphate pathway
ROS	reactive oxygen species
RyR	ryanodine receptor
sAC	soluble adenyl cyclase
Udx1	urchin dual oxidase
VL	vitelline layer

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