

pH Regulation and Excretion in Echinoderms

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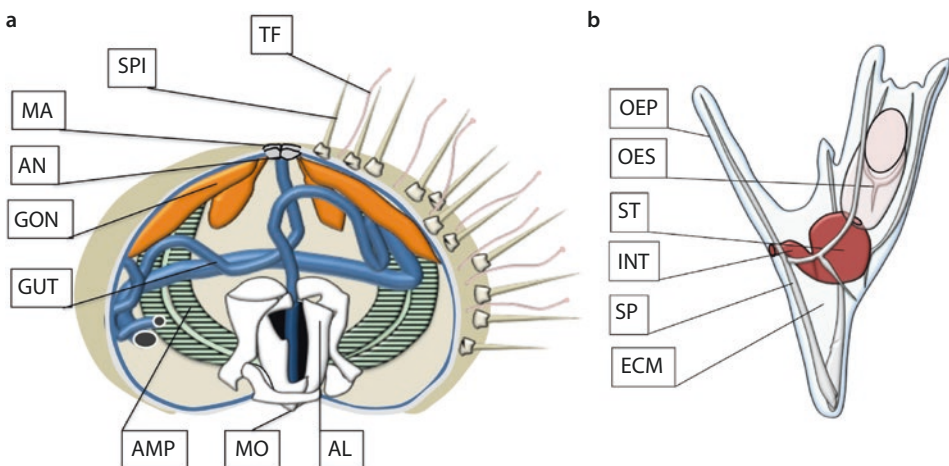
10.1 Adult Echinoderms

10.1.1 Extracellular Acid–Base Regulation

Echinoderms consist of five classes: Crinoidea (sea lilies), Asteroidea (sea stars), Ophiuroidea (brittle stars), Echinoidea (sea urchins), and Holothuroidea (sea cucumbers). Most of them are key stone species in many marine ecosystems and especially sea urchins and sea cucumbers are economically important organisms in many countries.

Adult echinoderms are characterized by their pentameric symmetry and an ambulacrarian vascular system (■ see Fig. 10.1 for an echinoid body plan). Some of the classes have heavily calcified tests, like sea urchins, brittle stars, and crinoids. Sea stars and sea cucumbers have calcified spines or test plates and are not as heavily calcified. All echinoderms have a so-called coelomic fluid, their blood derivates with coelomic cells that resemble higher animals' immune cells. The composition of the coelomic fluid is similar to that of seawater and is prone to acid–base disturbances when the animals encounter hypercapnic conditions or emersion. The coelomic fluid can be relatively easily sampled from the animals, and examinations regarding the extracellular acid–base status in echinoderms were primarily conducted on this fluid.

Among the five classes of echinoderms, there is data on extracellular acid–base status on four of them (no data on crinoids). Of these four classes of echinoderms, only Echinoidea and Ophiuroidea were reported to actively compensate for acid–base disturbances indicated by an accumulation of bicarbonate in response to elevated $p\text{CO}_2$ (Stumpp et al. 2012b; Calosi et al. 2013; Hu et al. 2014). The effects of CO_2 -induced seawater acidification on echinoderms have lately received considerable attention, as calcifying species were predicted to be particularly sensitive to ocean acidification. The maintenance of calcification is directly linked to acid–base regulatory capacities making this physiological process a key candidate to address sensitivities in echinoderms (Ries et al. 2009). Furthermore,



■ Fig. 10.1 Body plan of an adult sea urchin (a) and an echinopluteus larvae (b). Aristotle's lantern (AL); mouth (MO); ampullae (AMP); gut (GUT); gonads (GON); anus (AN); madreporite (MA); spines (SPI); tube feet (TF); extracellular matrix (ECM); spicule (SP); intestine (INT); stomach (ST); esophagus (OES); outer epithelium (OEP)

echinoderms are key stone species in many marine habitats, and vulnerability of these organisms to near-future climate change could have severe repercussions on populations and ecosystem stabilities.

Echinoidea

Sea urchins of the species *Strongylocentrotus droebachiensis* exposed to 1400 and 3800 μatm CO_2 were able to fully or partially compensate extracellular pH (pH_e) disturbances within 7–10 days of exposure to elevated $p\text{CO}_2$ (Dupont and Thorndyke 2012; Stumpp et al. 2012b) and were able to sustain this compensation for over 45 days. The maximum bicarbonate accumulation recorded in this species was 2.5 mmol L^{-1} above their control levels of 4.0–5.3 mmol L^{-1} HCO_3^- . Interestingly, the control levels of $[\text{HCO}_3^-]_e$ were already higher than that of seawater (around 2 mmol L^{-1}). Sea urchins of the species *Arbacia lixula* and *Paracentrotus lividus* also accumulated bicarbonate up to 3.5 mmol L^{-1} (from 2 mmol L^{-1} control) and 5–5.1 mmol L^{-1} (3.6–3.8 mmol L^{-1} control levels) in response to 1500 μatm and 2500 μatm CO_2 (Calosi et al. 2013; Collard et al. 2014b). With this increase in bicarbonate, both species were able to fully or partially compensate induced extracellular acid–base disturbances (Catarino et al. 2012). *Tripneustes ventricosus* accumulated up to a $[\text{HCO}_3^-]_e$ of 5.0 mmol L^{-1} (from 2.9 mmol L^{-1} control levels) in response to 2000 μatm CO_2 (Collard et al. 2014b). *Psammechinus miliaris* accumulated 0.5 mmol L^{-1} HCO_3^- from 1.8 mmol L^{-1} to 2.3 within 7 days of exposure to 2400 μatm CO_2 (Miles et al. 2007). These examples highlight that at least some sea urchins have a higher potential to actively regulate their extracellular pH than previously thought.

Nevertheless, there are also examples for sea urchins that do not compensate pH_e disturbances: The cidaroid pencil urchin *Eucidaris tribuloides* was not able to increase extracellular bicarbonate levels within 32 days of exposure to 2000 μatm CO_2 (Collard et al. 2014b). Data on irregular and/or infaunal sea urchins such as sand dollars or sea mice are still missing and may offer more conclusive patterns regarding acid–base physiology in different groups of echinoids.

In general, it seems that noncidaroid, regular sea urchins are able to accumulate up to 2.5 mmol L^{-1} bicarbonate in order to counteract acid–base disturbances. The mechanisms underlying bicarbonate accumulation, however, are insufficiently understood. Based on coelomic Mg^{2+} and Ca^{2+} concentration measurements in response to elevated $p\text{CO}_2$, an accumulation based on test dissolution was suggested (Miles et al. 2007). Contrary, bicarbonate concentrations in the coelomic fluid of control animals is already significantly higher than seawater in most studied sea urchins (e.g., 2–3 mmol L^{-1} above seawater $[\text{HCO}_3^-]_{\text{SW}}$ of 2–3 mmol L^{-1}) and favors the hypothesis, that already under control conditions an active HCO_3^- accumulation mechanism is employed. While temporary test dissolution may still contribute to the initial compensation phase in sea urchins, it seems unlikely that test dissolution is solely responsible for the observed reestablishment of pH_e homeostasis.

There are two tissues that could contribute to bicarbonate accumulation: the peritoneal epithelium that separates the sea urchins test from the coelomic fluid and the intestinal tissue. The peritoneal epithelium, though being tight for molecules larger than 0.3 kDa, is no barrier for ions and small molecules, such as HCO_3^- (Holtmann et al. 2013). Therefore, the organ that most likely contributes to the observed bicarbonate accumulation is the digestive system. The intestine prevents loss of bicarbonate ions by establishing a tight barrier for HCO_3^- ions (Holtmann et al. 2013). However, the mechanisms and a potential function of the intestine in acid–base regulation remain unexplored.

Ophiuroidea, Asteroidea, and Holothuroidea

There is only one study reporting extracellular acid–base status for an ophiuroid. The infaunal brittle star *Amphiura filiformis* has extracellular bicarbonate levels of 4–6 mmol L⁻¹ under control conditions (compared to 2 mmol L⁻¹ in seawater) and increased extracellular bicarbonate levels up to 8 mmol L⁻¹ when exposed to 1500 and 3200 μatm CO₂ (Hu et al. 2014). The compensation reaction happened within 24 h after onset of exposure and resulted in a full (1500 μatm) and partial (3200 μatm) recovery of pH_e. *A. filiformis* was able to sustain high bicarbonate levels during 14 days along the experimental period (Hu et al. 2014). Interestingly, transcript levels for V-type H⁺-ATPase were elevated in disk tissue, and not in the arm tissue, in response to CO₂ stress indicating that proton transport might also be an important mechanism for extra- and/or intracellular pH compensation.

The sea star *Asterias rubens* did neither accumulate bicarbonate in response to elevated pCO₂ (1200–3500 μatm, (Appelhans et al. 2012)) nor were they demonstrated to compensate acid–base disturbances in their coelomic fluid (Collard et al. 2014b) over an experimental duration ranging from 14 days to 10 weeks. The sea star *Leptasterias polaris* did also not compensate pHe disturbances during 7 days of exposure to 1275 μatm CO₂ (Dupont and Thorndyke 2012).

The sea cucumbers *Holothuria scabra* and *Holothuria parva* did not significantly accumulate bicarbonate to compensate their pHe in response to hypercapnic conditions (2000–3500 μatm) (Collard et al. 2014a) in the experimental period of 6–12 days that may, however, be too short an interval to initiate a compensation reaction. [HCO₃⁻]_e was still slightly higher (between 2.7 and 3.0 mmol L⁻¹) than in seawater (between 2.2 and 2.7 mmol L⁻¹).

These observations indicate that the ability to compensate for acid–base disturbances via active accumulation of HCO₃⁻ in body fluids is not a universal feature in all echinoderms but seems to be restricted to Echinoidea and Ophiuroidea. The fact that most holothurians and asteroids are less calcified than echinoids and ophiuroids can lead to speculations that acid–base regulatory abilities are correlated to the degree of calcification in echinoderms, with the exception of the pencil urchin *E. tribuloides*, which is a heavily calcified sea urchin even among sea urchins. In order to test this hypothesis, a comparative approach using a larger range of species and a standardized experimental design would be needed. Moreover, additional studies on the mechanistic basis of pHe regulation in echinoderms will represent an important future task to better understand the mechanisms of acid–base transport across gut epithelia in a so far unexplored animal group.

10.1.2 Nitrogen Excretion

It has been proposed that excretion of protons may be facilitated by the excretion of ammonia (for mechanistic details on the cellular level, see chapters on crustaceans and cephalopods). There are only a few studies examining the ammonia excretion rates in response to hypercapnia in echinoderms (Stumpp et al. 2012b; Hu et al. 2014).

It seems that in all studied echinoderm species, the compensation reaction for CO₂-induced acid–base disturbances is accompanied by a significant increase in NH₃/NH₄⁺ excretion rates. For example, *Strongylocentrotus droebachiensis* increased ammonia excretion rates by 70% in response to 2800 μatm CO₂ (Stumpp et al. 2012b). *Amphiura filiformis* significantly excreted 65% more ammonia under elevated pCO₂ conditions (3400 and 6600 μatm CO₂) (Hu et al. 2014). Species, e.g., *Holothuria scabra* and *Holothuria parva*,

with uncompensated perivisceral fluids did not increase their ammonia excretion in response to hypercapnic conditions (Collard et al. 2014a).

This observation suggests that pH_e regulatory mechanisms are linked to ammonia excretion as a mechanism to facilitate bicarbonate formation/accumulation and/or proton (equivalent) excretion. A potent mechanism to excrete protons is essential in species that are capable of actively accumulating HCO_3^- as the formation of HCO_3^- from CO_2 and H_2O is ultimately associated with the formation of H^+ that needs to be secreted from the animals. As the present body of knowledge regarding species-specific $\text{NH}_3/\text{NH}_4^+$ secretion characteristics and mechanisms for echinoderms are scarce, definite conclusions cannot be drawn from the present literature data.

10.2 Larval Echinoderms

Echinoderms generally reproduce by free-swimming larvae – with very few exceptions of brooding species. Larvae are either lecithotrophic or in most of the cases planktotrophic. Depending on the class, you find larvae with calcified endoskeletons (sea urchins and brittle stars) or larvae that are not calcified until the adult rudiment is formed (sea stars and sea cucumbers). The calcified structures, if present, are surrounded by a cytoplasmic sheet of primary mesenchyme cells, which is again embedded in a vast extracellular space between the epidermis and digestive tract. The extracellular space of echinoderm larvae is characterized by extensive almost cell-free gelatinous material occupying the primary body cavity and surrounding the calcifying primary mesenchyme cells (PMCs) in echinopluteus larvae (Strathmann 1989; Crawford 1990). The gel-like compounds of the primary body cavity are extremely flexible and the larval body is able to rebound to its original shape following muscle contraction (e.g., during swallowing of food particles). Thus, it is a highly energy-saving system for larval movement, allowing for large larvae with relatively little cellular material, a situation not unlike that in many Scyphomedusae (Strathmann 1989; Crawford 1990). The larval calcium carbonate structures, if present, combined with the gelatinous material support the larval morphology and orientation of the larvae in the water column (Pennington and Strathmann 1990). ■ See Fig. 10.1b for a larval body plan of an echinopluteus.

Thus, there are three morphological structures/compartments that are of interest for acid–base regulation: intracellular pH regulation in calcifying primary mesenchyme cells, the extracellular space between the ectoderm and endoderm, and the extracellular/luminal space of the digestive system. While there is genetic data available of whole larvae exposed to hypercapnic conditions, mechanistic data on acid–base regulation in echinoderms is, again, scarce and is within the echinoderms limited to larvae from sea urchins.

10.2.1 Intracellular Acid–Base Regulation in Calcifying Primary Mesenchyme Cells

Echinoderm larvae are considered to be particularly sensitive to seawater acidification and to the connected changes in calcium carbonate saturation state of seawater (Ω_{Cal}) since their internal skeleton is composed of high-magnesium calcite, the most soluble form of CaCO_3 (Beniash et al. 1997; Raz et al. 2003). However, sea urchin larvae are able to maintain calcification rates (when corrected for developmental or growth delay) even during

exposure to unrealistically high CO_2 partial pressures (0.35 kPa CO_2 , pH 7.25, Ω_{Cal} 1.02) (Martin et al. 2011). This indicates that the calcification machinery has substantial capacities to compensate for CaCO_3 dissolution due to CO_2 -induced reductions in seawater pH. The calcification process in sea urchin larvae has been relatively well investigated (e.g., Nakano et al. 1963; Sikes et al. 1981; Wilt 2002; Raz et al. 2003; Politi et al. 2008) and primary mesenchyme cells (PMCs) were identified as the cell type mediating calcification in sea urchin larvae. These cells are located within the extracellular matrix of the primary body cavity and form a syncytium covering the spicules of the pluteus larvae. This syncytial sheath covers the entire surface of the spicules and communicates with the extracellular environment of the primary body cavity (Decker et al. 1987; Beniash et al. 1997). Recent studies using a pharmacological approach demonstrated that several loop diuretics including azosemide, bumetanide, and furosemide could significantly inhibit spicule formation at concentrations that significantly inhibit the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ symporter (NKCC) (Basse et al. 2015). Using histological analyses it has been concluded that the impaired formation of spicules was due to an inhibition of cytoplasmic cord formation and maintenance in PMCs.

The high-magnesium calcitic spicules are formed through the production of a transient amorphous calcium carbonate (ACC) phase within vesicles in PMCs which is subsequently released into the spicular cavity (Beniash et al. 1997; Raz et al. 2003; Politi et al. 2004, 2008). To fuel the calcification process, bicarbonate is absorbed from the seawater (40%) as well as generated from metabolic CO_2 (60%) (Sikes et al. 1981). On the other hand, Ca^{2+} is exclusively obtained directly from the seawater (Nakano et al. 1963). Although the general principle of calcification is well understood, mechanistic information regarding transporters that facilitate Ca^{2+} or HCO_3^- uptake in PMCs is still limited. Several pharmacological studies suggested that Ca^{2+} channels and pumps are key players in the provision of Ca^{2+} for spicule formation (Hwang and Lennarz 1993; Gunaratne and Vacquier 2007). In order to remove protons generated during CaCO_3 precipitation, PMCs must possess efficient acid–base regulatory properties (McConnaughey and Whelan 1996; Buitenhuis et al. 1999). A recent study applied the intracellular pH dye acid (2,7-bis(2-carboxyethyl)-5-(and-6)-carboxy fluorescein (BCECF)) in order to investigate pH regulatory mechanisms in sea urchin PMCs (Stumpp et al. 2012a). Using pulses of $\text{NH}_3/\text{NH}_4^+$ solution, it has been demonstrated that PMCs have significant pH_i regulatory abilities. PMCs have a control pH_i of 6.9 and 20 mmol L^{-1} $\text{NH}_3/\text{NH}_4^+$ solution induced an intracellular alkalinization leading to an increase of pH_i by approximately 0.7 units. Washout of the $\text{NH}_3/\text{NH}_4^+$ solution induced an acidosis of approximately 0.7 pH units below the control value, followed by a pH recovery to control levels within 15 min. Rates of pH_i recovery after the $\text{NH}_3/\text{NH}_4^+$ pulse were significant but weak compared to recovery times reported for strong ion regulatory cells of vertebrates (Boron and De Weer 1976; Bleich et al. 1995; Hasselblatt et al. 2000; Boron 2004) and similar to those reported for crayfish neurons (~20 min, (Moody 1981)). These experiments indicated that PMCs that are involved in skeletogenesis in sea urchins have a significant acid–base regulatory capacity.

The rate of pH compensation following an $\text{NH}_3/\text{NH}_4^+$ -induced acidosis was further investigated in the presence of low- Na^+ and low- HCO_3^- artificial seawater solutions in order to test the potential role of these ions in mediating pH_i . These experiments clearly indicated that the compensation reaction after $\text{NH}_3/\text{NH}_4^+$ -induced acidosis in PMCs is highly dependent on Na^+ and HCO_3^- transport and suggest the involvement of Na^+ -dependent HCO_3^- import mechanisms during pH recovery. These are an indirect sink of ATP mediated through secondary active transporters (e.g., transporters of the SLC4

family, including $\text{Na}^+/\text{HCO}_3^-$ cotransporters and Na^+ -dependent $\text{Cl}^-/\text{HCO}_3^-$ exchangers) that are driven through ATP-dependent ion pumps such as the Na^+/K^+ -ATPase or the V-type H^+ -ATPase (Boron 2004). The existence of a pH regulatory machinery in PMCs of sea urchins is underlined by the identification of genes coding for ion transporters including Na^+/K^+ -ATPase (NKA), $\text{Na}^+/\text{HCO}_3^-$ cotransporters (NBC), H^+ -ATPases (HA), and Na^+/H^+ -exchangers (NHE) from the sea urchin PMC transcriptome (Zhu et al. 2001). However, since protons are generated during the intracellular calcification process, the identification and characterization of proton secretion mechanisms involving transporters such as the V-type H^+ -ATPase or Na^+/H^+ exchangers represent an important future task to better understand the mechanisms of calcification in echinoid PMCs.

10.2.2 Extracellular Acid–Base Regulation

Primary Body Cavity

Microelectrode and microfluorescence measurements of extracellular pH (pH_e) within the primary body cavity of *S. droebachiensis* pluteus larvae revealed that the fluid of the primary body cavity pH conforms to the surrounding seawater (Stumpff et al. 2012a). Given that the fluid composition of the primary body cavity is similar to seawater, this indicates that the internal $p\text{CO}_2$ within the animals is similar to that of seawater $p\text{CO}_2$. Usually, extracellular $p\text{CO}_2$ of adult, heterotrophic metazoans is at least 100 Pa higher than the seawater $p\text{CO}_2$ in order to establish a CO_2 diffusion gradient away from the organism (Melzner et al. 2009). The result would imply that sea urchin larvae do not have an outward directed CO_2 diffusion gradient due to metabolic CO_2 production. During acute exposure of larvae to elevated seawater $p\text{CO}_2$, pH_e decreased linearly with pH_{SW} indicating that the epidermis is extremely leaky to ions and small molecules. A transepithelial KCl potential of 0 mV of the ectodermal epithelium also suggests that the epithelium is not a specialized transporting epithelium (Stumpff et al. 2012a). In fact, diffusion experiments using different sizes of BCECF-substituted dextrans demonstrated that the ectodermal epithelium forms no barrier for molecules up to 10 kDa if sufficient time for diffusion is allowed (60 min). Molecules up to 4 kDa penetrate the epidermis within 15 min (Stumpff et al. 2012a). Gases and small ions can therefore be expected to be in equilibrium with seawater leading to good conditions for calcification at present conditions, but on the other hand being sensitive to environmental variability in pH or future ocean acidification.

Stomach Lumen

Sea urchin larvae have a tripartite gut consisting of a foregut (esophagus), midgut (stomach), and a hindgut (intestine). Food particles (e.g., microalgae) are collected and concentrated in the esophagus and then swallowed by opening of the anterior cardiac sphincter. Food is processed in the stomach and absorbed in the stomach as well as the intestine. While the pH of the primary body cavity is conformed with seawater, the luminal pH_e in the stomach of *S. droebachiensis* increased after feeding commenced from pH_e 8.9 at 5 days post fertilization to pH_e 9.5 at 9 days post fertilization (Stumpff et al. 2013) and up to pH 9.0 in *S. purpuratus* (Stumpff et al. 2015). Alkaline gastric pHs are known to occur in a range of insects and their larvae such as lepidopterans (Dow 1984) and dipteran larvae (Boudko et al. 2001). Among marine species alkaline digestive conditions were additionally found in the marine copepod *Calanus helgolandicus* (Pond et al. 1995) as well

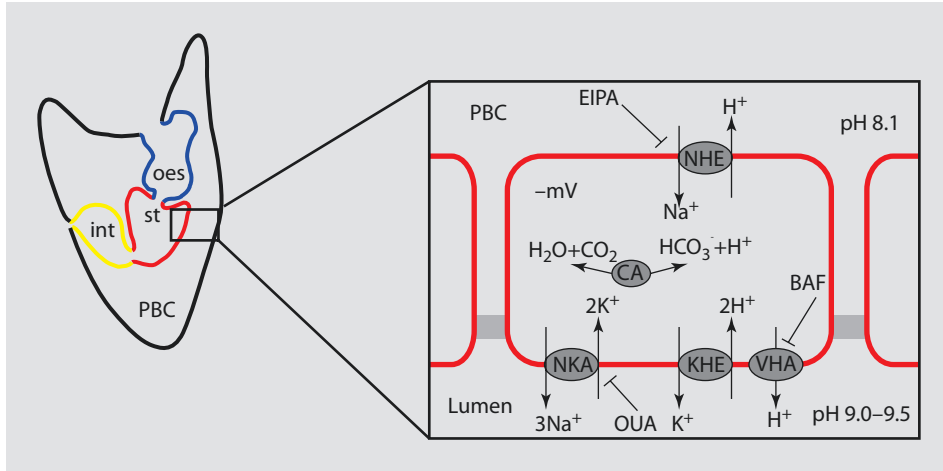
as in tornaria larvae of the hemichordate *Ptychodera flava* – reaching a pH of 10.5 in their stomach lumen (Stumpp et al. 2015). Alkaline digestive conditions may represent an evolutionary advantage for marine herbivorous larvae as alkaline conditions may facilitate breakdown of plant and algal proteins (Felton and Duffey 1991). Moreover, alkaline conditions higher than pH 9.5 in larval digestive systems can be regarded as an efficient defense mechanism against environmental pathogens because most marine protists and viruses are killed or growth inhibited by alkaline conditions exceeding pH 9.5 (Pedersen and Hansen 2003; Jończyk et al. 2011). However, these unique digestive features were proposed to be a key process that makes these larvae sensitive to climate change and the associated reductions in seawater pH (ocean acidification) by impairing digestion and exerting additional energetic costs to the larvae in future oceans (Stumpp et al. 2013) (for details, see next paragraph).

Chronic exposure of *S. droebachiensis* larvae to elevated $p\text{CO}_2$ resulted in a lower stomach lumen pH_c at 7 and 9 days post fertilization in two CO_2 treatments (1015 and 2460 $\mu\text{atm } p\text{CO}_2$). The data suggest that the environmental CO_2 induced acidification is exceeding the larval capacity of fully alkalize the stomach lumen to control levels. The drop in stomach pH_c of 0.25 and 0.4 pH units at the high $p\text{CO}_2$ level (2460 μatm at 7 and 9 dpf, respectively) is, however, much smaller than the environmental pH_{SW} drop of 0.67 pH units, possibly indicating an activation of compensatory mechanisms. Acute acidification experiments down to pH 7 in *S. purpuratus* resulted in a partial compensation reaction of the gastric pH within 3 h. This compensation reaction was likely mediated by cells of the stomach epithelium.

In contrast to the thin squamous epidermal cell layers, the echinopluteus' stomach epithelium consists of simple columnar cells (of 10–15 μm height) with microvilli extending into the stomach lumen (Burke 1981). At least two cell types are present in the stomach epithelium. Type I cells are characterized by a variety of different vacuoles and are expected to function in absorption and storage of nutrients as well as the secretion of digestive enzymes. Type II cells are assumed to phagocytose and digest whole algal cells (Burke 1981).

Immunohistochemical and in situ hybridization using antibodies and probes against specific ion transporters could demonstrate that ion pumps and transporters including Na^+/K^+ -ATPase (NKA) and V-type H^+ -ATPase (VHA) are highly expressed in specialized epidermal cells of the stomach and intestine. The colocalization of NKA, VHA, and Na^+H^+ exchangers revealed the subcellular localization of these proteins in stomach cells. While NKA and VHA are localized in the luminal membrane positive Na^+/H^+ exchanger (NHE), immunoreactivity was mainly found in the basolateral membrane (Stumpp et al. 2015). The mechanistic basis of gastric alkalization was further examined using H^+ -selective microelectrodes in combination with specific inhibitors for acid–base relevant ion transporters. These studies underlined the histological findings by demonstrating that gastric alkalization in sea urchin larvae is inhibited by ouabain and bafilomycin, specific inhibitors for the Na^+/K^+ -ATPase (NKA) and the V-type H^+ -ATPase (VHA), respectively. In addition, gastric alkalization was decreased by treatments with ethyl-isopropyl amiloride (EIPA, a specific inhibitor of Na^+ -dependent H^+ exchangers (NHEs)) as well as artificial seawater with reduced Na^+ (5 mmol L^{-1}) and K^+ (0 mmol L^{-1}) concentrations.

Gene expression analyses of genes encoding acid–base relevant transporters in pluteus upon challenge of the gastric pH regulatory machinery with acidified seawater identified genes coding for the transporters mediating gastric alkalization. During acute (1–3 h) challenge of the gastric pH regulatory machinery by exposing larvae to acidified conditions



■ **Fig. 10.2** First working model for gastric alkalization in sea urchin pluteus larvae. The stomach epithelium is rich in ion transporters that are capable of regulating gastric pH. The current model suggests that gastric alkalization is achieved by a basolateral (facing the primary body cavity, PBC) NHE that is fueled by a Na^+/K^+ -ATPase located in the luminal membrane. Protons that are excreted via NHE derive from the hydration of metabolic CO_2 via cytosolic carbonic anhydrases (CA) as well as from a luminal K^+/H^+ antiporter mechanism energized by luminal V-type H^+ -ATPase (VHA) (modified after Stumpp et al. 2015).

(pH 7.0), ion transport genes, including NKA, VHA, NHE3, and a KHE, were significantly up-regulated when compared to larvae kept under control conditions. Accordingly a first working model for gastric alkalization in sea urchin larvae has been proposed (Stumpp et al. 2015). Gastric alkalization in the sea urchin stomach is mainly generated through cationic exchange processes, leading to a net export of protons from the stomach lumen (summarized in ■ Fig. 10.2). NKA located in luminal membranes creates an electrochemical gradient, and a low intracellular $[\text{Na}^+]$ that drives basolateral excretion of H^+ via NHE in the primary body cavity (PBC). On the luminal side, H^+ is imported via a coupling of VHA and a putative electrogenic K^+/H^+ antiporter. A similar cationic-based alkalization mechanism driven by the VHA coupled to secondary active transport processes including K^+/H^+ antiport has been proposed for the midgut alkalization in lepidopteran insect larvae (Wieczorek et al. 1991). The employment of NHE localized in membranes facing the PBC that mediate excretion of H^+ in exchange for Na^+ represents an energetically favorable mechanism due to high external $[\text{Na}^+]$ ($\approx 460 \text{ mmol L}^{-1}$) compared to low intracellular $[\text{Na}^+]$ ($\approx 30 \text{ mmol L}^{-1}$) (Robertson 1949).

10.3 Effects of Near-Future Ocean Acidification on Echinoderms

Effects of simulated ocean acidification experiments on adult echinoderms generally included reduced growth and feeding rates, decreased fertility (measured as number of eggs per female or gonad growth), and occasionally reduced metabolic rates (Appelhans et al. 2012; Dupont and Thorndyke 2012; Stumpp et al. 2012b). This most likely leads to alterations in the energy budget that may be due to elevated costs for cellular and extracellular pH homeostasis as described in sea urchins and brittle stars. However, most studies employed experimental time frames between a few days and a few months, possibly

not allowing enough time for the animals to acclimate to the future scenario. For example, Dupont and colleagues demonstrated in one of the few long-term studies that the green sea urchin *S. droebachiensis* was able to acclimate to elevated $p\text{CO}_2$ conditions resulting in comparable fertility after 16 months. In contrast, animals that were acclimated for only 4 months displayed significantly reduced fertility (Dupont et al. 2012). Thus, in order to understand how ocean acidification will affect echinoderms in general, long-term studies of several months to years would yield valuable results in regard to acclimation and possibly adaptation potential.

Apart from a few exceptions with nonfeeding echinoderm larvae, i.e., *Crossaster papposus*, which displayed increased growth rates in response to seawater acidification (Dupont et al. 2010b), exposure of echinoderm larvae to elevated $p\text{CO}_2$ resulted in reduced growth rates (Dupont et al. 2010a) and in some cases high mortalities (Dupont et al. 2008). However, most studies were conducted on sea urchin larvae, since pluteus larvae with their calcified endoskeleton were readily identified as being especially sensitive to seawater acidification. Only two studies looked at noncalcifying, feeding marine larvae such as bipinnaria larvae of sea stars. Interestingly, despite the lack of calcified structures, exposure of sea star larvae to environmentally realistic increases in CO_2 levels could also demonstrate slowed larval growth (Gonzalez-Bernat et al. 2012; Byrne et al. 2013). This clearly indicates that other physiological processes than calcification may cause sensitivities to seawater acidification in marine larval stages.

Based on the findings of alkaline digestive systems in sea urchin larvae (and also hemichordate larvae (Stumpp et al. 2015)) and their sensitivity to lowered pH (Stumpp et al. 2013), the hypothesis of gastric pH homeostasis being a dominant factor determining sensitivity in echinoderm larvae arises. Apart from potential elevated costs for intracellular and/or extracellular pH regulation itself (Stumpp et al. 2012a, 2015), a lowered stomach pH might have negative consequences for larval energy acquisition. Digestive enzymes secreted into the gut to hydrolyze nutrients usually exhibit specific pH optima at which the enzymes are most efficient. Sea urchin larval total protease activity exhibited a pH optimum at pH 11 with a steady increase in activity from pH 8. A potential digestive pH drop of 0.3 pH units would thus translate into 8% less digestive efficiency. The digestive potential to digest algal cells measured in *in vivo* digestion assays supports the *in vitro* total protease measurements by demonstrating a significantly lower digestive potential in sea urchin larvae raised under future elevated $p\text{CO}_2$ conditions (Stumpp et al. 2013). Decreased digestive potential together with increased acid–base regulatory costs would inevitably result in modifications of the larval energy budget and could potentially explain the observed reduced growth rates in sea urchin plutei, as well as in noncalcifying but feeding echinoderm larvae, such as sea star larvae.

10.4 Conclusions

The present body of knowledge regarding pH regulatory abilities in echinoderms demonstrated that despite their relatively basal position among deuterostomes, these animals have evolved significant pH regulatory abilities. Although echinoderms were demonstrated to exhibit extracellular pH regulatory abilities, the organs and mechanisms involved remain unexplored. Few studies indicated that the pH compensatory mechanism involves accumulation of buffers (e.g., HCO_3^-) as well as the excretion of proton equivalents such as NH_4^+ . Although extracellular pH regulatory abilities were found in a range of

adult echinoids, their larval stages lack extracellular pH regulatory abilities. However, the regulation of an alkaline gastric pH homeostasis seems to play an important role in digestive function, which could be identified as an “Achilles’ heel” in the context of CO₂-induced seawater acidification.

The fact that echinoderms are an evolutionary ancient group, that has also survived acidification events during geological timescales, suggests that this animal group has the ability to successfully cope with environmental pH/pCO₂ fluctuations. In this context special attention should be dedicated to the ability of these animals to acclimate and to genetically or epigenetically adapt to changing environmental conditions. As larval stages were identified as the weakest ontogenetic link, these pelagic life stages deserve special attention. Thus, in a first step, a better understanding regarding the physiological processes in echinoderm larval stages are needed in order to better understand animal–environment interactions in this ecologically and economically important group.

Bibliography

- Appelhans JS, Thomsen J, Pansch C, Melzner F, Wahl M (2012) Sour times: seawater acidification effects on growth, feeding behaviour and acid-base status of *Asterias rubens* and *Carcinus maenas*. *Mar Ecol Prog Ser* 459:85–97
- Basse WC, Gutowska MA, Findeisen U, Stumpp M, Dupont ST, Jackson DJ, Himmerkus N, Melzner F, Bleich M (2015) A sea urchin Na⁺K⁺ 2Cl cotransporter is involved in the maintenance of calcification-relevant cytoplasmic cords in *Strongylocentrotus droebachiensis* larvae. *Comp Biochem Physiol A Mol Integr Physiol* 187:184–192
- Beniash E, Aizenberg J, Addadi L, Weiner S (1997) Amorphous calcium carbonate transforms into calcite during sea urchin larval spicule growth. *Proc R Soc Lond B* 264:461–465
- Bleich M, Köttgen M, Schlatter E, Greger R (1995) Effect of NH₄⁺/NH₃ on cytosolic pH and the K⁺ channels of freshly isolated cells from the thick ascending limb of Henle’s loop. *Pflügers Arch* 429:345–354
- Boron WF (2004) Regulation of intracellular pH. *Adv Physiol Educ* 28:160–179
- Boron WF, De Weer P (1976) Intracellular pH transients in squid giant axons caused by CO₂, NH₃, and metabolic inhibitors. *J Gen Physiol* 67:91–112
- Boudko DY, Moroz LL, Harvey WR, Linser PJ (2001) Alkalinization by chloride/bicarbonate pathway in larval mosquito midgut. *Proc Natl Acad Sci U S A* 98:15354–15359
- Buitenhuis ET, de Baar HJW, Veldhuis MJW (1999) Photosynthesis and calcification by *Emiliania huxleyi* (Prymnesiophyceae) as a function of inorganic carbon species. *J Phycol* 35:949–959
- Burke DR (1981) Structure of the digestive tract of pluteus larva of *Dendroaster excentricus* (Echinodermata: Echinoidea). *Zoomorph* 98:209–225
- Byrne M, Gonzalez-Bernat M, Doo S, Foo S, Soars N, Lamare M (2013) Effects of ocean warming and acidification on embryos and non-calcifying larvae of the invasive sea star *Patiriella regularis*. *Mar Ecol Prog Ser* 473:235–246
- Calosi P, Rastrick SPS, Graziano M, Thomas SC, Baggini C, Carter HA, Hall-Spencer JM, Milazzo M, Spicer JI (2013) Distribution of sea urchins living near shallow water CO₂ vents is dependent upon species acid-base and ion-regulatory abilities. *Mar Poll Bull* 30:470–484
- Catarino A, Bauwens M, Dubois P (2012) Acid-base balance and metabolic response of the sea urchin *Paracentrotus lividus* to different seawater pH and temperatures. *Environ Sci Poll Res* 19:2344–2355
- Collard M, Eeckhaut I, Dehairs F, Dubois P (2014a) Acid-base physiology response to ocean acidification of two ecologically and economically important holothuroids from contrasting habitats, *Holothuria scabra* and *Holothuria parva*. *Environ Sci Poll Res* 21:13602–13614
- Collard M, Srey A, Dehairs F, Dubois P (2014b) Euechinoidea and Cidarzoidea respond differently to ocean acidification. *Comp Biochem Physiol A* 174:45–55
- Crawford BJ (1990) Changes in the arrangement of the extracellular matrix, larval shape, and mesenchyme cell migration during asteroid larval development. *J Morphol* 206:147–161

- Decker GL, Morrill JKB, Lennarz WJ (1987) Characterization of sea urchin primary mesenchyme cells and spicules during biomineralization, in vitro. *Development* 101:297–312
- Dow JA (1984) Extremely high pH in biological systems: a model for carbonate transport. *Am J Physiol* 246:R633–R636
- Dupont S, Ortega-Martinez O, Thorndyke MC (2010a) Impact of near-future ocean acidification on echinoderms. *Ecotoxicology* 19:449–462
- Dupont ST, Dorey N, Stumpp M, Melzner F, Thorndyke MC (2012) Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. *Mar Biol*. Volume 160, Issue 8, pp 1835–1843. doi: [10.1007/s00227-012-1921-x](https://doi.org/10.1007/s00227-012-1921-x)
- Dupont ST, Havenhand JN, Thorndyke W, Peck L, Thorndyke MC (2008) Near-future level of CO₂-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *MEPS*, Volume 373, pp285–294
- Dupont ST, Lundve B, Thorndyke MC (2010b) Near future ocean acidification increases growth rate of the lecithotrophic larvae and juveniles of the sea star *Crossaster papposus*. *J Exp Zool* 314B:1–8
- Dupont ST, Thorndyke MC (2012) Relationship between CO₂-driven changes in extracellular acid-base balance and cellular immune response in two polar echinoderm species. *J Exp Mar Biol Ecol* 424–425:32–37
- Felton GW, Duffey SS (1991) Reassessment of the role of gut alkalinity and detergency in insect herbivory. *J Chem Ecol* 17:1821–1836
- Gonzalez-Bernat MJ, Lamare M, Barker M (2012) Effects of reduced seawater pH on fertilisation, embryogenesis and larval development in the Antarctic seastar *Odontaster validus*. *Polar Biol* 36:235–247
- Gunaratne HJ, Vacquier VD (2007) Sequence, annotation and developmental expression of the sea urchin Ca²⁺-ATPase family. *Gene* 397:67–75
- Hasselblatt P, Warth R, Schulz-Baldes A, Greger R, Bleich M (2000) pH regulation in isolated in vitro perfused rat colonic crypts. *Pflugers Arch* 441:118–124
- Holtmann WC, Stumpp M, Gutowska MA, Syré S, Himmerkus N, Melzner F, Bleich M (2013) Maintenance of coelomic fluid pH in sea urchins exposed to elevated CO₂: the role of body cavity epithelia and stereom dissolution. *Mar Biol* 160:2631–2645
- Hu MY, Casties I, Stumpp M, Ortega-Martinez O, Dupont ST (2014) Energy metabolism and regeneration are impaired by seawater acidification in the infaunal brittlestar *Amphiura filiformis*. *J Exp Biol* 217:2411–2421
- Hwang SP, Lennarz WJ (1993) Studies on the cellular pathway involved in assembly of the embryonic sea urchin spicule. *Exp Cell Res* 205:383–387
- Jończyk E, Klak M, Międzybrodzi R, Górski A (2011) The influence of external factors on bacteriophages—review. *Folia Microbiol* 56:191–200
- Martin S, Richier S, Pedrotti M-L, Dupont S, Castejon C, Gerakis Y, Kerros M-E, Oberhänsli F, Teyssié J-L, Jeffree R, Gattuso J-P (2011) Early development and molecular plasticity in the Mediterranean sea urchin *Paracentrotus lividus* exposed to CO₂-driven acidification. *J Exp Biol* 214:1357–1368
- McConnaughey TA, Whelan JF (1996) Calcification generates protons for nutrient and bicarbonate uptake. *Earth Sci Rev* 41:95–117
- Melzner F, Gutowska MA, Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, Bleich M, Pörtner HO (2009) Physiological basis for high CO₂ tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny. *Biogeosci* 6:2313–2331
- Miles H, Widdicombe S, Spicer JI, Hall-Spencer JM (2007) Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin *Psammechinus miliaris*. *Mar Poll Bull* 54:89–96
- Moody WJ (1981) The ionic mechanism of intracellular pH regulation in crayfish neurones. *J Physiol* 316:293–308
- Nakano E, Okazaki K, Iwamatsu T (1963) Accumulation of radioactive calcium in the larvae of the sea urchin *Pseudocentrotus depressus*. *Biol Bull* 125:125–136
- Pedersen MF, Hansen PJ (2003) Effects of high pH on the growth and survival of six marine heterotrophic protists. *Mar Ecol Prog Ser* 260:33–41
- Pennington JT, Strathmann RR (1990) Consequences of the calcite skeletons of planktonic echinoderm larvae for orientation, swimming, and shape. *Biol Bull* 179:121–133
- Politi Y, Arad T, Klein E, Weiner S, Addadi L (2004) Sea urchin spine calcite forms via a transient amorphous calcium carbonate phase. *Science* 306:1161–1164
- Politi Y, Metzler RA, Abrecht M, Gilbert B, Wilt FH, Sagi I, Addadi L, Weiner S, Gilbert PUPA (2008) Transformation mechanisms of amorphous calcium carbonate into calcite in the sea urchin larval spicule. *Proc Natl Acad Sci U S A* 105:17362–17366

- Pond DW, Harris RP, Brownlee C (1995) A microinjection technique using a pH-sensitive dye to determine the gut pH of *Calanus helgolandicus*. *Mar Biol* 123:75–79
- Raz S, Hamilton PC, Wilt FH, Weiner S, Addadi L (2003) The transient phase of amorphous calcium carbonate in sea urchin larval spicules: the involvement of proteins and magnesium ions in its formation and stabilization. *Adv Funct Mater* 13:480–486
- Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO₂-induced ocean acidification. *Geology* 37:1131–1134
- Robertson JD (1949) Ionic regulation in some marine invertebrates. *J Exp Biol* 26:182–200
- Sikes CS, Okazaki K, Fink RD (1981) Respiratory CO₂ and the supply of inorganic carbon for calcification of sea urchin embryos. *Comp Biochem Physiol A* 70:285–291
- Strathmann RR (1989) Existence and functions of a gel filled primary body cavity in development of echinoderms and hemichordates. *Biol Bull* 176:25–31
- Stumpp M, Hu MY, Casties I, Saborowski R, Bleich M, Melzner F, Dupont S (2013) Digestion in sea urchin larvae impaired under ocean acidification. *Nat Climate Change* 3:1044–1049
- Stumpp M, Hu MY, Melzner F, Gutowska MA, Dorey N, Himmerkus N, Holtmann WC, Dupont ST, Thorndyke MC, Bleich M (2012a) Acidified seawater impacts sea urchin larvae pH regulatory systems relevant for calcification. *Proc Natl Acad Sci U S A* 109:18192–18197
- Stumpp M, Hu MY, Tseng Y-C, Guh YJ, Chen YC, Yu JK, Su YH, Hwang PP (2015) Evolution of extreme stomach pH in bilateria inferred from gastric alkalization mechanisms in basal deuterostomes. *Sci Rep* 5:1–9
- Stumpp M, Trübenbach K, Brennecke D, Hu MY, Melzner F (2012b) Resource allocation and extracellular acid-base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO₂ induced seawater acidification. *Aquat Toxicol* 110–111:194–207
- Wieczorek H, Putzenlechner M, Zeiske W, Klein U (1991) A vacuolar-type proton pump energizes K⁺/H⁺ antiport in an animal plasma membrane. *J Biol Chem* 266:15340–15347
- Wilt FH (2002) Biomineralization of the spicules of sea urchin embryos. *Zool Sci* 19:253–261
- Zhu X, Mahairas G, Illies M, Cameron RA, Davidson EH, Etensohn CA (2001) A large-scale analysis of mRNAs expressed by primary mesenchyme cells of the sea urchin embryo. *Development* 128:2615–2627