Acid–Base Regulation in Aquatic Decapod Crustaceans

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6.1   Summary

Aquatic decapod crustaceans live in a highly variable and constantly changing environment, permanently challenging their physiological homeostasis. One of the key processes considered ensuring physiological performance and function is the maintenance of acid–base balance. This chapter aims to provide a comprehensive overview of the challenges for aquatic decapod crustaceans' acid–base homeostasis, as well as the current knowledge regarding the respective mechanisms for acid–base regulation. Like many other marine organisms including fish and cephalopods, aquatic decapod crustaceans are capable of counteracting an acidosis or alkalosis of their bodily fluids mainly by modulating their hemolymph bicarbonate levels in order to buffer the pH. In addition, they adjust the excretion of acid and/or base equivalents, respectively. It is evident that ion transport mechanisms at the level of the gill epithelium contribute substantially to these acid–base regulatory processes, including the modulation of gene (mRNA) expression levels of distinct gill epithelial transcripts like carbonic anhydrase, Rhesus-like protein, Na^+/K^+-ATPase, V-(H^+)-ATPase and Cl^-/HCO_3^--exchanger. As a result of recently generated data mainly from gill perfusion experiments, a novel hypothetical working model for branchial acid–base regulation is put forward. It ties in general ion as well as ammonia regulatory mechanisms and accounts for the obvious linkage between these three processes.

6.2   The Importance of Acid–Base Homeostasis in Aquatic Decapod Crustaceans

Maintaining acid–base balance is fundamental for all living organisms, including decapod crustaceans (Henry and Wheatly 1992). Only slight disturbances in the concentration of acid–base equivalents resulting in shifts of pH in the intra- or extracellular fluids may impair properties of essential proteins and their regulation (i.e. enzymes, Somero 1986; respiratory proteins, Riggs 1988; Truchot 1975a) and ultimately lead to a disruption of basic physiological functions. Consequently, securing whole animal acid–base homeostasis not only includes the maintenance of a constant intra- and extracellular pH but also needs mechanisms in place for its re-adjustments after an acid–base disturbance. Factors to disrupt acid–base homeostasis in decapod crustaceans might include a variety of intrinsic as well extrinsic parameters like the internal acid load due to exercise (e.g. Booth et al. 1984; Rose et al. 1998), shifts in $CaCO_3$ handling during the moulting process (e.g. Mangum et al. 1985) and fluctuations of environmental parameters like salinity (e.g. Whiteley et al. 2001), temperature (e.g. McMahon et al. 1978; Whiteley and Taylor 1990), pO_2/pCO_2 (e.g. De Fur et al. 1983; Urbina et al. 2013) and ammonia (e.g. Cheng et al. 2013; Martin et al. 2011). The regulation of acid–base balance in aquatic decapod crustaceans therefore is a complex interaction of physiological and biochemical processes including respiratory gas exchange, ion regulation and overall adjustments of metabolism (Henry and Wheatly 1992).

Most studies on acid–base regulation in decapod crustaceans to date concentrate on whole animal extracellular acid–base status and its responses upon disturbances (▶ see Sect. 6.3). Few studies investigated acid–base regulation on a cellular level, and therefore, little is known about direct trans-branchial transport of acid–base equivalents in decapod crustaceans (▶ see Sect. 6.4). While most available acid–base related data has been collected on brachyuran hyper-regulating crabs (e.g. the green crab *Carcinus maenas*, the
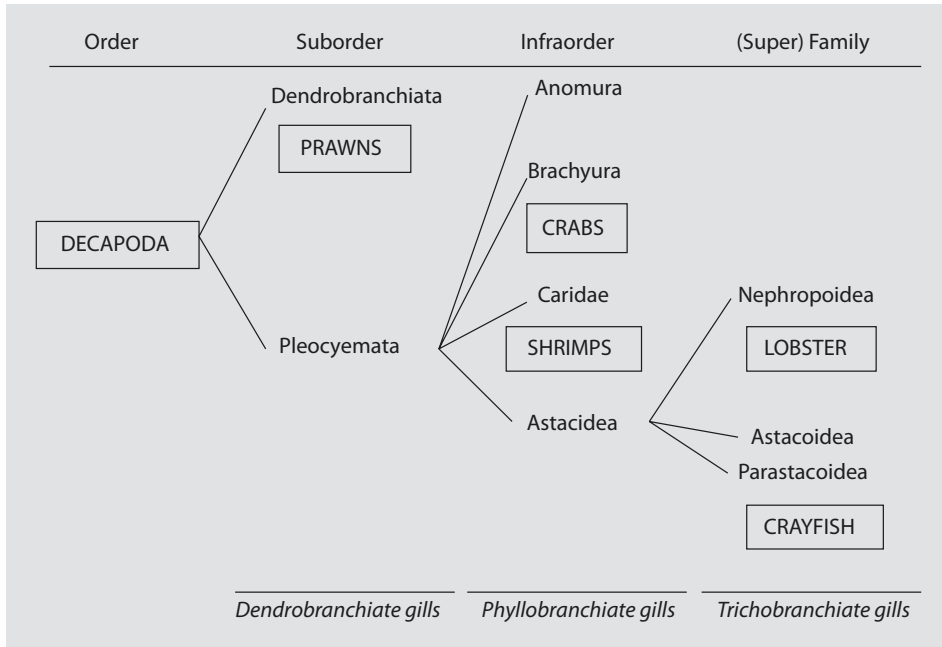
■ **Fig. 6.1** Overview of the different subgroups of decapod crustaceans discussed in this chapter. The nomenclature follows De Grave et al. (2009). To avoid confusion, members of the suborder Dendrobranchiata are referred to as "prawns" throughout the text, while "shrimps" solely refers to the infraorder Caridae

blue crab *Callinectes sapidus* and the Chinese mitten crab *Eriocheir sinensis*), this chapter aims to provide a broader review of the currently available literature on acid–base regulation also in the other major members of decapod crustaceans, namely, prawns (in this chapter referred to as synonym for penaeoid and sergestoid shrimp), Anomura, shrimp (caridean shrimp), lobster and crayfish (De Grave et al. 2009; ■ Fig. 6.1).

6.2.1 Tissues Involved in Acid–Base Regulation

In crustaceans, anisosmotic extracellular regulation (AER), or the osmotic and ionic buffering of the extracellular fluid in order to maintain (acid–base) homeostasis, is believed to be mainly driven by the gills, antennal glands and gut (McNamara and Faria 2012). A tissue-specific inventory of epithelial membrane transporters then translates the changes of extracellular adjustments into the cell to ensure the intracellular maintenance of acid–base balance (Freire et al. 2008).

■■ **Gills**
Similar to fish and cephalopods (▶ see Chap. 11), the majority of the acid–base-relevant ion regulatory apparatus of decapod crustaceans is located in their gill epithelia (Henry et al. 2012; Larsen et al. 2014, and references therein). Not only are the gills involved in respiratory and acid–base physiology, but they are the major organs also for ion regulation

and ammonia excretion, therefore linking all of these regulatory processes (Freire et al. 2008; Henry et al. 2012).

All decapod crustaceans possess paired gills that are covered by a fine chitinous cuticle, lined by a single-layered epithelium and attached to a basal lamina (Freire et al. 2008). Depending on taxa, the number of paired gills, their location of attachment and the arrangement of the gill lamellae (phyllobranchiate, trichobranchiate, dendrobranchiate, as indicated in ◨ Fig. 6.1) vary substantially, providing more or less gill surface amplification for ion and gas exchange processes between the external (environment) and the internal medium (hemolymph). For further details, the reader is referred to the extensive descriptions by Taylor and Taylor (1992).

According to their different life strategies (i.e. primary habitat/habitat changes), gill epithelia of decapod crustaceans exhibit specific characteristics that can vary even within the respective sub-/infraorder or (super) family. Acid–base status and regulation in decapod crustaceans have been shown to be linked to external salinity and NaCl regulation (i.e. in the freshwater crayfish *Astacus leptodactylus* (Ehrenfeld 1974), *C. sapidus* (Henry and Cameron 1982) and *E. sinensis* (Whiteley et al. 2001), and therefore the tightness of the gill epithelium consequently might also affect the animals' capability for acid–base regulation. While the gill epithelia of strong hyper-regulators like *E. sinensis* (Weihrauch et al. 1999) and freshwater crayfish (Wheatly and Gannon 1995) represent a tight epithelium (conductance for ions <5 mS cm^{-2}), the epithelium of weak hyper-regulators like *C. maenas* (Weihrauch et al. 1999) and the American lobster *Homarus americanus* (Lucu and Towle 2010) is much leakier and allows for increased intercellular transport of ions (conductance 40–60 mS cm^{-2}). Gills of osmoconforming crustaceans like *M. magister* (Hunter and Rudy 1975) or *Cancer pagurus* (Weihrauch et al. 1999) in contrast are highly permeable for ions (conductance > 200 mS cm^{-2}), and therefore these species are very limited in their capability to osmoregulate (Larsen et al. 2014).

Furthermore, specializations of gill epithelia can be seen at the ultrastructural level. Of the five different cell types found in decapod crustacean gill epithelia (thin cells, thick cells, flange cells, attenuated cells and pilaster cells; Freire et al. 2008), thin cells are generally believed to be associated with respiratory epithelia due to their low height (1–5 µM), a lack of extensive membrane folding and low number of mitochondria. Consequently, they have been considered to play an increased role in acid–base regulation rather than osmoregulation (Freire et al. 2008). Thin cells are found in all gills of osmoconforming crabs as well as the most anterior four to six pairs of gills of hyper-regulating crabs like *C. maenas* (Compere et al. 1989), *C. sapidus* (Copeland and Fitzjarrell 1968) and *E. sinensis* (Barra et al. 1983). Some thin cells were also observed in the gill epithelium of lobsters (Haond et al. 1998). In some hyper-regulating crabs like *C. maenas* (Compere et al. 1989) and *C. sapidus* (Copeland and Fitzjarrell 1968), thin cells are found to surround thick cells (also called ionocytes due to their supposed major role in ion transport) in the most posterior (osmoregulatory) pairs of gills, therefore indicating that acid–base regulatory properties might not be solely associated with the anterior gills in these species. To date, however, the direct site for acid–base regulation in euryhaline Brachyuran gills has not been confirmed, while osmoregulation has been demonstrated to be associated predominantly with the posterior gills (Henry et al. 2012) and ammonia with both anterior and posterior gills (Weihrauch et al. 1999; ► see Chap. 1).

The gill epithelia of lobsters (Haond et al. 1998), prawns (Bouaricha et al. 1994), shrimp (Freire and McNamara 1995) and freshwater crayfish (Morse et al. 1970) on the other hand are more homogeneous and possess so-called flange cells that exhibit features of both thin and thick cells of crabs and are therefore believed to incorporate both respiratory/acid–base and ion regulatory functions (Freire et al. 2008).

Even though most pilaster cells (resembling thin or thick cell criteria depending on their localization in the gill, Compere et al. 1989) in the epithelia of crabs and crayfish seem to inherit a mainly stabilizing function for the intra-lamellar septum, they are the exclusive sites for the vacuolar-type H^+-ATPase (V-(H^+)-ATPase) in *E. sinensis*, indicating an additional role for these cells in acid–base regulation in this species (Freire et al. 2008).

■■ Antennal Glands

Situated at the anterior end of the body at the base of the eyestalks, the paired antennal glands are mainly involved in the production (ultrafiltration) and ionic regulation of the urine to maintain extracellular water balance (Larsen et al. 2014). Therefore, they can be regarded as analogues of the nephron of the vertebrate kidney, the major acid–base regulatory organ in mammals and other terrestrial vertebrates (Weiner and Verlander 2013). Even though urine $[Na^+]$, $[K^+]$ and $[Cl^-]$ are adjusted upon disturbance of (acid–base) homeostasis, antennal glands are believed to rather contribute to the regulation of divalent cations like Ca^{2+} and Mg^{2+} based on the respective clearance ratios (Wheatly 1985).

Only a few studies have investigated the role of antennal glands in acid–base regulation in decapod crustaceans. In freshwater-acclimated blue crabs *C. sapidus*, net urinary acid–base and ammonia efflux were negligible and did not change significantly when animals were exposed to hypercapnia (2 % pCO$_2$; Cameron and Batterton 1978a). In the Dungeness crab *Metacarcinus magister* acclimated to dilute salinity (~20 ppt), the increase in antennal gland-mediated HCO_3^- efflux resulted in an increased alkalinization of the urine, but was accompanied by an increase in HCO_3^- reabsorption over time, likely to assist in HCO_3^- accumulation in the hemolymph (Wheatly 1985). However, in respect to the overall base output in response to dilute salinity acclimation, the antennal gland of *M. magister* contributed to only 10 % at best (Wheatly 1985). Also in the freshwater-acclimated euryhaline crayfish *Pacifastacus leniusculus* exposed to hyperoxia, an initial extracellular acidosis resulted in an increase in HCO_3^- reabsorption from the urine to buffer hemolymph pH, but in parallel, an acidification of the urine was observed mainly due to increased ammonia (NH_4^+) excretion (Wheatly and Toop 1989). Similar to the observations of hyposaline-acclimated *M. magister*, however, net H^+ efflux accounted for only 10 % of the whole animal response in this crayfish. Interestingly, antennal glands of *P. leniusculus* show a significantly higher activity of carbonic anhydrase (CA), the enzyme involved in the hydration of CO_2 to form H_2CO_3 and subsequently dissociate to H^+ and HCO_3^-, compared to the gills (Wheatly and Henry 1987). When acclimated to hypersaline conditions, however, CA activity was progressively reduced with increased salinity (up to 80 % at ~25 ppt).

In conclusion, the existing data suggest an overall negligible involvement of antennal glands in acid–base regulation in decapod crustaceans.

■■ Gut and Gut Diverticula

Besides the respective adjustment of urine flow, gut-mediated fluid absorption and secretion of digestive fluid have been shown to be ion dependent in both hypo- and hyper-regulating crustaceans and likely help in the regulation of the hemolymph composition (Mantel and Farmer 1983). Accordingly, crustacean gut epithelia have been shown to possess Na^+/K^+-ATPase that in addition to their function in ion regulation might also promote the uptake of nutrients (Chu 1987; Chung and Lin 2006; Mantel and Farmer 1983). Even though being directly exposed to the environment and showing evidence for the capability to take up/excrete small ions like Na^+, Cl^-, K^+, Ca^{2+} and SO_4^- (Ahearn 1978;

Mantel and Farmer 1983), a potential role of the gut in acid–base regulation has not been investigated to date.

In addition to the gut, the presence of an electrogenic, likely apically situated $2Na^+:1H^+$-exchanger in the hepatopancreas of lobster and freshwater prawns (Ahearn et al. 1990, 1994) would provide an important key player for acid–base regulation in this tissue. Similar as for the gut, however, a direct involvement for the hepatopancreas in maintaining acid–base homeostasis has not been investigated to date. Clearly, future studies need to be performed in order to characterize the potential roles of gut and hepatopancreas in crustacean acid–base regulation.

6.2.2   Preadaptation Through Life Strategies?

The estimated 14,000 species of aquatic decapod crustaceans can be found in nearly all water bodies of the world. As described earlier, the capability of inhabiting specific water bodies mainly depends on the crustaceans' regulatory capacity due to the characteristics of the gill epithelium as well as the general permeability of their carapace. Acid–base regulatory capabilities are therefore likely correlated with life history, genetic predisposition and physiological plasticity (Melzner et al. 2009). As metazoans with a relatively high metabolic rate and level of activity, a high capacity to adjust body fluid pH (also dependent on the relatively large fluid volume) and relatively little expressed calcified structures compared to other marine calcifiers like corals, echinoderms and molluscs (decapod), crustaceans are believed to cope better with changes in their environment than other marine invertebrates (Wittmann and Pörtner 2013).

The following sections are intended to roughly characterize the basic life strategies for key species of the major decapod crustacean groups that will be discussed in more detail concerning their acid–base regulatory capabilities in the subsequent chapters.

■ ■ Prawns
While most Dendrobranchiata (Penaeidae) are euryhaline hyper-/hypo-osmoregulators and found in the marine environment (e.g. *(Marsu)Penaeus japonicus* (Cheng et al. 2013); *(Lito)Penaeus vannamei* (Liu et al. 2015)), some species of Sergestidae are found in freshwater. Many species inhabit deep (offshore) waters, while most Penaeidae are mainly found in shallow inshore tropical and subtropical waters and estuaries. Some prawn species are known to bury in mud substrates during the daytime (Tavares and Martin 2010), challenging their acid–base homeostasis as described below.

■ ■ Anomura
While king crabs (Lithodidea) like the southern king crab *Lithodes santolla* are subtidal species that can be found between 5 and 700 m depth in temperate waters (Urbina et al. 2013), hermit crabs (Paguroidea) like *Pagurus bernhardus* (De la Haye et al. 2011) and Porcelain crabs (Galatheoidea) like *Petrolisthes cinctipes* (Carter et al. 2013) are commonly found in the intertidal zone, potentially being trapped in rocky tide pools experiencing large spatial and temporal changes in abiotic parameters as discussed below.

■ ■ Crabs
With over 6700 species in 93 families, brachyuran crabs constitute to *ca*. 50 % of all decapod crustaceans (Ng et al. 2008). Accordingly, all imaginable life strategies and habitat uses are exhibited by this infraorder, including terrestrial species. Some of the most

thoroughly investigated crabs are the osmoconforming Dungeness crab *M. magister*, the weak hyper-osmoregulating green crab *C. maenas* and the closely related blue crab *C. sapidus*, as well as the strong hyper-osmoregulating Chinese mitten crab *E. sinensis*. Like prawns and Anomura, they are oftentimes trapped in tide pools (Truchot and Duhamel-Jouve 1980) and some species bury in the sediment (Bellwood 2002).

■ ■ **Shrimp**
Besides brachyuran crabs (palaemonid), shrimps are the most diverse of the decapod groups with a great inter- and intraspecific variability in osmoregulatory capabilities. While most species can be found in freshwater and are strong hyper-regulators (i.e. genus *Macrobrachium*), some species inhabit estuarine (brackish) and even marine waters (i.e. genus *Palaemon*, *Palaemonetes*) and are hyper-/hypo-osmoregulators (Freire et al. 2003; McNamara and Faria 2012). Some shrimp species are associated with the intertidal zones and therefore more shallow waters (i.e. *Crangon crangon*, Almut and Bamber 2013) or are amphidromous and occupy those habitats during their early life stages (e.g. *Pandalus borealis*, Hammer and Pedersen 2013; *Macrobrachium olfersii*, Freire and McNamara 1995; McNamara and Lima 1997). Other shrimps are deep-water dwellers (e.g. *P. borealis*, Hammer and Pedersen 2013).

■ ■ **Lobster**
For the longest time, lobsters, especially the commercially important lobsters of the genus *Homarus* and the Norway lobster *Nephros norvegicus*, have traditionally been considered to be osmoconforming, stenohaline (salinity > 25 ppt) and limited to coastal and offshore habitats down to 700 m depth (Chapman 1980; Cooper and Uzmann 1980; Dall 1970). More recent research, however, identified them to be present also in estuarine and intertidal regions where they experience short-term fluctuation in salinity and other abiotic parameters (Charmantier et al. 2001). Additionally, the reproduction of lobsters seems to be dependent on higher water temperatures that lead to the animals' migration into different habitats and consequently, their exposure to different environmental conditions that potentially challenge acid–base homeostasis (Charmantier et al. 2001).

■ ■ **Crayfish**
Crayfish belong to the only decapod superfamily that is almost entirely found in freshwater (Reynolds et al. 2013). However, many crayfish species depend on the connection to the ocean in order to breed and therefore have a limited capability to osmo- and ion-regulate (Pequeux 1995). A remarkable number of crayfish build complex burrows in which they spend most of their life (Crandall and Buhay 2008; Guiasu 2002). Others are defined as stream or lake/pond/large river dwellers or are obligated cave dwellers (Crandall and Buhay 2008). Due to the very different chemistry of freshwater (low total osmolarity of ~4 mOsm/mainly $CaCO_3$ vs. 1000 mOsm/mainly NaCl in marine environments), ion regulation in crayfish is challenged and they maintain a lower, yet still hyper-osmotic extracellular ionic concentration and a lower carapace permeability for ions and water compared to marine decapod crustaceans (Wheatly and Gannon 1995).

6.2.3 **Challenging Acid–Base Homeostasis**

There are several factors that can challenge the acid–base regulatory machinery of animals. As (opportunistic) omnivores, all decapod crustaceans experience regular internal

acid loads due to the *catabolism of proteins* and the resulting build-up of extracellular ammonia (mostly NH_4^+ at physiological pH, Weiner and Verlander 2013) that can affect extra- as well as intracellular acid–base homeostasis (Larsen et al. 2014). The response of decapod crustaceans upon *exercise (functional/internal hypoxia)* on the other hand results in a build-up of lactate and CO_2 in the hemolymph, therefore delivering H^+ and challenging extracellular acid–base regulation (Henry et al. 1994; see below).

Besides these intrinsic sources of acid–base disturbances, many of the mainly benthic aquatic decapods experience regular fluctuations of the abiotic parameters pH, pCO_2/pO_2, salinity and temperature in their surrounding environment. In intertidal zones, estuaries and water bodies like the Baltic Sea with restricted connection to the well-buffered open ocean, naturally recurrent elevated pCO_2 (*hypercapnia,* ~234 Pa vs. normal levels of 39 Pa) and changes in *pH* (7.5–8.2) and *temperature* (3.3–18.7 °C), as well as *salinity* (14.5–21.5 ppt), challenge acid–base homeostasis on a regular basis. Additionally, decapods in these shallow water environments are prone to be trapped in tide pools, where they experience even more drastic changes in all abiotic parameters (Truchot 1988), including extremely low levels of oxygen (*hypoxia*; Truchot and Duhamel-Jouve 1980). In extreme cases, these tide pools fall dry so that decapods are *exposed to air.* As mentioned above, many decapod crustacean species hide in burrows and caves or bury in the sediment to avoid predators (Larsen et al. 2014). With only limited water circulation around them, metabolic ammonia builds up around the animals, consequently exposing them to *high environmental ammonia* (HEA, Weihrauch et al. 2004a, b), another challenging factor for acid–base homeostasis mainly for osmoconforming crabs like *M. magister* (Martin et al. 2011). Furthermore, pH has been shown to decrease to as low as 6.5 already within the first few centimetres of sand and mud substrates, accompanied by elevated CO_2 levels of up to 1,600 Pa (Widdicombe et al. 2011).

Besides these naturally occurring challenges for acid–base homeostasis, global climate change and its impacts on acid–base regulation of decapod crustaceans and other invertebrates have become of greatest concern (Whiteley 2011). On the one hand, the anthropogenic increase of atmospheric pCO_2 and its oceanic uptake will result in a decrease of the surface ocean pH of up to 0.3 units by the year 2100 (corresponding to pCO_2 of 1000 ppm; IPCC 2013) and up to 1.4 units by the year 2300 (corresponding to a pCO_2 of 8000 ppm; Caldeira and Wickett 2005), a process termed ocean acidification (IPCC 2013). Even though crustaceans are predicted to be less sensitive to ocean acidification than other invertebrates, still one third of all investigated species in a current meta-analysis by Wittmann and Pörtner (2013) were negatively affected at an environmental pCO_2 of 851 and 1,370 µatm (scenario RCP8.5., Meinshausen et al. 2011). On the other hand, the increase of ocean surface temperature of up to 3 °C by the year 2100 as predicted by the Intergovernmental Panel on Climate Change (Collins et al. 2013) might pose an additional challenge for acid–base homeostasis in decapod crustaceans and even to shifts in whole ecosystem ecology and animal distributions (Walther et al. 2002). As a result, so-called dead zones and oxygen minimum zones (zones of depleted or low oxygen) are markedly increasing due to the anthropogenic pollution and the resulting increase in algal blooms and likely also due to a decrease in water circulation resulting from global warming (Mora et al. 2013). In combination, ocean acidification and warming negatively affected crustacean growth and potentially survival, but had no severe effects on calcification (Harvey et al. 2013).

6.3 Whole Animal Acid–Base Homeostasis and Regulation

6.3.1 Hemolymph Acid–Base Status

In decapod crustaceans, acid–base homeostasis on the whole animal level is described best by the carbonate system characteristics of the extracellular fluid. A disturbance of acid–base homeostasis can be of metabolic (shifts in aerobic/anaerobic metabolism and production of organic acids) or respiratory (shifts in respiratory CO_2) origin and leads to a decrease (acidosis) or increase (alkalosis) in hemolymph pH if not compensated for. Typically, these fluctuations are depicted in a Davenport diagram as shown in ◘ Fig. 6.2 (Davenport 1974).

Besides a small contribution of non-carbonate buffers like the respiratory pigments and other hemolymph proteins, adjustments in the hemolymph carbonate speciation allows for the buffering of the extracellular fluid upon an acid–base disturbance, according to following equation:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$
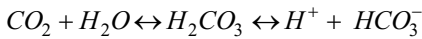
Due to the low solubility of O_2 in water, aquatic animals need to establish a high flow rate of the external medium over their major gas exchange surfaces in order to ensure sufficient oxygenation. Aquatic decapod crustaceans are therefore very restricted in adjusting ventilation rates in order to regulate CO_2 flow. With a pK value of 6.2, extracellular CO_2 is mainly dissociated into H^+ and HCO_3^- at average physiological pH. Therefore, acid–base disturbances may be counteracted using primarily ion regulatory mechanisms at the gills (Henry et al. 2012; Truchot 1988).

◘ Tables 6.1, 6.2, 6.3, 6.4, 6.5, 6.6 and 6.7 give an overview of hemolymph acid–base characteristics of decapod crustaceans under control conditions, as well as changes
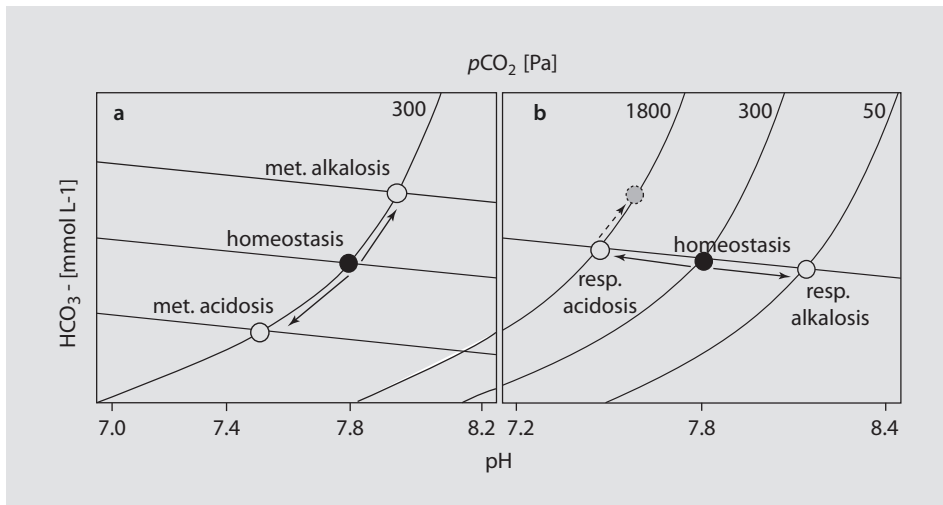
◘ **Fig. 6.2** Davenport diagram for disturbances in acid–base homeostasis. (**a**) Metabolic and (**b**) respiratory components of acid–base disturbance. Indicated values are fitted roughly to represent an average decapod crustacean as listed in ◘ Tables 6.1, 6.2, 6.3, 6.4, 6.5, 6.6 and 6.7. Diagrams are reproduced according to Davenport (1974). *Filled circles*, acid–base homeostasis; *open circles*, status after disturbance. *Thin horizontal lines* indicate the non-carbonate buffer capacity of the hemolymph. *Thin curved lines* indicate CO_2 isopleths. The *grey circle* indicates a potential partial compensation by accumulation of HCO_3^- (see text for details)

◘ Table 6.1 Physiological, whole animal response of decapod crustaceans upon exposure to air

Species	Group	References	Condition	Water salinity [ppt]	Water/air T [°C]	Hemolymph pH	Hemolymph pCO_2 [Pa]	Hemolymph HCO_3^- [mM]	Hemolymph TCO_2 [mM]	Lactate [mM]
Lithodes santolla	2	Urbina et al. (2013)	Control	30	12	7.65	–	–	–	–
			50 h air	30	12	6.78	–	–	–	–
Pachygrapsus crassipes	3	Burnett and McMahon (1987)	Control	32	23	7.8	306	–	5	1.0
			12 h air	N/A	23	7.7	1300	–	19	0.5
Eurytium albidigitum	3	Burnett and McMahon (1987)	Control	32	23	7.7	270	–	4	1.0
			8 h air	N/A	23	7.3	1500	–	9	0.5
Hemigrapsus nudus	3	Burnett and McMahon (1987)	Control	32	11	7.80	330	–	9	–
			4 h air	N/A	11	7.95	530	–	15	–
Neohelice granulata	3	Luquet and Ansaldo (1997)	Control	"BW"	20	7.82	280	–	–	–
			6 h air	N/A	20	7.75	310	–	–	–
Carcinus maenas	3	Simonik and Henry (2014)	Control	32	10	7.70	–	–	7.8	1.1
			6 h air	N/A	10	7.57	–	–	9.9	7.1

6

Species	Group	Reference	Treatment	Salinity	Temperature	pH				
Carcinus maenas	3	Truchot (1975b)	Control	35	15	7.82	150	–	3.86	–
			9 h air	N/A	15	7.59	680	–	9.67	–
			115 h air	N/A	15	7.79	590	–	13.79	–
Cancer productus	3	De Fur and McMahon (1984)	Control	32–35	10	7.84	320	–	10	0.9
			4 h air	N/A	10	7.62	510	–	13	9.0
Scylla serrata	3	Varley and Greenaway (1992)	Control	35	25	7.70	400	–	8	1.25
			6 h air	N/A	25	7.58	740	–	10	1.04
			7 days air	N/A	25	7.79	830	–	19	1.04
Homarus gammarus	5	Whiteley and Taylor (1990)	Control	32–35	15	7.8	130	4.1	–	0.41
			3 h air	32–35	10	7.6*	330*	6.6*	–	2.12*
			24 h air	32–35	20	7.8	800*	18.2*	–	3.24*
Homarus gammarus	5	Taylor and Whiteley (1989)	Control	35	15	7.78	400	9.0	–	1.8
			14 ha air	N/A	15	7.64*	1200	15.8	–	6.2
Austropotamobius pallipes	6	Taylor and Wheatly (1981)	Control	0	15	7.90	400	6.9	–	0.55
			4 h air	N/A	15	7.45*	1130*	6.9	–	8.28*
			24 h air	N/A	15	7.79	1040*	15.4*	–	0.57

Group definitions: (2) Anomura, (3) crabs, (5) lobster and (6) crayfish. Where available from the study, asterisks indicate significant differences from controls

Table 6.2 Physiological, whole animal response of decapod crustaceans upon changes in environmental pO_2 (hyper- or hypoxia)

Species	Group	References	Condition	Water pO_2 [kPa]	Water salinity [ppt]	Water T [°C]	Hemolymph pH	Hemolymph pCO_2 [Pa]	Hemolymph HCO_3^- [mM]	Hemolymph TCO_2 [mM]	Lactate [mM]
Carcinus maenas	3	Burnett and Johansen (1981)	Control	19.3 ?	35	10	7.85	450	–	13	–
			66 h	2.7–3.3	35	10	8.05*	170*	–	7*	–
			Control	19.3 ?	17	10	7.95**	425	–	15.9**	–
			22 h	2.7–3.3	17	10	8.29*	95*	–	6.2*	–
Carcinus maenas	3	Hill et al. (1991)	Control	21	30	10	7.86	240	6.52	8.2	1
			30 min	0	30	10	8.09*	190	–	8.1	3
			12 h	0	30	10	7.84	190	3.17*	5	18*
Palaemon elegans	4	Taylor and Spicer (1991)	Control	19.3 ?	32	10	7.79	–	–	5.4	0.55
			6 h mod	4	32	10	7.93*	–	–	1.9*	5.00*
			6 h sev	1.3	32	10	7.95*	–	–	5.3	0.73
Palaemon serratus	4	Taylor and Spicer 1991	Control	19.3 ?	32	10	7.8	–	–	5.1	0.6
			6 h mod	4	32	10	8.0*	–	–	1.5*	6.7*
			6 h sev	1.3	32	10	8.1*	–	–	4.9	1.2

6

Species		Reference	Condition								
Astacus leptodactylus	6	Dejours and Beekenkamp (1977)	Control	3.2	0	13	7.85	330	5.9	–	–
			44 days (hyper)	80	0	13	7.65	800	9	–	–
Pacifastacus leniusculus	6	Wheatly et al. (1991)	Control	–	0	12	7.83	330	7	–	–
			6 h (hyper)	67	0	12	7.55*	800	10*	–	–
			48 h (hyper)	67	0	12	7.73	730	13*	–	–
Austropotamobius pallipes	6	Wheatly and Taylor (1981)	Control	19.3	0	15	7.90	405	–	7.5	0.40
			24 h low	8.4	0	15	7.96	230	–	5.0	0.49
			24 h mod	4.8	0	15	7.98	195	–	4.0	0.97
Orconectes rusticus	6	Wilkes and McMahon (1982)	Control	16.5	0	15	7.78	480	–	5.84	–
			24 h	6–7.3	0	15	7.98*	140*	–	2.30*	–
			6 days	6–7.3	0	15	7.90*	270*	–	3.20*	–

Group definitions: (3) crabs, (4) shrimp and (6) crayfish. Asterisks indicate significant differences from controls, where available. Double asterisks indicate values significantly different between controls. Question marks indicate a value taken from a cross-reference within the paper

mod moderate, *sev* severe

Table 6.3 Physiological, whole animal response of decapod crustaceans upon changes in environmental pCO_2 (hypercapnia)

Species	Group	References	Condition	Water pCO_2 [kPa]	Water pH	Water salinity [ppt]	Water T [°C]	Hemolymph pH	Hemolymph pCO_2 [Pa]	Hemolymph HCO_3^- [mM]	Hemolymph TCO_2 [mM]
Callinectes sapidus	3	Henry et al. (1981)	Control	–	–	25	26	7.78	270	–	5
			48 h	2	–	25	26	7.61	2100	–	20
Callinectes sapidus	3	Cameron (1978)	Control	0.04	–	–	–	7.96	530	–	10
			15 min	1	–	–	–	7.65	1470	–	12
			44 h	1	–	–	–	7.80	1200	–	19
Carcinus maenas	3	Appelhans et al. (2012)	Control	0.066	8.06	15	13	7.82	380	8	–
			10 w mod	0.126	7.84	15	13	7.83	490	10	–
			10 w sev	0.351	7.36	15	13	7.81	550	12	–
Carcinus maenas	3	Fehsenfeld and Weihrauch (2013)	Control	0.054	7.7	10	14	7.9	263	6.6	–
			7 days	0.324	7.0	10	14	7.9	344	7.9	–
Carcinus maenas	3	Fehsenfeld and Weihrauch (2016a)	Control	0.09	7.76	32	13.2	7.87	156	5.5	–
			48 h	9.40	6.85	32	13.8	7.76*	688*	18.7*	–

6

Carcinus maenas	3	Truchot (1975a)	Control	0.04	7.8	35	17	7.79	180	3.9	–
			24 h	0.31	7.4	35	17	7.71	730	13.7	–
Necora puper	3	Spicer et al. (2007)	Control	0.02	7.98	34	15	7.90	190	6.6	–
			24 h	0.08	7.30	34	15	7.82	360	10.8	–
			24 h	1.11	6.70	34	15	7.97	1190	27.4	–
			24 h	6.04	6.05	34	15	7.59	4520	55.9	–
Necora puper	3	Small et al. (2010)	Control	0.074	7.85	35	17	7.73	190	2.63	–
			30 days sev	1.250	6.69	35	17	7.53	880	8.66	–
Metacarcinus magister	3	Hans et al. (2014)	Control	0.049	8.1	32	14	7.93	133	4.9	–
			7 days	0.330	7.4	32	14	8.01	402*	14.9*	–
Metacarcinus magister	3	Pane and Barry (2007)	Control	–	7.90	34	10	7.82	265	6	–
			90 min	1.013	7.08	34	10	7.42*	1000*	8*	–
			24 h	1.013	7.08	34	10	7.75	900*	18*	–
Pandalus borealis	4	Hammer and Pedersen (2013)	Control	0.052	8.1	35	7	7.76	250	5.1	–
			16 days	0.920	6.9	35	7	7.60	1000*	13.0*	–

(continued)

Table 6.3 (continued)

Species	Group	References	Condition	Water pCO_2 [kPa]	Water pH	Water salinity [ppt]	Water T [°C]	Hemolymph pH	Hemolymph pCO_2 [Pa]	Hemolymph HCO_3^- [mM]	Hemolymph TCO_2 [mM]
Palaemon elegans	4	Dissanayake et al. (2010)	Control	0.10	8.0	32	15	7.70	–	?	–
			14 days	0.31	7.5	32	15	7.55	–	Δ1.0	–
			30 days	0.38	7.5	32	15	7.80	–	Δ1.8	–
Palaemon serratus	4	Dissanayake et al. (2010)	Control	0.12	8.0	32	15	7.7	–	?	–
			14 days	0.32	7.5	32	15	7.7	–	Δ1.2	–
			30 days	0.37	7.5	32	15	8.2	–	Δ2.4	–

Group definitions: (3) crabs and (4) shrimp. Asterisks indicate significant differences from controls, where available. Question marks indicate unavailable data; accordingly, differences in hemolymph HCO_3^- are given as the difference in comparison to controls

w weeks, *mod* moderate, *sev* severe

◘ **Table 6.4** Physiological, whole animal response of decapod crustaceans upon changes in environmental temperature

Species	References	Condition	Water T [°C]	Water salinity [ppt]	Hemo-lymph pH	Hemo-lymph pCO_2 [Pa]	Hemo-lymph HCO_3^- [mM]	Lactate [mM]
Carcinus maenas	Truchot (1973)	Control	15	20	7.75	335	6.6	–
		4 days	5	20	7.90	225	7.3	–
		4 days	10	20	7.75	280	6.5	–
		4 days	21	20	7.63	425	5.9	–
		4 days	26	20	7.53	545	5.5	–
Carcinus maenas	Howell et al. (1973)	Control	15	32	7.79	625	13.2	–
		7 days	5	32	8.07	305	16.7	–
		7 days	10	32	7.86	590	16.0	–
		7 days	20	32	7.77	560	10.2	–
Callinectes sapidus	Howell et al. (1973)	Control	15	32	7.77	320	5.9	–
		7 days	5	32	8.03	465	18.8	–
		7 days	10	32	7.80	425	9.2	–
Callinectes sapidus	Cameron and Batterton (1978a)	Control	10	0	7.94	280	10.35[a]	–
		7–14 days	27	0	7.74	400	7.60[a]	
Metacarcinus magister	McMahon et al. (1978)	Control	12	32	7.84	225	5.83	1.5
		10 days	7	32	7.91	235	7.42	1.4
		10 days	17	32	7.73	250	4.47	1.5

All studies have been conducted on brachyuran crabs
[a]Values are for total carbon

Table 6.5 Physiological, whole animal response of decapod crustaceans upon environmental salinity

Species	Group	References	Condition	Water salinity [ppt]	Water pH	Water pCO_2/pO_2 [kPa]	Water T [°C]	Hemolymph pH	Hemolymph pCO_2 [Pa]	Hemolymph HCO_3^- [mM]
Callinectes sapidus	3	Henry and Cameron (1982)	Control	35	–	–	–	7.75	320	4.2
			24 h	12	–	–	–	7.84	400	9.0
Eriocheir sinensis	3	Truchot (1992)	Control	35	8.1	0.06	15	7.89	280	7.9
			3 days	0	8.4	0.06	15	8.02*	495*	16.0*
			Control	0	8.4	0.06	15	7.96	425	12.2
			24 h	35	8.1	0.06	15	7.96	240*	8.0*
Eriocheir sinensis	3	Whiteley et al. (2001)	Control	35	–	–	12	7.76	460	7.1
			6 h	12	–	–	12	7.50*	290	4.0
			24 h	12	–	–	12	7.94	310	9.0
Necora puber	3	Whiteley et al. (2001)	Control	35	–	–	12	7.82	300	7
			6 h	24	–	–	12	7.80	500	9
Carcinus maenas	3	Truchot (1981)	Control	35	8.19	0.039	15	7.85	215	5.5

Species	Group	Reference	Time							
Pacifastacus leniusculus	6	Wheatly and McMahon (1982)	48 h	12	7.85	0.040	15	8.08*	200	8.0*
			24 h	35	8.19	0.039	15	7.65*	190	3.0*
			Control	0	–	–	15	7.95	365	8.47
			48 h	8	–	–	15	8.00	180	5.73
			48 h	16	–	–	15	7.96	140	4.76*
			48 h	32	–	–	15	7.83*	90	2.83*

Group definitions: (3) crabs and (6) crayfish. Asterisks indicate significant differences from controls, where available

Table 6.6 Physiological, whole animal response of decapod crustaceans upon exercise (forced movement)

Species	Group	References	Condition	Water salinity [ppt]	Water T [°C]	Hemolymph pH	Hemolymph pCO_2 [Pa]	Hemolymph TCO_2 [mM]	Lactate [mM]
Metacarcinus magister	3	McDonald et al. (1979)	Control	27	8	7.94	230	8.3	0.69
			20 min	–	–	7.52	430	–	9.90
Callinectes sapidus	3	Booth et al. (1984)	Control	31	20	7.67	470	5.7	2
			30 min	31	20	7.24	800	3.6	15
Carcinus maenas	3	Hamilton and Houlihan (1992)	Control	31	15	7.83	210	5.2	3.9
				31	15	7.76*	250	4.3	9.7*
Homarus americanus	5	Rose et al. (1998)	Control	32	14	7.78	268	4.80	0.20
			30 min slow	32	14	7.64*	507*	5.51*	1.55*
			30 min fast	32	14	7.42*	603*	5.74*	1.29*

Group definitions: (3) crabs and (5) lobster. Asterisks indicate significant differences from controls, where available

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■ **Table 6.7** Physiological, whole animal response of decapod crustaceans upon a combination of different environmental stressors

Species	Group	References	Condition	Water pCO_2/pO_2 [kPa]	Water pH	Water T [°C]	Water salinity [ppt]	Hemolymph pH	Hemolymph pCO_2 [Pa]	Hemolymph HCO_3^- [mM]	Hemolymph TCO_2 [mM]	Lactate [mM]
Metapenaeus joyneri	1	Dissanayake and Ishimatsu (2011)	Control	0.04	8.2	15/20/25	32	7.65	–	–	–	–
			10 days HC	1.00	8.2	15	32	7.73	–	–	–	–
			10 days HC+T	1.00	8.2	20	32	7.79	–	–	–	–
			10 days HC+T	1.00	8.2	25	32	7.80 (2 d)	–	–	–	–
Hyas araneus	3	Harms et al. (2014)	Control	0.045	8.2	5	32	8.05	250	2.8	–	–
			10 weeks HC mod	0.100	7.8	5	32	8.00	350*	4.2	–	–
			10 weeks HC sev	0.190	7.6	5	32	7.75*	430*	4.0	–	–
			Control+T	0.037	8.2	10	34	7.98	210	1.4	–	–
			10 weeks mod HC+T	0.095	7.9	10	34	7.95	430*	5.1*	–	–

(continued)

6

◻ Table 6.7 (continued)

Species	Group	References	Condition	Water pCO_2/pO_2 [kPa]	Water pH	Water T [°C]	Water salinity [ppt]	Hemolymph pH	Hemolymph pCO_2 [Pa]	Hemolymph HCO_3^- [mM]	Hemolymph TCO_2 [mM]	Lactate [mM]
			10 weeks sev HC+T	0.204	7.5	10	34	7.70*	430*	3.0	–	–
Necora puber	3	Rastrick et al. (2014)	(0) control	0.041	–	10	35	7.84	290	6.6	–	1.0
			(1) 2 weeks temp	0.041	–	15	35	7.89	300	7.1	–	1.0
			(2) 2 weeks HC	0.101	–	10	35	7.87	380	9.2*	–	1.0
			(3) 2 weeks HC+T	0.101	–	15	35	7.95	370	11.0*	–	1.0
			Control: 3 h air (0)	0.041	–	10	N/A	7.3**	1100**	7.0	–	8.0**
			3 h air of (1)	0.041	–	15	N/A	7.3	1600	12.0	–	7.5
			3 h air of (2)	0.101	–	10	N/A	7.5	1100	14.0	–	9.0
			3 h air of (3)	0.101	–	15	N/A	7.3	2100	14.0	–	10.0
Cancer productus	3	De Fur et al. (1980)	Control	18	–	10	32	7.89	280	–	9.0	–
			4 h air	–	–	10	N/A	7.62	1100	–	13.6	–

Species	N	Reference	Condition	61–68	–	10	32	7.80	1100	–	20.0	–
Chionoecetes tanneri	3	Pane and Barry (2007)	24 h hyperoxia	–	7.85	3	34	7.8	240	5	–	–
			Control high O$_2$	1.013	7.08	3.5	34	7.4*	535*	5	–	–
			24 h HC, at high O$_2$	–	7.08	3	34	8.00	240	9.5	–	–
			Control low O$_2$	1.013	7.08	3.5	34	7.65*	665*	11.5	–	–
			24 h HC, at low O$_2$									

Group definitions: (1) prawns and (3) crabs. Asterisks indicate significant differences, where available. Double asterisks indicate values significantly different between controls

HC hypercapnia, *mod* moderate, *sev* severe

induced by various stressors as described in ▶ Sect. 6.2.3 and below. Due to the vast number of publications on acid–base disturbances, we do not claim for the list to be complete, but rather tried to give representative examples for as many different species as possible. In case multiple studies were available for the same species, we attempted to include the most relevant publication(s) that was (were) comparable to the other studies. Data was partly extracted from graphs and values transformed into the units as depicted in the tables where applicable. If significantly different, values are given for the time point of maximum effect as well as from the end of the incubation period.

Under control conditions, all decapod crustaceans maintain their hemolymph pH typically between 7.7 and 8.0. Hemolymph pCO_2 levels, however, can vary quite substantially between crustacean species and are typically low (*ca.* 200–500 Pa) due to the almost complete dissociation to H^+ and HCO_3^- at physiological pH, but high enough likely to enable diffusion out of the body along the gradient between the extracellular fluid and the environment (*ca.* 40 Pa; Henry et al. 2012; Melzner et al. 2009). Prawns like the Japanese tiger prawn *Penaeus japonicus* seem to be an exception: These decapod crustaceans have a slightly lower than average hemolymph pH (7.5) and, accordingly, a higher pCO_2 (ca. 600 Pa; Cheng et al. 2013). Similar to hemolymph pCO_2, levels of hemolymph HCO_3^- have also been observed to vary between species and typically average between 4 and 9 mmol L^{-1}. As indicated in ◪ Tables 6.1, 6.2, 6.3, 6.4, 6.5, 6.6 and 6.7, control levels for hemolymph $[HCO_3^-]$ as high as 14 mmol L^{-1} have been observed in some studies, but these values have to be treated with caution as they may indicate that animals were in premoult rather than intermoult stages (▶ see Sect. 6.3.2).

■ ■ Exposure to Air (◪ Table 6.1)

The primary consequence for most aquatic decapod crustaceans of emerging from water is the collapse of their gills, physically impairing gas exchange processes. Many crabs are therefore retaining branchial water in their gill chambers likely to facilitate CO_2 diffusion (Burnett and McMahon 1987). Due to the higher solubility of O_2 in air compared to water, however, some decapod crustaceans like *Pachygrapsus crassipes* (Burnett and McMahon 1987) or *C. maenas* (Simonik and Henry 2014) are capable of extracting O_2 from the air and voluntarily move out of the water to offset acid–base disturbances resulting from other stressors like hypoxia (Wheatly and Taylor 1979).

Generally, exposure to air results in a pronounced respiratory acidosis with a pH drop of 0.1–0.2 units (in crabs) up to 0.5–0.7 units (in Anomura and crayfish), a two- to fivefold increase in hemolymph pCO_2 and a significant two- to threefold elevation of hemolymph HCO_3^- in all investigated decapod crustaceans (◪ Table 6.1).

It seems, however, that there are marked species-specific differences in compensating for the experienced acidosis. While some crabs (De Fur and McMahon 1984; Simonik and Henry 2014), lobsters (Taylor and Whiteley 1989; Whiteley and Taylor 1990) and crayfish (Taylor and Wheatly 1981) seem to switch partly to anaerobic metabolism and therefore experience an additional metabolic acidosis with a pronounced increase in hemolymph lactate levels, other crabs like *Eurytium albidigitum* (Luquet and Ansaldo 1997) and *Pachygrapsus crassipes* (Burnett and McMahon 1987) seem to undergo a metabolic depression (*E. albidigitum*) or maintain or even increase their aerobic metabolism (*P. crassipes*). Additionally, some crab species were observed to increase their strong ion difference (SID) likely via ion exchange processes at the gill in response to emersion (Burnett and McMahon 1987; Luquet and Ansaldo 1997; Truchot 1979), which is interpreted to help offset the experienced acidosis (Stewart 1978). Consequently, *C. maenas* (Truchot 1975b),

S. serrata (Varley and Greenaway 1992), *H. gammarus* (Whiteley and Taylor 1990), *A. pallipes* (Taylor and Wheatly 1981), *P. crassipes* and *H. nudus* (Burnett and McMahon 1987) are capable of fully compensating for the pH drop resulting from the experienced acidosis, while *E. albidigitum* is not (Burnett and McMahon 1987).

■ ■ Hyper-/Hypoxia (◘ Table 6.2)

Due to its low solubility in water compared to air, oxygen has to be considered one of the limiting factors in the aquatic environment (Dejours 1975). Hence, only subtle changes in water pO_2 result in immediate alterations of ventilation rates in aquatic decapod crustaceans in order to be able to maintain aerobic metabolism (Jouve-Duhamel and Truchot 1983; Truchot 1988). Consequently, hyperventilation as observed in moderate hypoxic conditions simultaneously leads to an increase in branchial CO_2 excretion and therefore respiratory alkalosis (elevated pH and lower HCO_3^-), while reduced ventilation in a (moderate) hyperoxic environment ultimately leads to accumulation of hemolymph pCO_2 and hence a respiratory acidosis (lower pH and higher HCO_3^-), as can be seen in ◘ Table 6.2. In shrimp and *C. maenas*, the increase in hemolymph lactate during moderate hypoxia and anoxia, respectively, indicates that in these decapod crustaceans, a metabolic component seems to be present that might explain the reduced levels of total carbon/HCO_3^-, but did not affect the actual increase in hemolymph pH (Taylor and Spicer 1991). Interestingly, when the same shrimp species *P. elegans* and *P. serratus* were exposed to a severe hypoxia (<2 Pa), lactate levels stayed constant and total carbon was not affected (Taylor and Spicer 1991).

■ ■ Hypercapnia (◘ Table 6.3)

In contrast to hypoxia, exposure to elevated environmental pCO_2 does not drive a ventilation response in decapod crustaceans due to its very similar solubility in air and water (Henry et al. 2012; Jouve-Duhamel and Truchot 1983). Nonetheless, exposure to hypercapnia leads to a respiratory acidosis marked by a rapid drop in hemolymph pH of up to 0.4 units and substantial increases in pCO_2 (two- to fourfold) in all investigated decapod crustaceans (◘ Table 6.3). Elevated extracellular pCO_2 is believed to be maintained in order to ensure an outwardly directed CO_2 gradient for the diffusion-based excretion of metabolic CO_2 (Melzner et al. 2009). Most decapod crustaceans are capable of fully compensating for the respiratory acidosis by accumulating HCO_3^- in their hemolymph to buffer excess protons, likely via active ion regulatory processes at the gill. While some species are capable of maintaining or even increasing their metabolic rate in response to hypercapnia (*C. maenas*, Appelhans pers. communication), others experience a metabolic depression (e.g. *M. magister*, Hans et al. 2014; *P. borealis*, Hammer and Pedersen 2013). Interestingly, green crabs *C. maenas* that are acclimated to full-strength seawater (32–35 ppt; Truchot 1975c; Fehsenfeld and Weihrauch 2016a) seemed to accumulate more CO_2 in their hemolymph than brackish-water acclimated specimen (Appelhans et al. 2012; Fehsenfeld and Weihrauch 2013). In contrast to brackish-water crabs, the resulting respiratory acidosis in the seawater-acclimated crabs was not fully compensated for after 24 h, and hemolymph pH decreased. This example indicates that acid–base and osmoregulation might indeed be closely linked in this species.

■ ■ Temperature (◘ Table 6.4)

It has been shown for poikilotherm animals such as decapod crustaceans that temperature correlates inversely with hemolymph pH in order to maintain extracellular H^+/OH^- ratios

to ensure a constant net charge of proteins (Howell et al. 1973; Truchot 1973). In parallel, hemolymph pCO_2 seems to generally stay constant/increase only slightly with increasing temperature, while [HCO_3^-] and/or total carbon (C_T) decreases more drastically. The authors of the respective studies (Cameron and Batterton 1978b; Truchot 1973) attributed the changes in C_T to active regulation of HCO_3^- via ion exchanges at the gills in order to compensate for the acidosis, rather than solution of the carapace or passive processes alone (Henry et al. 2012).

▪ ▪ **Salinity** (◨ Table 6.5)

Generally, acclimation to low salinity results in a metabolic alkalosis in all investigated decapod crustaceans, characterized by an increase in hemolymph pH at relatively stable pCO_2 and a significant increase in [HCO_3^-]. Conversely, when freshwater crayfish (Wheatly and McMahon 1982), freshwater *E. sinensis* (Truchot 1992) or brackish-water acclimated *C. maenas* (Truchot 1981) were acclimated to full-strength seawater, they developed a metabolic acidosis characterized by a decrease in hemolymph pH and HCO_3^-. In this case, however, an additional slight respiratory alkalosis (decrease in pCO_2) was observed in parallel, likely compensating for the respiratory acidosis. Throughout the time course of different salinity acclimations, species can exhibit specific alterations to this general pattern. An initial respiratory acidosis, for example, was observed in *C. maenas* upon acclimation to dilute salinity before switching into the expected metabolic alkalosis (Truchot 1981), and in *E. sinensis* a transient respiratory acidosis marked by a spontaneous drop in pH was only present at days 6 (Truchot 1992).

While Henry and Cameron (1982) attributed the observed increase in hemolymph [HCO_3^-] following acclimation of *C. sapidus* to dilute salinity to the additionally observed change in the strong ion difference (SID), no equivalent observation was made in brackish-water acclimated *E. sinensis* in the study by Whiteley et al. (2001). In contrast, Truchot (1981, 1992) suggested metabolic adjustments correlated to isosmotic intracellular regulation in the cells to be responsible, resulting in either a measureable efflux of base or acid into the environment. These observations reveal the complex nature of acid–base disturbances upon different salinity acclimations, and consequently, the reasons for the observed metabolic alkalosis and acidosis are not yet fully understood.

▪ ▪ **Exercise** (◨ Table 6.6)

Hemolymph lactate levels are typically held lower than 1 mmol L^{-1} and negligible in undisturbed decapod crustaceans, but can increase more than tenfold in crabs that experience a metabolic acidosis due to exercise (forced movement). Furthermore, the experienced acidosis is characterized by an immediate drop in pH of up to 0.4 units and a twofold increase in hemolymph pCO_2, therefore also resembling characteristics of a respiratory acidosis. Interestingly, exercised lobsters seem to be able to avoid anaerobic metabolism during exercise for the most part and experience primarily a respiratory acidosis without the substantial rises in hemolymph lactate observed in other crustaceans (Rose et al. 1998).

In *M. magister* (McDonald et al. 1979), *C. maenas* and *C. sapidus* (Booth et al. 1984), the proton concentration in the hemolymph was observed to be lower than could be expected from the accumulated lactate, given that both are produced in equimolar quantities during glycolysis (Hochachka and Mommsen 1983). Due to the observed drastic increase in ammonia excretion, Booth et al. (1984) concluded that at least part of the protons are excreted as NH_4^+ via ion exchange processes at the gill epithelium.

■ ■ **Combined Stressors** (■ Table 6.7)

As is clear by the previous sections, extracellular acid–base regulation in response to environmental disturbances can be quite complex despite some common principles. While the discussed studies isolated one stressor at a time and investigated its effects on the respective species' acid–base characteristics, environments are rarely that "simple" and a combination of simultaneous stressors seems much more likely, especially in tide pools (Truchot 1988) or in the face of ongoing global climate change (IPCC 2013).

For example, when combined with an increase in water temperature, hypercapnia resulted in a respiratory alkalosis in *N. puber* that was not observed when crabs were exposed to either one of the stressors alone (Rastrick et al. 2014). Even though the increase in HCO_3^- upon hypercapnia and high-temperature acclimation rendered the crabs more resistant to short periods of subsequent emersion, they still experienced the same magnitude of acidosis, and recovery from these stressors was significantly attenuated. In a different example, prior acclimation of green crabs to hypercapnia enabled them to avoid an uncompensated hypercapnic acidosis that was induced by low environmental alkalinity in normocapnic-acclimated animals (Truchot 1984). In *C. productus*, the respiratory acidosis usually observed following exposure to hyperoxia was not observed in crabs that were first exposed to air (De Fur et al. 1980). Finally, *P. joyneri* exposed to a combination of elevated temperature and hypercapnia no longer experienced an acidosis as to be expected by studies on other decapod crustaceans, but exhibited an alkalosis (Dissanayake and Ishimatsu 2011).

Even though studies on combined environmental stressors are rare, the existing data indicates alarming differences in the acid–base responses of decapod crustaceans in comparison to single-stressor studies. Therefore, it would be desirable for future research to focus on a more holistic and realistic approach.

6.3.2 Calcification, $CaCO_3$ and Moulting

Interestingly, as one of the most important physiological processes, growth in crustaceans is closely linked to the whole animal acid–base status and regulation. Due to their hard and inflexible exoskeleton, decapod crustaceans depend on a series of moults in order to grow. During the different pre-, post- and intermoult stages that compose the complex moult cycle (Mangum et al. 1985), the connectives between the living tissue and the extracellular cuticle are loosened and water uptake ensures the shedding of the old and the expansion of the new carapace. The exoskeleton contains the majority of the organismal $CaCO_3$ that is mobilized during the moult in order to soften this structure and either excreted into the environment or stored in gastroliths for the new exoskeleton (Ahearn et al. 2004). Generally, decapod crustaceans experience a pronounced premoult alkalosis (increase in hemolymph HCO_3^-) in order to compensate for a concomitant acidosis of mainly metabolic origin (increase in hemolymph lactate) after successful exuviation (Mangum et al. 1985). Interestingly, this HCO_3^- seems not to originate from mobilization of the exoskeletal stores. Even though an early study by Robertson (1960) detected a seemingly HCO_3^--correlated increase in hemolymph $[Ca^{2+}]$ and $[Mg^{2+}]$ in premoult *C. maenas*, later studies on the blue crab *C. sapidus* did not detect a change in hemolymph $[Ca^{2+}]$ but observed a decrease in $[Cl^-]$ instead, indicating a direct Cl^-/HCO_3^--exchange with the environment as the source of the extracellular HCO_3^- (Cameron and Wood 1985; Cameron 1978; Henry et al. 1981; Mangum et al. 1985). As a response to air exposure,

however, crayfish (Wheatly and Gannon 1995), the anomuran porcelain crabs *Petrolisthes laevigatus* (Lagos and Cáceres 2008) and *Petrolisthes violaceus* (Vargas et al. 2010) and subpopulations of the brachyuran crab *Cyclograpsus cinereus* (Lagos et al. 2014) and *N. granulata* (Luquet and Ansaldo 1997) were able to mobilize exoskeletal Ca^{2+}/HCO_3^- stores in response to the acid–base disturbance.

Interestingly and in contrast to other invertebrate marine calcifiers like mussels (Beniash et al. 2010; Michaelidis et al. 2005) and corals (Langdon et al. 2000; ▶ Chap. 7), calcification of the carapace in response to hypercapnia (ocean acidification) seems to increase in the red rock cleaner shrimp *Lysmata californica* (Taylor et al. 2015), the prawns *(Lito)Penaeus occidentalis* and *P. monodon* (Wickins 1984), female Anomuran red king crab *Paralithodes camtschaticus* (Long et al. 2013), as well as the crab *C. sapidus*, the lobster *H. americanus* and the prawn *P. plebejus* (Ries et al. 2009). The robustness of the crustacean carapace is believed to be due to its increased amount of calcite, the less soluble form of $CaCO_3$ (Taylor et al. 2015), as well as its complete coverage with a relatively thick organic epicuticle (Ries et al. 2009), and the crustaceans' generally high capability for acid–base regulation. While an increase in calcification might sound advantageous, it potentially has negative effects on the crustaceans' moulting frequency (Wickins 1984) and crypsis/predator defence (Taylor et al. 2015). The metabolic investment and possible allocation of energy resources due to an increased calcification might also lead to other negative impacts in these crustaceans that might reduce the overall fitness (i.e. metabolic depression and reduced growth; Taylor et al. 2015). An increase in calcification as well as an increase in metabolic costs in premoult was already observed in very early life stages (zoea I larval stage) of the Anomuran red king crab *P. camtschaticus* (Long et al. 2013) and the brachyuran great spider crab *Hyas araneus* (Schiffer et al. 2013).

Only few decapod crustaceans like the velvet swimming crab *N. puber*, however, might also exhibit a dissolution of their exoskeleton in response to high levels of ocean acidification (Spicer et al. 2007). Also in late European lobster larvae, exposure to hypercapnia resulted in significantly lower carapace mass as well as less mineralization in response to hypercapnia (Arnold et al. 2009), as well as a delay in the first moult cycles (Keppel et al. 2012).

6.4   Gill Epithelial Acid–Base Regulation

6.4.1   Gill Epithelial Transporters Involved in Acid–Base Regulation

While the numerous studies on acid–base homeostasis in aquatic decapod crustaceans mainly have focussed on describing the whole animal acid–base status in response to diverse environmental stressors as described above, to date only a few investigations have commented on the actual regulatory mechanisms involved in these processes. These few studies indicate that the high acclimation potential of decapod crustaceans in response to environmental changes can be attributed mainly to ion exchange processes in the gill epithelium (see above). As the major site for osmoregulation and ammonia excretion (reviewed by Henry et al. 2012; Larsen et al. 2014; Weihrauch et al. 2004b), the gills possess many epithelial membrane transporters that are likely also involved in acid–base regulation. Indirect evidence was drawn from the observation that changes in hemolymph acid–base equivalents (H^+/HCO_3^-) were accompanied by changes in the strong ion

difference (Na^+/Cl^-), when decapod crustaceans were exposed to air and dilute salinity (Burnett and McMahon 1987; Ehrenfeld 1974; Henry and Cameron 1982; Luquet and Ansaldo 1997; Truchot 1979) or when carbonic anhydrase, the enzyme responsible for the conversion of CO_2 into H_2CO_3 and subsequent dissociation to H^+ and HCO_3^-, was blocked (Burnett et al. 1981; Henry and Cameron 1983; Henry et al. 2003). While the crustacean gill epithelium has been subject to many investigations of membrane transporters involved in osmoregulation and ammonia excretion (for reviews see Henry et al. 2012; Larsen et al. 2014; Weihrauch et al. 2004a, b), hardly anything is known about the respective mechanisms for acid–base regulation. A recent set of gill perfusion experiments on anterior gills of seawater-acclimated *C. maenas*, however, shed some light on the gill transporter inventory potentially involved in branchial acid–base regulation and its linkage to ammonia regulation (see also section above) in this species (Fehsenfeld and Weihrauch 2016a).

◪ Figure 6.3a–d represents the current working models for osmoregulation, ammonia excretion and general acid–base regulation, as well as a new, more specific model for acid–base regulation and its overlap/link to ammonia excretion in the model organism *C. maenas*.

Trans-branchial active NaCl transport in moderate hyper-osmoregulators such as *C. maenas* (Riestenpatt et al. 1996) and *N. granulata* (Lucu and Siebers 1987; Onken et al. 2003) is fairly well characterized. As can be seen in ◪ Fig. 6.3a, basolateral Na^+/K^+-ATPase and Cl^--channels, as well as apical $Na^+/K^+/2Cl^-$-cotransporter supported by apical and basolateral K^+-channels, are key players in this osmoregulatory mechanism. A number of studies also indicated an alternative pathway for NaCl uptake via apical Na^+/H^+- and HCO_3^-/Cl^- -exchangers, linked to the actions of a carbonic anhydrase (Henry et al. 2003; Lucu 1990; Onken et al. 2003; Tresguerres et al. 2008), therefore directly linking NaCl transport to the transport of acid–base equivalents. In *N. granulata*, however, a basolateral Na^+/H^+-exchanger seems to promote intracellular Na^+ uptake in exchange for H^+ rather than being situated apically (Tresguerres et al. 2008).

Overlapping with the model for NaCl transport, basolateral Na^+/K^+-ATPase and Cs^+/Ba^{2+}-sensitive K^+-channels have also been shown to be involved in ammonia excretion through the gills of *C. maenas* (Weihrauch et al. 1998, 2004a, b; ◪ Fig. 6.3b). In addition to the general Ba^{2+}-sensitive K^+-channels, a ZD7288-sensitive K^+-channel of the hyperpolarization activated cyclic nucleotide-gated potassium channel family (HCN) has recently been identified to contribute to NH_4^+ regulation over the gill epithelium of *C. maenas* (Fehsenfeld and Weihrauch 2016b).

Additionally, a cytoplasmic V-(H^+)-ATPase and a functional microtubule network have been hypothesized to promote ammonia excretion over the apical membrane via NH_3 trapping and transport in acidified vesicles in this species (Weihrauch et al. 2002), potentially linking ammonia excretion with acid–base regulation.

In comparison to the models for osmoregulation and ammonia excretion, the hypothetical model for general crustacean acid–base regulation after Freire et al. (2008) as seen in ◪ Fig. 6.3c is much more speculative. When considered in correlation with ammonia excretion as seen in ◪ Fig. 6.3d, however (▶ see also Sect. 6.5), potential pathways become more comprehensive. The most significant key player in *C. maenas*' branchial acid–base regulation (although not affecting ammonia excretion), as identified in a recent gill perfusion study applying pharmaceuticals to block specific transporters in *C. maenas* gills, was a potential basolateral Na^+/HCO_3^--cotransporter (Fehsenfeld and Weihrauch 2016a). A recently identified basolateral Na^+/HCO_3^--exchanger in the squid *Sepioteuthis lessoniana* (Hu et al. 2014, ▶ Chap. 11) has also been postulated to be important for acid–base regulation in the euryhaline crab *N. granulata* (Tresguerres et al. 2008).

A strictly apical distribution of V-(H^+)-ATPase as hypothesized in the model of Freire et al. (2008) and depicted in ◰ Fig. 6.3c has only been identified in freshwater (and terrestrial) crustaceans (Tsai and Lin 2007), including the red crab *Dilocarcinus pagei* (Weihrauch et al. 2004a, b) and *E. sinensis* (Onken and Putzenlechner 1995; Tsai and Lin 2007), as well as many freshwater fish (Gilmour and Perry 2009), to generate an electrochemical gradient over the apical membrane to drive Na^+ uptake (Larsen et al. 2014; Weihrauch et al. 2001). While an apical V-(H^+)-ATPase seems unlikely to be present in sea- and brackish-water acclimated decapod crustaceans for osmoregulatory purposes, an apical presence cannot be excluded to be involved in acid–base regulation. The pharmacological studies by Fehsenfeld and Weihrauch (2016a) on the isolated perfused gill and the immunohisto-chemical localization (Weihrauch et al. 2001), however, indicated a significant contribution of a cytoplasmic V-(H^+)-ATPase that – together with the Rhesus-like protein – has been hypothesized to be involved in ammonia trapping in acidified vesicles as suggested by Weihrauch et al. (2002), therefore promoting the excretion of both, NH_3 and H^+.

Supporting the findings of the above-mentioned gill perfusion study in *C. maenas* gills (Fehsenfeld and Weihrauch 2016a), a study by Siebers et al. (1994), identified baso-lateral Na^+/K^+-ATPase (NKA) to be involved in branchial acid–base regulation. K^+-channels on the other hand provide a backflow of K^+ into the hemolymph. As mentioned earlier, both structures provide additional transport of NH_4^+ due to its similar size and charge compared to K^+ (Skou 1960; Lignon 1987; Weihrauch et al. 1998; Choe et al. 2000). Additionally, the HCN-like potassium channel recently identified to be involved in NH_4^+ movements over the gill epithelium of *C. maenas* as mentioned above has also been shown to be involved in branchial acid–base regulation in the respective study (Fehsenfeld and Weihrauch 2016b).

In fish, the Na^+/K^+-ATPase generates the electrochemical gradient over the basolateral membrane that is then the major driving force for the excretion of H^+ via apical Na^+/H^+-exchanger (NHE) in acid excretory epithelial cells (Choe et al. 2005; Edwards et al. 2002). While a potential electrogenic NHE ($2Na^+/1H^+$) has been identified to be present in crus-tacean gills (Shetlar and Towle 1989), an apical localization as suggested by studies on *Cancer antennarius* and *P. cinctipes* (Hunter and Kirschner 1986) and *C. maenas* (Weihrauch et al. 1998) is not clear to date due to the interference of the employed phar-maceuticals (amiloride) with the cuticle (Onken and Riestenpatt 2002; Weihrauch et al. 2002). A basolateral NHE that promotes H^+ excretion into the hemolymph, however, has been observed in *N. granulata* (Tresguerres et al. 2008). An additional cytoplasmic distri-bution of NHE that is potentially involved in both ammonia and proton excretion via vesicles is supported by phylogenetic analysis of diverse NHEs as conducted by Fehsenfeld and Weihrauch (2016a). Also in gills of *N. granulata*, Tresguerres et al. (2008) identified an apical Cl^-/HCO_3^--exchanger that would provide an apical exit of HCO_3^- as indicated in the proposed model (◰ Fig. 6.3d). In the gills of *C. maenas*, two isoforms of branchial carbonic anhydrase have been identified, a cytoplasmic and a membrane bound isoform (Boettcher et al. 1990; Serrano and Henry 2008). Supported by findings of the study of Siebers et al. (1994), carbonic anhydrase played a role in branchial acid–base regulation as observed in the inhibitor study by Fehsenfeld and Weihrauch (2016a).

The described epithelial transport processes closely resemble mechanisms that are observed in the mammalian kidney. While the crustacean epithelial ion regulatory mecha-nisms based on ◰ Fig. 6.3a has been compared to the thick ascending limb of the mamma-lian kidney in the past (Riestenpatt et al. 1996), the proposed new model for branchial acid–base regulation and its link to ammonia regulation as seen in ◰ Fig. 6.3d additionally resembles features of the mammalian kidney collecting duct (Weiner and Verlander 2013).

6.4.2 Genetic Responses to Acid–Base Disturbance

Two microarray and transcriptomic studies have identified changes in (mRNA) expression levels of gill epithelial transporters upon environmental disturbances that helped identify some of the candidate genes involved in acid–base regulation.

Interestingly, exposure to hypercapnia (400 Pa for 7 days) did not seem to elucidate a typical stress response in posterior gills of osmoregulating green crabs. Applying microarray and quantitative real-time experiments, Fehsenfeld et al. (2011) observed generally only subtle changes in mRNA expression levels among over 4400 genes in *C. maenas* and did not identify any changes in heat-shock proteins resembling direct indicators for stress. Instead, the data suggested an increased contribution of vesicular membrane transport, indicating that the proposed vesicular transport for active ammonia excretion (Weihrauch et al. 2002; ◘ Fig. 6.3b) might indeed contribute to $H^+(NH_4^+)$ excretion and therefore acid–base regulation in this species. Additionally, most of the annotated common ion transporters of the gill epithelium were not differentially expressed with the exception of a significant upregulated calcium-activated chloride channel and the downregulated Cl^-/HCO_3^- exchanger of the SLC 4 family, as well as a downregulated glycosyl-phosphatidylinositol-linked carbonic anhydrase VII. Interestingly, these genes were also affected by acclimation of green crabs to dilute salinity (Towle et al. 2011). One of the most downregulated transcripts in the hypercapnia/microarray study, the hippocampus abundant gene transcript or 1 (initially falsely annotated as a hyperpolarization activated nucleotide-gated potassium channel), has been confirmed to have significantly reduced mRNA expression levels in posterior gill 7 after 7 days of hypercapnia, as well as HEA-acclimation (Fehsenfeld and Weihrauch, unpublished data). A very similar response (downregulation) was observed in HCN as identified in the recent study of Fehsenfeld and Weihrauch (2016b). Both genes are therefore interesting novel candidate genes for further studies in respect to branchial acid–base regulation.

In a different study, changes in branchial mRNA expression levels of a number of important epithelial transporters were monitored by quantitative real-time PCR after acclimation of *C. maenas* to hypercapnia (Fehsenfeld and Weihrauch 2013). Similar to the results of the microarray study mentioned above, only subtle changes in mRNA expression were observed in individual gills, but the experiments indicated a role for the Rhesus-like protein, Na^+-K^+-ATPase and glycosyl-phosphatidylinositol-linked carbonic anhydrase VII in branchial acid–base regulation, as well as potentially the Na^+/H^+-exchanger and anion exchanger.

A different picture is generated in the branchial response of the great spider crab *Hyas araneus* upon exposure to different levels of environmental pCO_2 combined with varying temperatures (Harms et al. 2014). While Na^+/K^+-ATPase was upregulated following hypercapnia alone and hypercapnia in combination with temperature, mRNA levels of V-(H^+)-ATPase and carbonic anhydrase were only significantly elevated upon moderate and severe hypercapnia (Harms et al. 2014). Additionally, changes in genes involved in metabolism indicated an enhanced aerobic metabolism in response to moderate hypercapnia, while severe hypercapnia induced a metabolic depression. Specifically, decreased trehalose metabolism of the gills seems to be a common response of hypercapnia as well as temperature acclimation in *H. araneus*.

Similar to the response of *C. maenas* (Fehsenfeld et al. 2011), analysis of the Gene Ontology terms (GO-terms) indicated a restructuring of the gill epithelium and/or the cytoskeleton upon hypercapnia in *H. araneus* (Harms et al. 2014), a phenomenon that can also be observed upon acclimation to dilute salinity in posterior gills of *C. maenas* (Compere et al. 1989). In contrast to *C. maenas*, however, gill epithelia of *H. araneus* seem to undergo a pronounced stress response that includes the elevation of genes involved in intracellular oxidative stress defence, including a number of peroxidases.
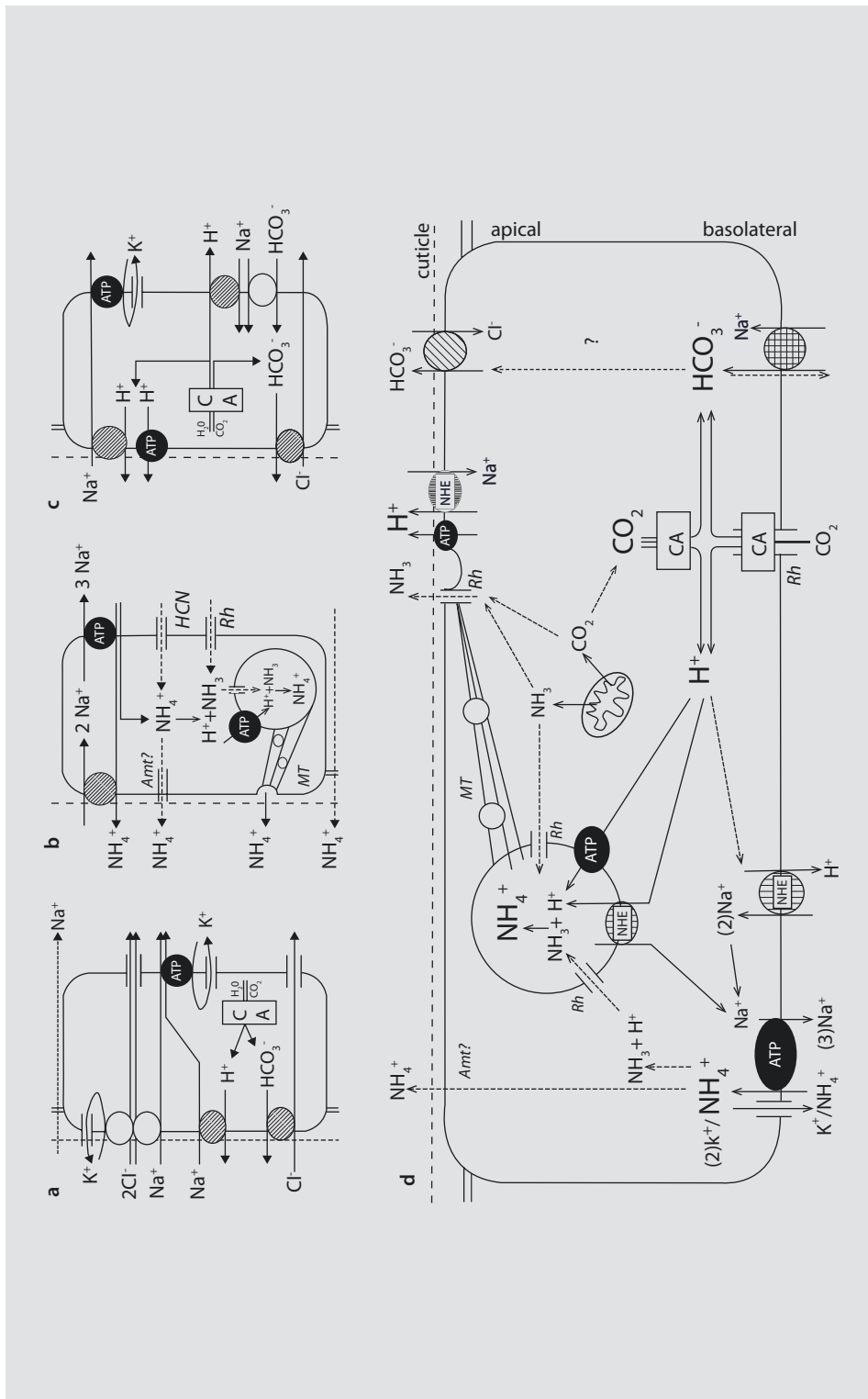
◘ **Fig. 6.3** Current working models for branchial ion regulatory processes in decapod crustaceans. (**a**) Osmoregulation, depicted after Onken et al. (2003); (**b**) ammonia excretion in the weak hyper-osmoregulator *C. maenas*, depicted after ► Chap. 1; and (**c**) general hypothetical model for acid–base regulation, depicted after Freire et al. (2008). (**d**) Depicts a recently developed model for acid–base regulation and its linkage with ammonia regulation in *C. maenas* after Fehsenfeld and Weihrauch (2016a). Potential overlaps in the transporter inventory of all models are basolateral Na⁺/K⁺-ATPase, basolateral K⁺-channels (including a member of the HCN family, Fehsenfeld and Weihrauch 2016b) and potential apical Na⁺/H⁺-exchanger. (**a, c, d**) Also share a potential apical HCO_3^-/Cl⁻-exchanger. Light dashed lines to the left (**a–c**) and top (**d**) indicate the cuticle covering the apical membrane. Notice the different orientation of panel D. *Blue colour* indicates the involvement of the respective transporter only in acid–base, but not in ammonia excretion. *Bold* and *bigger letters* indicate proposed major routes for transport of respective acid-base equivalents. *Amt* ammonia transporter, *ATP* ATPase, *CA* carbonic anhydrase, *NHE* Na⁺/H⁺-exchanger, *MT* microtubule network, *Rh* Rhesus-like protein

6.5   Linking Acid–Base to Ammonia Regulation

Even though ammonia excretion in decapod crustaceans has been the focus of many studies, the potential importance of ammonia regulatory patterns in respect to acid–base regulation has not been acknowledged to date. Generally, ammonia exists in a pH-dependent equilibrium between the weak base NH_3 and its acidic form NH_4^+. With a pKa of 9.15, most ammonia is present as NH_4^+ at physiological pH (Weiner and Verlander 2013). Due to its physical properties, ammonia (and therefore ammonia excretion) might therefore very well contribute to acid–base homeostasis as an additional hemolymph buffer beside the carbonate system. Being the primary waste product of protein catabolism, NH_3/NH_4^+ levels are ultimately linked to the overall metabolic rate of the organism. As mentioned earlier, metabolic rates of decapod crustaceans are individually adjusted when experiencing external stressors that simultaneously also affect acid–base homeostasis. In response to hypoxia, for example, metabolism and hemolymph ammonia decreased significantly in *N. norvegicus* (Hagerman et al. 1990). A similar response was seen in *M. magister* when exposed to hypercapnia and included also a significant decrease in ammonia excretion rates (Hans et al. 2014). In *C. maenas* on the other hand, hemolymph ammonia as well as whole animal ammonia excretion increased significantly upon exposure to hypercapnia in both full-strength seawater and brackish-water acclimated specimen (Fehsenfeld and Weihrauch 2013; Fehsenfeld and Weihrauch 2016a). Interestingly, NH_4^+ excretion by individual gills of brackish-water acclimated *C. maenas* closely mirrored their H^+ excretion, indicating that acid excretion over the gill epithelium was mainly accomplished by NH_4^+ excretion (Fehsenfeld and Weihrauch 2013). Blocking basolateral V-(H^+)-ATPase, Na^+/K^+-ATPase and general K^+-channels (Fehsenfeld and Weihrauch 2016a), as well as the recently identified transporter HCN (Fehsenfeld and Weihrauch 2016b) by transporter-specific pharmaceuticals, simultaneously affected branchial NH_3/NH_4^+ excretion as well as the excretion of acid–base equivalents in this decapod crustacean.

Furthermore, as an identified key player in branchial ammonia excretion in crustaceans (Weihrauch et al. 2004b; Martin et al. 2011), Rhesus proteins have recently been strongly suggested to not only mediate NH_3 but to also act as CO_2 channels in human red blood cells (Endeward et al. 2008; Kustu and Inwood 2006; Musa-Aziz et al. 2009; Soupene et al. 2002, 2004) and fish gills (Perry et al. 2010). Interestingly, this protein is significantly downregulated in *C. maenas* in anterior gill 4 in response to hypercapnia (Fehsenfeld and Weihrauch 2013), as well as in posterior gills in response to both hypercapnia and high environmental ammonia (HEA; Fehsenfeld, pers. communication), but is significantly upregulated in HEA-acclimated *M. magister* (Martin et al. 2011), clearly indicating its role in acid–base regulation and providing a link to ammonia regulation in these decapod crustaceans.

6.6   Conclusion

Thanks to the numerous descriptive studies, we presently have a very good understanding of how various environmental factors influence acid–base homeostasis in aquatic decapod crustaceans. Accomplished mainly via adjustments of extracellular HCO_3^- concentrations and the correlated excretion of acid- and/or base equivalents, possibly connected to changes in the strong ion difference, crustaceans are capable of efficiently counteracting acid–base disturbances. Even though the collected data support a major role for

the gill epithelium and the respective ion exchange processes in this organ in acid–base homeostasis, information on the actual mechanisms contributing its regulation are sparse. Studies on gill epithelial transporters involved in branchial acid–base regulation, however, deliver strong indications for a close link between ion and especially ammonia and acid–base regulation in the crustacean gill. The proposed mechanisms therefore resemble closely what is observed in the mammalian kidney, specifically in the thick ascending limb and the collecting duct. Future work needs to verify the localization of most of the respective proposed transporters in the crustacean gill epithelium and possibly other organs.

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