To Burnett
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Chapter 9

Osmoregulatory, Digestive, and Respiratory Physiology

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INTRODUCTION

The blue crab *Callinectes sapidus* offers an opportunity to ask physiological questions of an animal capable of thriving in a variety of aquatic environments. Its tolerance to changes in salinity, for example, is matched only rarely by other species. Similarly, its ability to adjust its respiratory function to the demands of active swimming, hypoxia, and air exposure is particularly remarkable. In this chapter, we seek to integrate many of the experiments that attest to the blue crab's physiological powers, focusing where possible on the mechanistic aspects of each adaptation to environmental challenge. In a few cases, we can bring molecular biology to bear on the question, but much remains to be accomplished before we truly understand how this animal functions.

OSMOREGULATION

One of the most remarkable features of the physiology of the blue crab is its ability to tolerate and indeed thrive in a broad range of environmental salinities. Apparently healthy individuals are found in fresh water environments as well as hypersaline lagoons (Hedgepeth 1967; Mangum and Amende 1972). In contrast, other species within this genus (e.g., *Callinectes similis*) may possess diminished tolerance to low salinities (Engel 1977; Piller et al. 1995; Guerin and Stickle 1997). A comparison of the osmoregulatory physiology of *C. sapidus* with *C. similis* reveals that the hemolymph of both organisms is essentially isosmotic with the medium in salinities above 27[1]. Thus, in high salinities, the hemolymph osmolality tracks that of the medium, suggesting that net uptake or excretion of osmolytes is not occurring. Below 27 salinity, both species are capable of maintaining a hyperosmotic hemolymph (Mantel 1967; Ballard and Abbott 1969; Guerin and Stickle 1997). However, *C. sapidus* shows substantially stronger osmoregulatory ability with respect to not only the blood-medium difference but also the extent to which regulation is maintained into very dilute salinities (Engel 1977; Mangum and Towle 1977; Piller et al. 1995) (Fig. 1).

What osmoregulatory mechanisms does *C. sapidus* possess that are lacking or less strongly expressed in some closely related species? Because the major osmolyte in crab hemolymph is NaCl, an investigation of the processes by which the blue crab transports Na^+ and Cl^- will likely reveal important insights (reviewed by Mantel and Farmer 1983; Lucu 1990, 1993; Towle 1990). Intact *C. sapidus*

[1] Salinity is presented as a pure ratio with no dimensions or units, according to the Practical Salinity Scale (UNESCO 1985).

acclimated to fresh water exchange Na^+ and Cl^- with the medium and exhibit a net influx of both ions (Cameron 1978a, 1979). Amiloride, an inhibitor of Na^+/H^+ exchange and epithelial Na^+ channels, blocks Na^+ influx in these experiments with little effect on Cl^- transport. Thiocyanate, a competitive inhibitor of Cl^- transport, reduces Cl^- influx with little effect on Na^+ transport (Cameron 1979). Thus, in studies with intact crabs, the transport mechanisms for Na^+ and Cl^- appear to operate independently.

The physiological function of the organ or organs responsible for NaCl regulation in brachyuran crabs is the subject of a large number of studies. Although the digestive and renal systems are specialized for a variety of transport processes, the gill epithelium has received the greatest emphasis with respect to osmoregulatory ion transport.

Ion Transport Systems in the Gill

Gill Structure

Each of the eight paired gills of blue crabs is described as phyllobranchiate, with about 8 lamellae per mm of gill length (Aldridge and Cameron 1982; Taylor and Taylor 1992) (Fig. 2). The lamellae, in contact with the external medium, are perfused internally with hemolymph via afferent and efferent blood vessels (Johnson 1980). Each of the lamellae is composed of a cuticular envelope lined by a single layer of epithelial cells that encloses a hemolymph space. Pillar cells containing extensive microtubules separate the two epithelial layers (Fig. 3). The lamellar structure, with its single layer of epithelial cells, provides an elegantly simple interface between the environment and internal milieu, serving a multifunctional role in gas exchange and ion transport as well as acid-base regulation and nitrogen excretion. The circulatory system of *C. sapidus,* which has

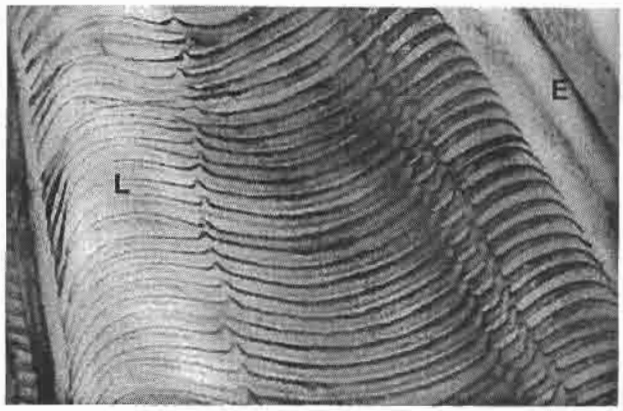

Figure 2. Scanning electron micrograph of gill lamellae from the blue crab *C. sapidus*. Lamellae [L] and efferent blood vessel [E] are shown. (x35). Courtesy D.E. Lovett.

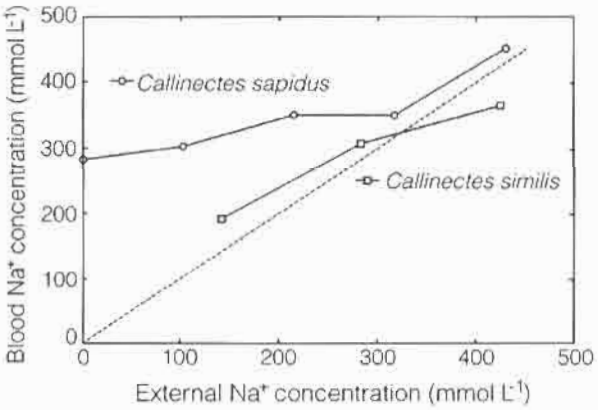

Figure 1. Regulation of hemolymph Na^+ in the blue crab *Callinectes sapidus* (-o-,Mantel 1967) and the lesser blue crab *Callinectes similis* (-□-, Piller et al. 1995) as a function of external Na^+ concentration. Dashed line represents isoionic values.

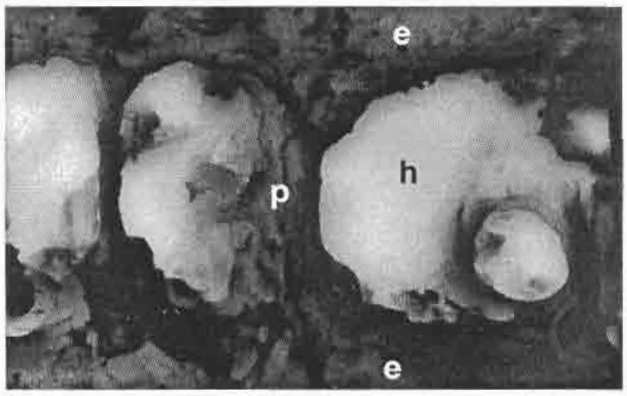

Figure 3. Scanning electron micrograph of interior of a single gill lamella of *C. sapidus* showing pillar cells [p] and epithelial layers [e] defining a hemolymph space [h]. The external cuticle layers are not visible in this micrograph. (x2,500). Courtesy D.E. Lovett.

been recently mapped (McGaw and Reiber 2002; see also McGaw and Reiber, Chapter 5), reveals a system of highly developed arteries dividing into smaller capillary-like vessels that ramify profusely within individual organs and a return network of vessels or sinuses that are discrete channels.

The epithelial cells are of two major types: thin (0.5-1.5 μm) cells believed to function primarily in gas exchange and thick (8-10 μm) cells believed to function in ion transport (Copeland and Fitzjarrell 1968; Towle and Kays 1986) (Fig. 4). The apical membrane of thin cells is closely apposed to the internal surface of the cuticle and the basolateral membrane is modestly elaborated with folds that run parallel with the cuticle. Diffusion distance between environment and hemolymph is minimized. In contrast, the apical membrane of thick cells is conspicuously folded, as is the basolateral membrane with its perpendicular folds nearly reaching the apical region of the epithelial cell. Abundant mitochondria are closely associated with the basolateral membrane of thick cells, producing a cell type that is typical of ion-transporting cells in a wide variety of epithelia.

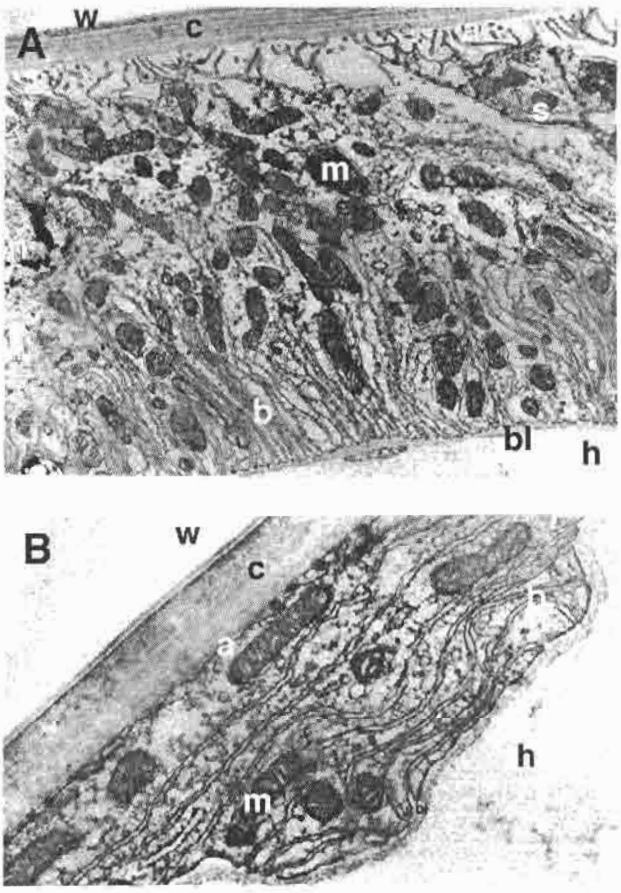

Figure 4. Transmission electron micrographs of gill epithelium from *C. sapidus*. (A) Section of thick epithelium (x13,900). (B) Section of thin epithelium (x31,000). Apical membrane [a]; basolateral membrane [b]; basal lamina [bl]); cuticle [c]; hemolymph space [h]; mitochondrion [m]; nucleus [n]; septate junction [s]; water space [w]. From Towle and Kays (1986).

Thick cells in the gill epithelium of the blue crab are restricted to the posterior four gill pairs and are organized in a discrete patch in each lamella adjacent to the afferent blood vessel (Copeland and Fitzjarrell 1968). This patch of thick cells proliferates when crabs are transferred from high to low salinities, with fresh-water-acclimated crabs showing a substantially greater area of thick cells compared with seawater-acclimated animals (Copeland and Fitzjarrell 1968; Aldridge and Cameron 1982). The expansion of the patch area is initiated by the 5th day after transfer to low salinity and is complete by the 7th day (MacDonald and Towle, unpubl. data) (Fig. 5).

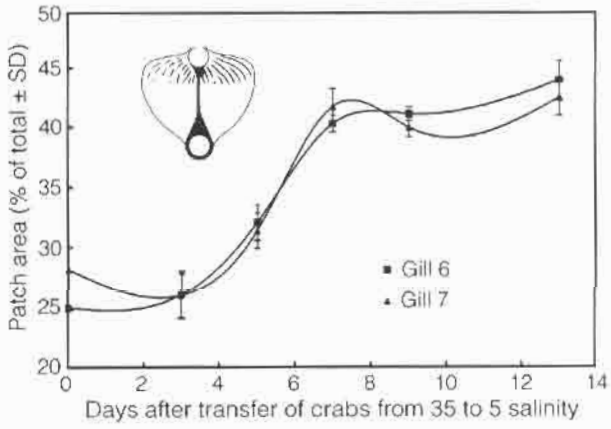

Figure 5. Increase in area of thick epithelium in gill lamellae of *C. sapidus* transferred from 35 to 5 salinity (MacDonald and Towle, unpublished). Areas of three representative lamellae from gills 6 (■) and 7 (▲) (of 8 total) of 3 to 4 male crabs were measured according to the method of Aldridge and Cameron (1982). Inset showing shaded region of thick epithelium is from Copeland and Fitzjarrell (1968).

Na^++K^+-ATPase

The plentiful mitochondria in the thick epithelial cells provide chemical energy in the form of ATP for transport and other energy-requiring processes. Among the major users of ATP in the gill of *C. sapidus* is the sodium pump, or Na^++K^+-ATPase. This transmembrane protein is restricted to the basolateral membrane of gill epithelial cells (Towle and Kays 1986) where it has access to the cytosol of the cell as well as the hemolymph in the blood space. Hydrolysis of intracellular ATP by the Na^+ + K^+-ATPase is coupled to the ejection of Na^+ ions from cytosol to hemolymph in exchange for K^+ or NH_4^+ ions moving from hemolymph to cytosol (Towle and Hølleland 1987).

Although the stoichiometry of the blue crab Na^++K^+-ATPase is not known, the sodium pump of other cells typically exchanges three Na^+ ions for two K^+ or NH_4^+ ions and is thus electrogenic, producing a charge difference across the basolateral membrane. This charge difference, as well as the Na^+ gradient itself, can be used as the driving force for a variety of other transport systems, including transporters situated at the apical membrane in contact (via the cuticle) with the external environment. Note that ions in the external environment do not have direct access to the Na^++K^+-ATPase, but rather must first enter the epithelial cell by other mechanisms.

That the gill of *C. sapidus* is capable of net Na^+ uptake has been shown by experiments with isolated perfused gills, in which the internal medium can be varied independently of the external medium. In the absence of an electrochemical gradient, where internal and external media are identical, net Na^+ influx can be demonstrated (Mantel 1967). Under conditions resembling those in crabs acclimated to low salinity, where internal and external media are not identical, net Na^+ influx can also be shown. In the latter case, the addition of the reducing agent glutathione was necessary to demonstrate net influx against a large concentration gradient, from an external medium containing 1 to 80 mmol Na^+ L^{-1} across the gill epithelium into an internal medium containing 273 mmol Na^+ L^{-1} (Burnett and Towle 1990). Influx of Na^+ in this preparation was strongly dependent on the external Na^+ concentration, exhibiting a K_m = 23 mmol Na^+ L^{-1} (Fig. 6), and could be inhibited by external amiloride or internal ouabain (an inhibitor of Na^++K^+-ATPase). Metabolic poisons reduced gill ATP content to 13% of controls and Na^+ influx to 20 to 30% of controls (Burnett and Towle 1990).

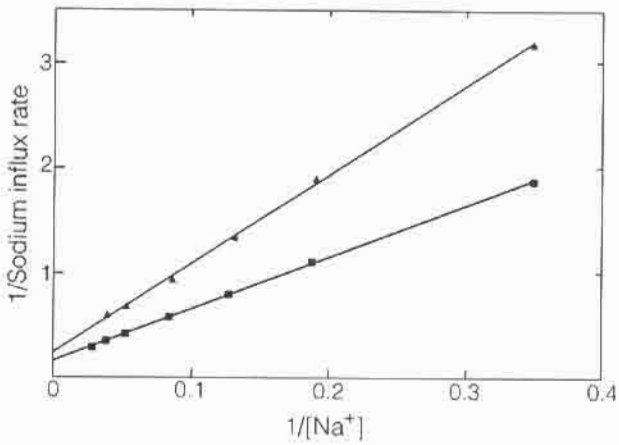

Figure 6. Dependence of the rate of $^{22}Na^+$ influx on Na^+ concentration in the external medium in isolated perfused posterior gills of *C. sapidus*, displayed as a double reciprocal (Lineweaver-Burke) plot. Data show two different gill preparations. Calculated K_m for Na^+ is 23 mmol Na^+ L^{-1}. Redrawn from Burnett and Towle (1990).

The conclusion from these studies is that Na^+ uptake across the blue crab gill requires a supply of ATP and is mediated, at least in part, by the Na^++K^+-ATPase situated in the basolateral membranes of the thick epithelial cells. Because the sodium pump is restricted to the basolateral membrane of these cells, it functions only in the transfer of Na^+ ions from the intracellular space to the hemolymph. The mechanism by which Na^+ enters the gill epithelial cell from the external milieu across the apical membrane is less clear. Three candidate transporters possibly associated with the apical membrane are receiving attention: a Na^+/H^+ exchanger, an epithelial Na^+ channel linked to a vacuolar-type H^+-activated ATPase, and a $Na^+/K^+/2$ Cl^- cotransporter (Fig. 7).

Sodium/Hydrogen Exchanger

A family of Na^+/H^+ exchangers has been identified in animal cells, including six isoforms in mammalian cells alone. The Na^+/H^+ exchanger protein is composed of a single polypeptide chain that traverses the lipid bilayer of the plasma membrane 10 to 12 times. The isoforms differ in their sensitivity to amiloride and amiloride analogs, their subcellular location, and their expression level in specific tissues (reviewed by Orlowski and Grinstein 1997). The Na^+/H^+ exchangers are believed to function in pH regulation, volume regulation, transepithelial Na^+ movement, and response to growth factors in a variety of animal cells. Their role in epithelial cells of crustacean gill is just beginning to be investigated.

A functionally unique Na^+/H^+ exchanger has been described in crustacean epithelial cells, namely one that apparently exchanges two sodium ions for one hydrogen ion, unlike the 1:1 stoichiometry observed for all vertebrate Na^+/H^+ exchangers. Such an electrogenic exchanger has been demonstrated in membrane vesicle preparations from gills of portunid crabs (*C. sapidus* and the green crab *Carcinus maenas*) (Maiolo 1988; Shetlar and Towle 1989) and from hepatopancreas of the American lobster *Homarus americanus* (Ahearn and Clay 1989) and prawn *Macrobrachium rosenbergii* (Ahearn et al. 1990). Analysis of the properties of the electrogenic exchanger from *C. maenas* gill reveals sigmoid kinetics with an apparent K_m of 34 mmol L^{-1} (Shetlar and Towle 1989) (Fig. 8), similar to the value of 23 mmol L^{-1} observed for Na^+ uptake by intact blue crab gill (Burnett and Towle 1990). In lobster hepatopancreas, the electrogenic exchanger is localized in the apical membrane of epithelial cells, poised for Na^+ uptake from the lumen of this tubular tissue (Kimura et al. 1994). Its subcellular location in crab gill is not known.

Molecular biological analysis has revealed a Na^+/H^+ exchanger in gills of *C. sapidus* and *C. maenas* similar in amino acid sequence to the mammalian Na^+/H^+ exchangers (Newton et al. 1996; Towle et al. 1997a). Starting with RNA extracted from posterior gills of *C. maenas* and reverse transcribed to cDNA, a Na^+/H^+ exchanger fragment

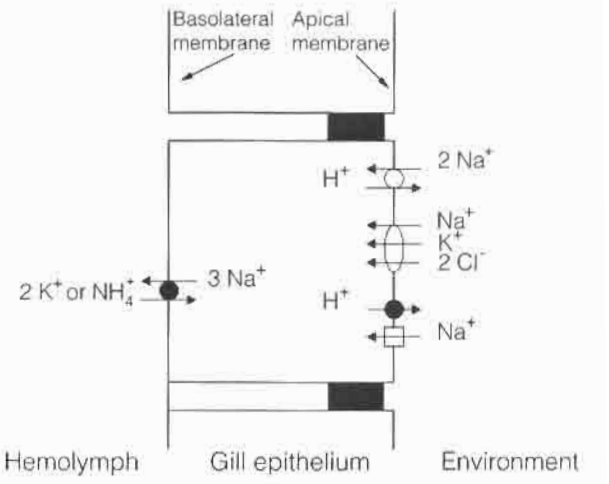

Figure 7. Model of thick epithelial cell in posterior gills of *C. sapidus* showing candidate transporters associated with uptake of Na^+ from the ambient medium. Solid circles: Na^++K^+-ATPase (basolateral membrane) and V-type H^+-ATPase (apical membrane). Open circle: Na^+/H^+ exchanger. Oval: $Na^+/K^+/2Cl^-$ cotransporter. Square: epithelial Na^+ channel. Based on Towle (1997).

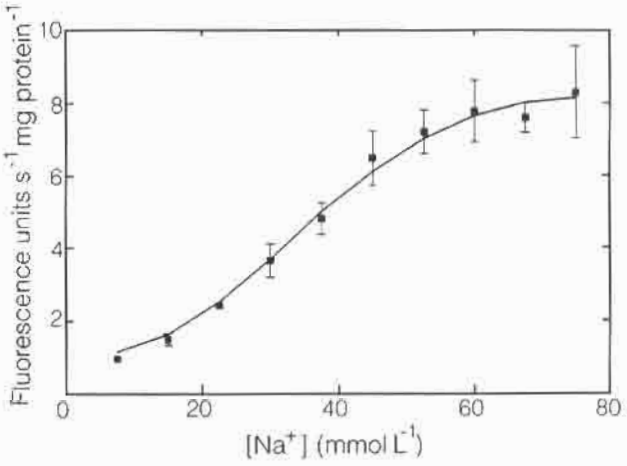

Figure 8. Sigmoid kinetics of Na^+/H^+ exchange by membrane vesicles from posterior gills of the green crab *C. maenas*. The calculated K_m for Na^+ is 34 mmol L^{-1}. Hill analysis revealed a Na^+:H^+ stoichiometry of 1.99, indicating that the crustacean Na^+/H^+ exchanger is electrogenic. Redrawn and used with permission from Shetlar and Towle (1987). Similar kinetics were observed for vesicles prepared from gills of *C. sapidus* Maiolo (1988). Mean ± S.E.

was successfully amplified using the polymerase chain reaction. Subsequent amplification and sequencing revealed a complete 2595-base-pair cDNA sequence encoding a 673-amino-acid protein (Genbank Accession Number U09274) (Towle 1997; Towle et al. 1997a). Sequencing a putative Na^+/H^+ exchanger fragment from *C. sapidus* indicated a 90% amino acid similarity with the *C. maenas* exchanger (Newton et al. 1996).

The exchanger cloned from crab gill is very strongly expressed in both posterior and anterior gills of *C. maenas* (Towle et al. 1997a) and *C. sapidus* (Newton et al. 1996), with much lower levels of expression in muscle, hepatopancreas, heart, and hypodermis (Fig. 9). Such tissue-specific expression may reflect a specialization for Na^+ uptake from dilute salinities, consistent with an effect of amiloride on sodium uptake by intact crabs and perfused gills (Cameron 1979; Burnett and Towle 1990). However, it is not yet known whether the cloned exchanger is responsible for the electrogenic Na^+/H^+ exchange measured in gill membrane vesicles, because its stoichiometry has not been established despite extensive attempts with the *Xenopus* oocyte expression system (Towle et al. 1997a).

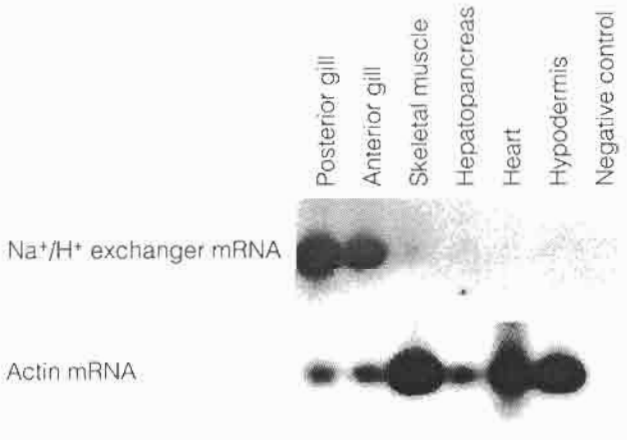

Figure 9. Expression of Na^+/H^+ exchanger mRNA in tissues of *C. maenas* compared with the "housekeeping" gene for actin, determined via quantitative polymerase chain reaction analysis. Redrawn from Towle et al. (1997a). Note that the exchanger gene appears to be most strongly expressed in anterior and posterior gills. Similar data were obtained for gills of *C. sapidus* (Newton et al. 1996).

If an electrogenic Na^+/H^+ exchanger were situated in the apical membrane of gill epithelial cells, then treatment with the inhibitor amiloride would be expected to disturb voltage gradients not only across the apical membrane but also across the epithelium as a whole. Indeed, in perfused gills of *C. sapidus* (Burnett and Towle, unpubl. data) and *C. maenas* (Siebers et al. 1987), amiloride treatment hyperpolarized the transepithelial potential, supporting an electrogenic process of Na^+ uptake at the apical membrane. However, an electrogenic Na^+/H^+ exchanger is not the only candidate to mediate such a response to amiloride; epithelial Na^+ channels inhibited by amiloride could function in this way as well (Zeiske et al. 1992).

Epithelial Na^+ Channels Coupled to Vacuolar-type H^+-ATPase

Epithelial Na^+ channels have been implicated in Na^+ uptake from fresh water by frog skin (Ehrenfeld and Klein 1997) and fish gill (Perry 1997). Coupling the function of these channels to a vacuolar-type (V-type) H^+-ATPase in the apical membrane is thought to produce a potential-driven exchange of Na^+ for H^+ that is independent of any Na^+/H^+ exchanger protein. According to this idea, the V-type H^+-ATPase ejects hydrogen ions from the cytosol across the apical membrane, producing a transmembrane voltage potential (inside negative) that would drive the uptake of sodium ions from the medium through Na^+ channels into the cytosol. Such coupling may provide for Na^+ uptake from very dilute media where Na^+/H^+ exchangers may not function because of their modest affinity for Na^+. Alternatively, the inside-negative potential may drive the efflux of chloride ions through basolateral Cl^- channels, a mechanism proposed to explain a likely role of V-type H^+-ATPase in energizing Cl^- uptake across gills of the Chinese crab *Eriocheir sinensis* (Onken and Putzenlechner 1995).

Although no molecular evidence so far exists for the presence of epithelial sodium channels or chloride channels in crab gill, molecular cloning experiments have demonstrated the presence of a V-type H^+-ATPase B subunit mRNA in gills of both *C. sapidus* and *C. maenas* (Weihrauch and Towle 1997).

A complete cDNA sequence has been determined for the V-type H^+-ATPase B subunit from *C. maenas* (Genbank Accession Number AF189779) (Weihrauch et al. 2001). Expression of the V-type H^+-ATPase B subunit mRNA in *C. maenas* gill does not respond to salinity reduction, and in fact appears to be greater in anterior gills than the ion-transporting posterior gills (Weihrauch et al. 2001). Thus, at least in *C. maenas*, the V-type H^+-ATPase does not appear to be intimately involved in transbranchial ion uptake. Whether V-type H^+-ATPase expression in *C. sapidus* gills is responsive to salinity variation is not yet known.

$Na^+/K^+/2\ Cl^-$ Cotransporter

The third major candidate for apical Na^+ uptake is the $Na^+/K^+/2\ Cl^-$ cotransporter, suggested as an important participant in Na^+ transport across isolated gills of *C. maenas* (Riestenpatt et al. 1996). The $Na^+/K^+/2\ Cl^-$ cotransporter of vertebrate cells consists of a single polypeptide chain predicted to contain 12 membrane-spanning regions (Kaplan et al. 1996). It mediates the electroneutral transport of the three ionic species unidirectionally, driven by a chemical gradient of one of the ions. Secretory and absorptive $Na^+/K^+/2\ Cl^-$ cotransporter isoforms have been described and cloned from several vertebrate species, initially by Forbush and colleagues (Xu et al. 1994). The secretory form is localized in the basolateral membrane and the absorptive form is localized in the apical membrane of epithelial cells (Haas 1994; Mount et al. 1998).

Molecular experiments designed to identify $Na^+/K^+/2\ Cl^-$ cotransporter mRNA in gills of *C. maenas* and *C. sapidus* produced positive results initially only with *C. sapidus* templates (Towle et al. 1997b). Subsequent experiments, however, have demonstrated its presence in *C. maenas* as well (Wiehrauch and Towle, unpublished). Sequence analysis of the blue crab $Na^+/K^+/2\ Cl^-$ cotransporter indicates a strong homology with the vertebrate $Na^+/K^+/2\ Cl^-$ cotransporters rather than the related Na^+/Cl^- and K^+/Cl^- cotransporters (Genbank Accession Number AF190129) (Towle 1998; Fig. 10). Polymerase chain reaction experiments indicate that the $Na^+/K^+/2\ Cl^-$ cotransporter gene is expressed

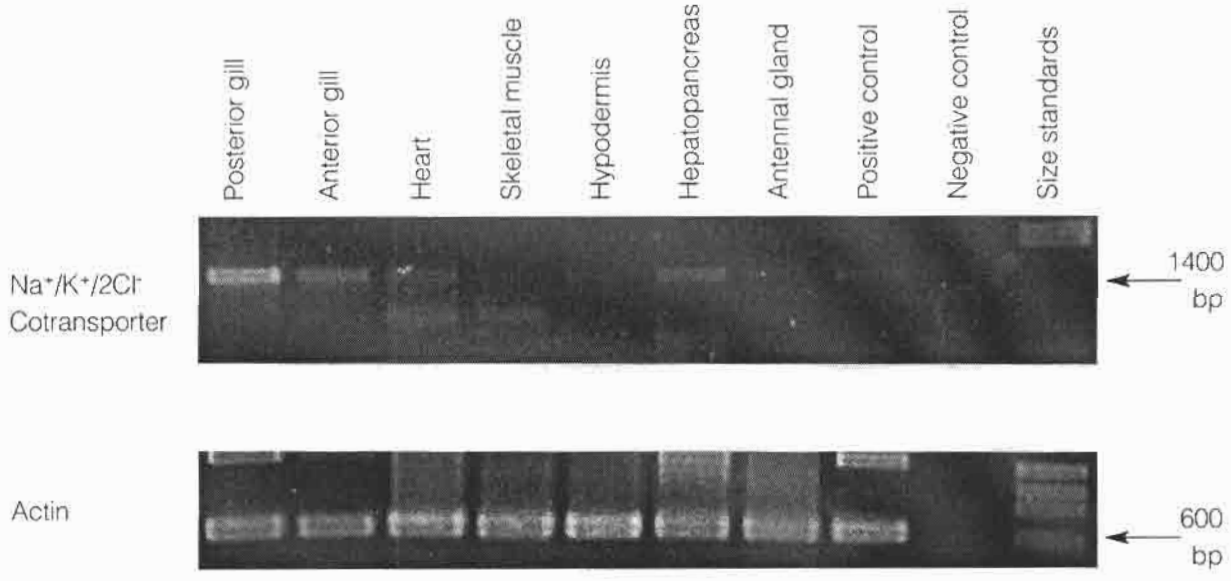

Figure 10. Amplification by the polymerase chain reaction of a $Na^+/K^+/2\ Cl^-$ cotransporter cDNA fragment from tissues of *C. sapidus* compared with actin. Sequencing confirmed that the 1400 base-pair [bp] band represents $Na^+/K^+/2\ Cl^-$ cotransporter. Note that the cotransporter gene appears to be most strongly expressed in posterior gill compared with anterior gill and other tissues. Data from Towle (1998).

most highly in the posterior gill, with lower levels of expression in the anterior gill, hepatopancreas, and heart (Fig. 10). Expression of the cotransporter thus parallels that of the Na^+/H^+ exchanger.

Chloride Transporters

Apart from the preliminary molecular studies of the $Na^+/K^+/2\ Cl^-$ cotransporter, little is known about transport mechanisms for chloride in the crab gill. Membrane vesicles from posterior gills of *C. sapidus* exhibit HCO_3^--dependent uptake of Cl^- that is not dependent on Na^+. Chloride transport in this system is blocked by inhibitors of anion exchange but not by amiloride, furosemide (an inhibitor of $Na^+/K^+/2\ Cl^-$ cotransporter), or ouabain (Lee and Pritchard 1985). The K_m for Cl^- of this Cl^-/HCO_3^- exchange system is 15 mmol L^{-1}, sufficient for Cl^- uptake from nearly fresh water. It is not known whether the rate of Cl^-/HCO_3^- exchange responds to salinity change.

A second candidate in anion transport across the gill is an HCO_3^--stimulated ATPase, described in microsomal membrane preparations from gills of *C. sapidus* (Lee 1982) and the fiddler crab *Uca minax* (DePew and Towle 1979). This HCO_3^--stimulated ATPase is blocked by thiocyanate and acetazolamide (at high concentrations) but not by ouabain or vanadate, inhibitors of $Na^+ + K^+$- ATPase. Specific activity of the HCO_3^--stimulated ATPase was reported to increase 2.5-fold upon acclimation of crabs from 35 to 7 salinity (Lee 1982). No molecular studies have been reported for either of these possible chloride transporters.

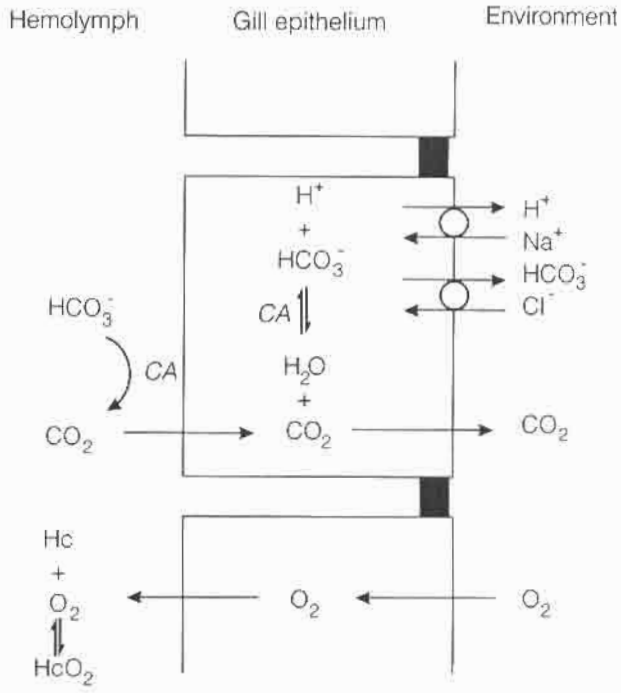

Figure 11. The transport of CO_2 across the gill of *C. sapidus* is facilitated by carbonic anhydrase situated in the basolateral membrane of gill epithelial cells. The large pool of hemolymph bicarbonate is dehydrated to form CO_2, which diffuses into the gill cells. There, the CO_2 may become rehydrated by the action of intracellular carbonic anhydrase or it may diffuse across the apical membrane into the ambient seawater. Bicarbonate ions and hydrogen ions generated by soluble carbonic anhydrase within the gill cells may provide counterions for Na^+/H^+ and Cl^-/HCO_3^- exchangers in the apical membrane.

Carbonic Anhydrase

An enzyme that plays a central role in ion transport across the gill of blue crabs is carbonic anhydrase (Fig. 11). A membrane-bound form of carbonic anhydrase, likely associated with the basolateral membrane of gill epithelial cells, is poised to facilitate CO_2 excretion by the gill (Burnett 1984; Burnett and McMahon 1985; Henry 1987), thus participating in acid-base regulation (see below). A separate cytoplasmic form of carbonic anhydrase, in contrast, possesses properties that suggest a critical role in blue crab ion regulation:

- The cytoplasmic carbonic anhydrase is found predominantly in the posterior (ion-transporting) gills, while the membrane-bound form is more evenly distributed between anterior and posterior gills (Henry 1988a).
- The cytoplasmic carbonic anhydrase increases in both total and specific activity upon dilution of the external salinity from 850 to 100 mOsm kg^{-1} (Henry and Cameron 1982).
- Injection of acetazolamide, a carbonic anhydrase inhibitor, disrupts ion regulation in dilute salin-

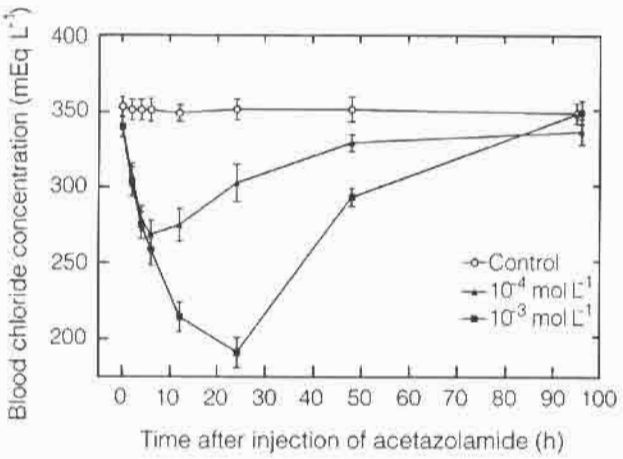

Figure 12. Effect of the carbonic anhydrase inhibitor acetazolamide on Cl^- concentrations in hemolymph of *C. sapidus* acclimated to 250 mOsm salinity. Data are presented for two concentrations (▲, 10^{-4} mol L^{-1}; ■, 10^{-3} mol L^{-1}) of injected acetazolamide compared with saline-injected controls (o). Mean ± S.E. Redrawn from Henry and Cameron (1983).

ity but has little effect on oxygen uptake or carbon dioxide excretion (Henry and Cameron 1983) (Fig. 12).

The conclusion from these studies is that cytoplasmic carbonic anhydrase supports ion regulation in crab gills by providing H^+ and HCO_3^- (from the hydration of CO_2), ions that serve as counterions for branchial Na^+/H^+ and Cl^-/HCO_3^- exchange processes (Henry 1984, 1996).

Recent molecular experiments have identified carbonic anhydrase mRNA sequences in gills of *C. sapidus* and *C. maenas* (Gehnrich et al. 1999). Reduced salinity elicits a dramatic increase in carbonic anhydrase mRNA expression, paralleling (and presumably preceding) the noted increase in carbonic anhydrase activity. The observed changes in carbonic anhydrase gene expression further confirm that this enzyme is intimately involved with active ion uptake across the gill of euryhaline crabs. However, the weakly osmoregulating *C. similis* demonstrates salinity-related activation of gill carbonic anhydrase and Na^++K^+-ATPase in a fashion similar to the strongly osmoregulating *C. sapidus* (Piller et al. 1995). Thus, the more modest ability of *C. similis* to regulate blood osmolytes may be related to differences in gill permeability or to limited expression or function of apical transporters, rather than to limitations of carbonic anhydrase or Na^++K^+-ATPase expression.

Regulation of Branchial Ion Transport

The movement of blue crabs from high salinity environments into estuaries is accompanied not only by the activation of ion uptake mechanisms but also by reduction of water permeability in the gills (Berlind and Kamemoto 1977; Cantelmo 1977; Robinson 1982). The recorded increase in abundance of thick ("ion-transporting") cells in the gill lamella would result in an enhanced capability for ion uptake (Copeland and Fitzjarrell 1968; Aldridge and Cameron 1982), at the same time reducing overall permeability of the gill epithelium. This cell proliferation apparently results not from an enhanced mitotic rate of pre-existing thick cells but from differentiation of precursor cells (Genovese et al. 2000).

Most experiments investigating the control of gill ion transport in blue crabs have focused on the sodium-pumping Na^++K^+-ATPase. The specific activity of this enzyme in the basolateral membrane is known to increase upon acclimation of blue crabs to dilute environments (Fig. 13) (Towle et al. 1976; Neufeld et al. 1980; reviewed by Towle 1993). A portion of the increased Na^++K^+-ATPase activity would arise simply from the proliferation of thick cells in the gill lamella. That is, the lamellar area containing a predominance of thick cells doubles with a time course of 5 to 7 d after transfer to low salinity. Regions of thick epithelium have about seven times more Na^++K^+-ATPase activity per mg total protein than thin cells (Towle and Kays 1986). It is thus not surprising that acclimation to fresh water or dilute salinities is accompanied by enhanced Na^++K^+-ATPase activity measured in homogenates or microsomes from posterior gills (Towle et al. 1976; Neufeld et al. 1980).

It has not been clear whether the well-documented enhancement of Na^++K^+-ATPase activity in crabs transferred from high to low salinity is the

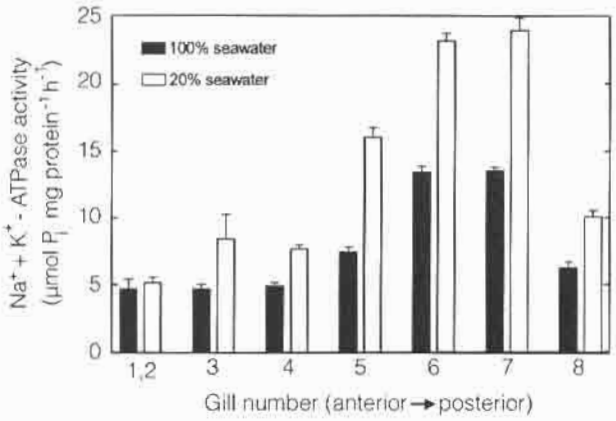

Figure 13. Na^++K^+-ATPase specific activity in homogenates of individual gills from *C. sapidus* acclimated to 100% (■) or 20% (□) seawater. Redrawn from Neufeld et al. (1980). Mean ± S.E. Note the doubling of enzyme activity in the posterior gills upon acclimation to dilute salinity.

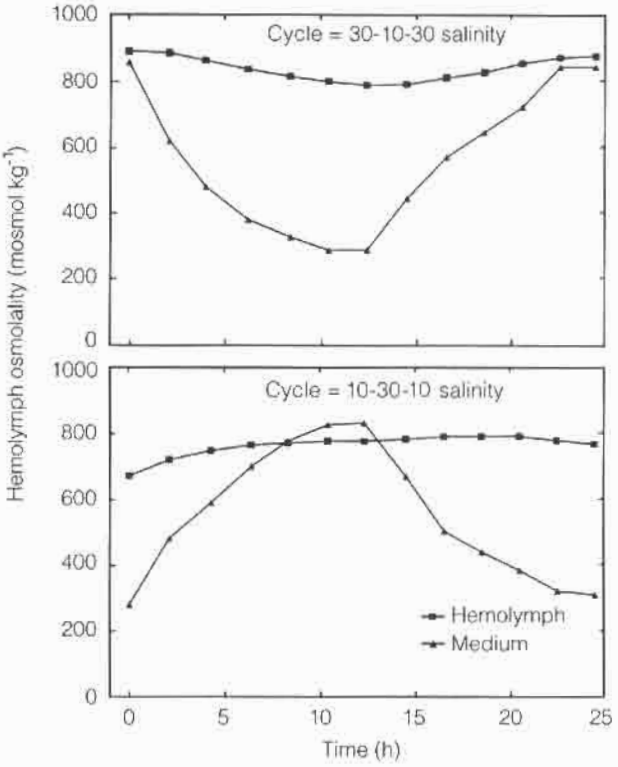

Figure 14. Regulation of hemolymph osmolality (■) in *C. sapidus* subjected to two different tidal regimens of external osmolality (▲). Tidal cycles, represented as salinity values, were initiated by gradually changing the salinity at 0 h and returning to the original salinity at 12 h. Mean ± S.E. Redrawn from Findley and Stickle (1978) with kind permission of Springer Science and Business Media.

result of activation of pre-existing enzyme or de novo synthesis of new enzyme. Recent molecular studies have shown that both mRNA and protein of the Na^++K^+-ATPase α-subunit accumulate to substantially higher levels in blue crabs acclimated to reduced salinity (Lovett et al. 2003). Thus the noted long-term changes in Na^++K^+-ATPase activity appear to be the result of upregulation of Na^++K^+-ATPase gene transcription, resulting in synthesis of new enzyme.

Increased Na^++K^+-ATPase activity in low salinities places a greater demand on the ATP supply system of gill cells. Part of this demand may be met through the action of arginine kinase, the major phosphagen kinase responsible for temporal ATP buffering in invertebrates. The enzymatic activity of arginine kinase doubles in gills of *C. sapidus* transferred from high to low salinity, although the mRNA content appears not to change (Kotlyar et al. 2000). The resulting doubling of ATP buffering capacity would support the enhanced pumping rate of Na^++K^+-ATPase.

The rate of oxygen consumption of excised *C. sapidus* gills tends to be higher in animals acclimated to 5 salinity compared to 35 salinity, and the ATP content tends to be lower (Engel et al. 1975). Intact blue crabs subjected to tide-related salinity fluctuation in the laboratory exhibit an inverse correlation between salinity and oxygen uptake while hemolymph NaCl concentrations barely vary (Findley et al. 1978; Findley and Stickle 1978) (Fig. 14). This finding suggests that ATP utilization by the Na^++K^+-ATPase, and thus pumping rate, are under dynamic short-term control. Indeed, there appears to be a component of ATPase activity that responds rapidly to salinity change (Towle et al. 1976; Lucu and Flik 1999).

Several lines of evidence suggest that the level of Na^++K^+-ATPase activity in ion-transporting cells of crab gills is regulated by the neuroendocrine system (Kamemoto 1991). Extracts of the pericardial organ of *C. sapidus* enhance Na^+ uptake by isolated gills, possibly via an induced increase of cyclic AMP

within gill epithelial cells (Lohrmann and Kamemoto 1987). The effect of pericardial organ extract, which contains dopamine, octopamine, and serotonin, could be mimicked by the first two amines (Kamemoto 1991). In *C. maenas*, injection of dopamine or cyclic AMP into intact crabs in full seawater increased gill Na^++K^+-ATPase activity and Na^+ uptake (Sommer and Mantel 1988, 1991). However, direct measurements of gill cyclic AMP in *C. maenas* revealed a negative correlation with Na^++K^+-ATPase activity (Lucu and Flik 1999).

Injection of hemolymph from hyperosmoregulating crabs into individuals in full seawater produced a small but significant increase in gill Na^++K^+-ATPase activity (Savage and Robinson 1983) but the nature of the hemolymph factor is not known. An interesting possibility was provided by an examination of methyl farnesoate concentrations in *C. maenas* subjected to 10 or 32 salinity. In crabs acclimated to the upper salinity, methyl farnesoate concentration in the hemolymph was 2.5 ± 0.8 ng ml^{-1}. Upon transfer to 10 salinity, concentrations increased to 32.7 ± 7.7 ng ml^{-1} within 8 h. The reversal experiment demonstrated a decline to basal levels within 6 h (Lovett et al. 1997). Although the response of methyl farnesoate to salinity change suggests that it may be involved with the hyperosmoregulatory response in *C. maenas*, it is not known if the activity of any gill transport system responds to such treatment.

Another clue to the nature of Na^++K^+-ATPase regulation has come from studies of polyamines and their direct effects on activity of the ATPase. Putrescine directly inhibits Na^++K^+-ATPase by raising its apparent K_m for Na^+. Moreover, concentrations of putrescine in gills of *C. sapidus* acclimated to 10 salinity are less than half those found in 35 salinity (Lovett and Watts 1995). The lowered concentration of this polyamine may therefore relieve inhibition of Na^++K^+-ATPase, providing a more permissive condition for enhanced transepithelial Na^+ movement.

Finally, preliminary molecular evidence suggests that an atrial natriuretic peptide is produced in the heart tissue of the blue crab (Poulos et al. 1995). This peptide causes a reduction in sodium transport in collecting ducts of the vertebrate kidney; whether it affects transport in crustacean gill remains to be investigated.

Ammonia Excretion by the Gill

Blue crabs transferred from 35 salinity to lower salinities show major increases in the blood level of ammonia, likely the consequence of deamination of amino acids leaving the cells (Mangum et al. 1976). The rate of ammonia excretion also increases under these conditions. In blue crabs living in fresh water, it is clear that the gills are the major site of ammonia excretion (Cameron and Batterton 1978). Although the un-ionized form of ammonia, NH_3 is traditionally believed to be freely diffusible through cellular membranes, the excretion of ammonia is not a simple process. The equilibrium between NH_3 and its ionized form, NH_4^+, is affected by pH, likely changing within and between cellular compartments that may vary considerably in their H^+ concentrations. In addition, measurements of NH_3 diffusion through lipid bilayers suggests that biological membranes may indeed present a significant permeability barrier to NH_3, dependent upon membrane fluidity (Lande et al. 1995).

With regard to the ionized form of ammonia, NH_4^+, the Na^++K^+-ATPase is capable of using NH_4^+ as an effective substitute for the counterion K^+ in supporting the uptake of $^{22}Na^+$ into membrane vesicles prepared from blue crab gills (Towle and Holleland 1987). Situated in the basolateral membrane of gill cells, the Na^++K^+-ATPase may thus mediate clearance of ionized ammonia from the blood into the cytosol of those cells. Indeed, the Na^++K^+-ATPase inhibitor ouabain has been shown to inhibit ammonia efflux by 44 to 47% when perfused through isolated gills of *C. maenas* (Lucu et al. 1989; Weihrauch et al. 1998).

The exit of ammonia from the cytosol across the apical membrane (and cuticle) could occur by diffusion of NH_3 or NH_4^+ (Kormanik and Cameron 1981) or it could occur by Na^+/NH_4^+ exchange perhaps mediated by the Na^+/H^+ exchanger. In one study with intact crabs in 17 or 35 salinity, the Na^+/H^+ exchanger inhibitor amiloride inhibited ammonia efflux by 63 to 67% (Pressley et al. 1981),

while in another study with crabs in 28 to 30 salinity, amiloride was ineffective (Kormanik and Cameron 1981). It may be helpful to use a more specific amiloride analog to differentiate between these two findings (Kleyman and Cragoe 1988). In perfused *C. maenas* gills, apical amiloride (0.1 mmol L^{-1}) reduced ammonia efflux by 55% (Weihrauch et al. 1998), supporting a role for cation transporters in this process.

An interesting alternative pathway is offered by the finding that the V-type H^+-ATPase inhibitor bafilomycin blocks ammonia excretion by perfused gills of *C. maenas* (Weihrauch et al. 1998). The flux of ammonia across cell membranes may thus respond to a proton gradient established by the V-type H^+-ATPase.

Ion Transport Systems in the Antennal Gland

The antennal gland of *C. sapidus* is composed of two principal regions, a coelomosac containing podocytes and a tubular labyrinth containing epithelial cells with microvilli and abundant basal mitochondria (Johnson 1980). The labyrinth is connected directly to the transparent bladder. Although the antennal gland is specialized for ultrafiltration of the hemolymph and probably for secretion of specific metabolites and xenobiotics, it is incapable of producing an anisosmotic urine with respect to the blood. Concentrations of the principal electrolytes (Na^+, K^+, and Cl^-) are only slightly different in blood and urine of blue crabs in fresh water (Cameron and Batterton 1978). The volume output of the antennal gland, however, responds very quickly to a dilution of environmental salinity. The rate of urine production doubles when crabs are moved from 35 to 17.5 salinity, balancing the osmotic uptake of water that occurs in reduced salinity (Robinson 1982).

Blue crabs in fresh water excrete a volume of urine equivalent to about 20% of their body weight per day, representing 31% of the total chloride efflux and 41% of the total sodium efflux from the animal (Cameron and Batterton 1978). Urinary output of ammonia is negligible, contributing just 1 to 2% of the total ammonia excretion, and net acid or base excretion in urine is also negligible (Cameron and Batterton 1978). The antennal gland of *C. sapidus* in fresh water thus appears to serve as a whole-body volume-regulating organ, contributing to major losses of monovalent ions that must be counterbalanced by ion uptake at the gill.

Ion Transport Systems in the Gut

Net absorption of Na^+ across the isolated midgut is sensitive to inhibition by ouabain, implicating Na^++K^+-ATPase as the driving force (Chu 1987). Indeed, the midgut of *C. sapidus* contains substantial levels of Na^++K^+-ATPase activity (Towle and Mangum 1985). However, no augmentation of Na^+ uptake across the midgut was noted in animals subjected to reduced salinities, suggesting that this tissue does not play an important role in controlling hemolymph Na^+ levels under conditions of osmoregulatory stress (Chu 1987).

Volume Regulation

In salinities below 27, *C. sapidus* regulates its blood osmotic concentration within a rather narrow range, declining only about 16% as the external osmotic concentration approaches zero (Mangum and Towle 1977). Consequently, a nearly constant osmotic environment is maintained within the animal, minimizing the osmotic impact of dilute (<27 salinity) environments on cells, with one notable exception. The epithelial cells of the branchial lamellae are separated only by a cuticular layer from the external environment and are in nearly direct contact with hemolymph, thus experiencing an osmotic gradient of as much as 600 mosmol L^{-1} in fresh water animals (Mangum and Amende 1972). The physiological and morphological specializations that permit branchial cells to function under these conditions are not at all understood.

The osmotic gradients between water and hemolymph that are observed in salinities below 27 disappear in salinities above 27, where the hemolymph osmotic concentration closely tracks that of the external medium (Fig. 1). As salinity rises above 27, the resulting osmotic stress on the internal

tissues will lead to loss of cell water and consequent reduction in cell volume. Conversely, if a crab is transferred from full-strength seawater (35 salinity) to a lower salinity, cells will tend to gain water and swell. Isolated nerve or muscle cells from *C. sapidus* readily demonstrate cell volume regulation in hypoosmotic conditions but do not regulate very successfully in hyperosmotic conditions over a 4-h experiment (Lang and Gainer 1969a, b; Gérard and Gilles 1972b) (Fig. 15).

Individual cells of *C. sapidus* are thought to respond to osmotic stress by regulating organic osmolytes, primarily the "non-essential" amino acids taurine, alanine, proline, and glycine (Gérard and Gilles 1972a; Engel 1977). In contrast to the inorganic salts NaCl and KCl, these osmolytes have minimal effect on intracellular enzyme function and thus may vary over a broad range without affecting metabolic activity (Bowlus and Somero 1979; Yancey et al. 1982). The most common free amino acid in claw muscle cells of *C. sapidus* is glycine, the concentration of which ranges from 199 to 256 mmol kg^{-1} tissue water, accounting for over 60% of the total free amino acid content of these cells. In

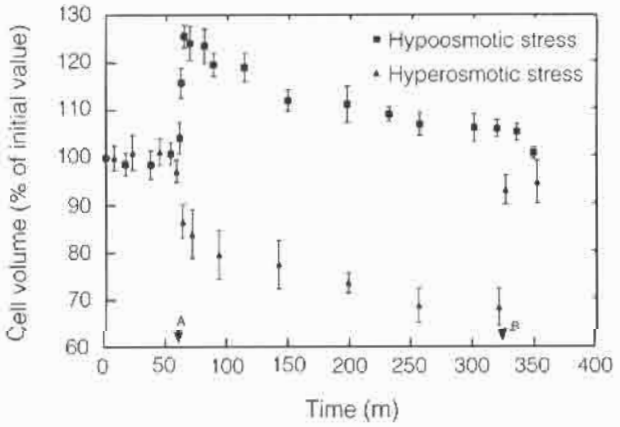

Figure 15. Volume regulation of isolated axons of *C. sapidus* subjected to hypoosmotic (■) or hyperosmotic (▲) treatment. Volume during the control period was considered as 100%. Mean ± S.E. Axons were maintained in 1100 (■) or 825 (▲) mosmol L^{-1} saline, then transferred at arrow A to 825 (■) or 1100 (▲) mosmol L^{-1} saline. At arrow B, the experimental solutions were returned to initial conditions. Redrawn from Gérard and Gilles (1972b).

nerve cells, an "undetermined ninhydrin-positive substance" along with alanine accounts for about 65% of the total organic osmolytes (Gérard and Gilles 1972a).

Free amino acids have been reported to accumulate in specific tissues of blue crabs living in full seawater, showing a corresponding decrease in 50% seawater. Such is the case in hepatopancreas, nerve, and muscle cells. Interestingly, free amino acid levels in gill cells are only about 20% of those in nerve and muscle cells, and do not change between full seawater and 50% seawater (Gérard and Gilles 1972a). It is not known whether the gill cells, which may be subjected to large transcellular osmotic gradients, contain substantial amounts of osmolytes other than salts and free amino acids.

A question exists as to whether the intracellular changes in free amino acid concentrations are the result of metabolic activity or membrane transport (Schoffeniels 1976). In blue crabs subjected to an abrupt decline in external salinity, higher concentrations of free amino acids appear in the hemolymph, likely exported by cells undergoing volume regulation (Lang 1987). In blue crab megalopae experiencing hyperosmotic stress, the rate of proline synthesis triples within 3 to 6 h after a shift from 17.5 to 35 salinity, then declines to control levels, resulting in an accumulation of proline within the cells (Burton 1992). Thus, accumulation of free amino acids appears to result from synthetic processes whereas their decline results from membrane transport.

DIGESTION

The gut is divided into three parts: the foregut, the midgut, and the hindgut (see Kennedy and Cronin, Chapter 3). Epithelia that are ectodermal in origin line the foregut and the hindgut. The midgut and its diverticula or ceca, including the hepatopancreas, are endodermal in origin. Unlike the foregut and the hindgut, the midgut lacks a cuticular lining. Johnson (1980) has written an excellent and thorough account of the anatomy and the histology of the gut of the blue crab.

In the foregut, a short esophagus is followed by a

large globular cardiac stomach and then a smaller pyloric stomach. The process of digestion is initiated by the cutting and tearing of food by the mandibles and other oral appendages. Food particles then pass into the muscular esophagus and peristalsis sends them to the cardiac stomach and then to the pyloric stomach. Parts of the cuticle in the cardiac stomach are thickened and calcified into ossicles that serve as support and as the masticatory apparatus (Johnson 1980). The pyloric stomach is a complicated valved structure in which food particles are filtered through a press and a gland filter. Only very fine food particles and liquid can pass into the ducts of the hepatopancreas (also known as the midgut gland) where final digestion and most absorption are thought to take place. The paired ducts of the hepatopancreas and the paired anterior midgut ceca arise from the anterior extremity of the midgut at the junction of the pyloric stomach. A singular highly coiled posterior cecum arises in the midgut at its posterior extremity (Johnson 1980). The hepatopancreas is thought to function in the absorption of nutrients, the storage of lipids, and the production of digestive enzymes. Unfortunately, few studies of nutrient or ion transport systems in blue crab hepatopancreas have been reported, although many such studies on *H. americanus* describe a variety of potentially relevant transport systems (Ahearn 1988; Ahearn et al. 1992). The functions of the midgut ceca are less clear.

The midgut is a straight tube that passes under the heart and the pericardial sinus. It is lined with columnar epithelial cells possessing extensive apical microvilli, numerous mitochondria, and intracellular vesicles (Johnson 1980). The midgut has been shown to absorb glucose through a Na^+-dependent pathway that can be blocked by metabolic inhibitors and ouabain (Chu 1986). However, the low rate of glucose transport across the midgut suggested that this tissue is not very important in total glucose absorption, leaving the hepatopancreas as the most likely major player in nutrient uptake. The midgut is thought to enclose material in the lumen in a peritrophic membrane. This material along with the peritrophic membrane is ultimately excreted.

Movement of material spiked with barium (15% by mass) through the digestive system of *C. sapidus* has been followed by radiography (McGaw and Reiber 2000). Food moved from the esophagus into the stomach within 15 min and was visible in the midgut after 2 h. After 6 h, material had reached the hindgut although some was still present in the stomach. Contents of the stomach were emptied between 8 and 10 h after ingestion and the entire digestive system was cleared after 18 h. McGaw and Reiber (2000) also measured circulatory variables as food passed through the gut. Heart rate increased as soon as the crab detected food and it remained elevated for 16 to 18 h after the food was ingested. The elevated heart rate was responsible for an increase in hemolymph flow (cardiac output), although cardiac stroke volume did not change significantly. Hemolymph flow increased in the sternal, anterolateral, and hepatic arteries, but remained unchanged in the anterior and posterior aorta. McGaw and Reiber (2000) associated the increase in hemolymph flow with the use of chelae and mouthparts in feeding, contraction of the visceral muscle surrounding the gut, and mobilization of enzymes in the hepatopancreas. Oxygen uptake increased significantly, reaching maximal levels 4 h after feeding, and decreasing slowly thereafter (McGaw and Reiber 2000). The increase in oxygen uptake is known as the specific dynamic action and is associated with the increased metabolic cost associated with digestion and absorption.

THE REQUIREMENT FOR OXYGEN

The demand for oxygen in *C. sapidus* has been reported by a number of investigators (Table 1). In well-aerated water between 20° and 25°C and between 20 and 35 salinity, oxygen uptake ranges from 2.08 to 9.2 μmol O_2 g wet weight^{-1} h^{-1}. Oxygen uptake increases at low salinity (Table 1) and decreases when crabs are exposed to air (O'Mahoney and Full 1984). Swimming activity causes a dramatic increase in oxygen uptake (Booth et al. 1982). After a crab molts, oxygen uptake increases significantly and gradually returns to premolt levels

Table 1. Oxygen uptake in *Callinectes sapidus* in a variety of different conditions.

	Conditions	O_2 Uptake ($\mu mol\ g^{-1}\ h^{-1}$)	Reference
	Salinity and Temperature		
Juveniles	7 salinity, 25°C	5.95	King 1965
	35 salinity, 25°C	3.83	
Adult	25-35 salinity, 20°-28°C	2.45	Batterton and Cameron 1978
Adult intermolt	10 salinity, 20°C	2.40	Findley et al. 1978
	30 salinity, 20°C	2.08	
Adult intermolt	10 salinity, 22°-25°C	4.59	Sabourin 1984
	30 salinity, 22°-25°C	4.47	
Juveniles, 2-6 g, male & female	27 salinity, 13°C	0.53	Leffler 1972
	27 salinity, 20°C	3.01	
	27 salinity, 27°C	2.34	
	27 salinity, 34°C	4.87	
Adult females, 125-200 g, from Chesapeake Bay, intermolt	32-34 salinity, 10°C	1.27	Robert and Gray 1972
	32-34 salinity, 20°C	3.41	
Adult males, 150-220 g, intermolt	10 salinity, 10°C	2.03	Laird and Haefner 1976
	30 salinity, 10°C	1.57	
	10 salinity, 24°C	3.71	
	30 salinity, 24°C	3.67	
Adult males, 134-200 g, intermolt	32-35 salinity, 5°C	0.55	Mauro and Mangum 1982a
	32-35 salinity, 15°C	2.56	
	32-35 salinity, 25°C	4.88	
	Air Exposure		
Submerged	35 salinity, 24°-26°C	3.28	O'Mahoney and Full 1984
Air exposure		1.15	
	Swimming		
Rest	32-33 salinity, 20°C	2.97	Booth et al. 1982
Exercise	"	7.72	
	Molting		
Intermolt	30-35 salinity, 24°-26°C	9.2	Mangum et al. 1985
D1	"	7.4	
D2	"	6.0	
D3	"	5.2	
D4	"	6.5	
Molt	"	no data	
A2	"	5.7	
B1 early	"	4.5	
B1 late	"	3.5	

(Mangum et al. 1985). Oxygen uptake doubles when the ambient temperature rises from 15° to 25°C and responds dramatically (Q_{10} = 4.92) when temperature is increased from 5 to 15°C (Mauro and Mangum 1982a). There are no known differences between oxygen uptake in male and female crabs (Laird and Haefner 1976).

Mechanisms of Gas Exchange

The mechanisms of the uptake of oxygen and the excretion of carbon dioxide in *C. sapidus* are well understood. A ventilatory stream of water is generated in each gill chamber by scaphognathites, or gill bailers, which are flattened exopodites located on the second maxilla (see Kennedy and Cronin, Chapter 3). The scaphognathite oscillates in a narrow channel, pushing water anteriorly through the upper lateral corners of the buccal frame and out in front of the head. This pumping action generates negative pressures within the branchial chamber. In response to this suction, water enters the branchial chambers primarily through openings at the base of the chelipeds, the Milne-Edwards' openings (Milne-Edwards 1839).

Water passes upward through the lamellae of the gills, then exits the crab anteriorly. The flow of water is countercurrent to the flow of hemolymph through the gills. The gills, eight on each side of the blue crab, are of the phyllobranchiate variety in which hemolymph flows from an afferent vessel in thin sheets toward a larger efferent vessel (reviewed by Taylor and Taylor 1992). The total surface area of the gills is 710 $mm^2\ g^{-1}$ (Aldridge and Cameron 1982), a value similar to that (770 $mm^2\ g^{-1}$) found in *C. maenas* (Hughes et al. 1969). The ventilation rate in a resting blue crab in well-aerated water at 25°C is 555 to 723 ml $kg^{-1}\ min^{-1}$ and 53% of the oxygen is extracted (Batterton and Cameron 1978).

Deoxygenated hemolymph accumulates in the infrabranchial sinuses located near the bases of the legs and enters the gills via the afferent vessel. This prebranchial hemolymph is considered to be mixed venous ($\bar{v}$) hemolymph and is low in oxygen and high in carbon dioxide. In quiescent blue crabs, the oxygen pressure of prebranchial blood ($P\bar{v}O_2$) is 8 to 20 torr (1 torr = 1 mm Hg) (Mangum and Weiland 1975; Booth et al. 1982; Mangum et al. 1985). The hemocyanin in prebranchial hemolymph is 42 to 53% saturated with oxygen, ensuring a significant venous oxygen reserve. As the hemolymph passes through the gills, the hemocyanin becomes fully saturated with oxygen and the Po_2 rises to between 35 and 100 torr. There are no paired values for Pco_2 in prebranchial and postbranchial hemolymph available. However, prebranchial-postbranchial differences in pH suggest that Pco_2 drops as hemolymph flows through the gill, yielding a small but measurable increase in hemolymph pH (Mauro and Mangum 1982a). The pH change across the gills is similar to that found in other decapod crustaceans.

Thus, in the resting blue crab oxygen is transported by a combination of mechanical pumps, the scaphognathite and the heart, and the oxygen-combining properties of its hemocyanin. The scaphognathite beating frequency in a resting crab is 94 beats min^{-1}, producing a branchial ventilation rate of 490 ml $kg^{-1}\ min^{-1}$ (Booth et al. 1982). From 40 to 60% of the oxygen in the inhalant water is extracted (Batterton and Cameron 1978; Booth et al. 1982; Mauro and Mangum 1982a). In a study of the relationship between the scaphognathite beat frequency and the branchial ventilation rate, Booth et al. (1982) concluded that scaphognathites do not act as fixed volume pumps and that the stroke volume decreases slightly as the scaphognathite beating rate increases. Furthermore, as in many other crustaceans, resting blue crabs exhibit ventilatory pauses where ventilation activity ceases or is greatly reduced (Booth et al. 1982).

Heart rate in a resting blue crab at 20° to 25°C is between 57 and 174 beats min^{-1} (deFur and Mangum 1979; Booth et al. 1982; Mauro and Mangum 1982a; Sabourin 1984). Cardiac output has been estimated from measurements of oxygen uptake and the prebranchial and postbranchial oxygen concentration difference. This estimate is derived from the Fick equation (eqn 1) in which Ca_{O_2} and $C\bar{v}O_2$ represent arterial and venous oxygen concentrations respectively. Cardiac output in a resting blue crab is 151 ml $kg^{-1}\ min^{-1}$ (Booth et al. 1982).

$$\text{Cardiac Output} = \frac{\text{Oxygen Uptake}}{Ca_{O_2} - C\bar{v}_{O_2}} \quad (1)$$

Carbon dioxide is excreted primarily through the gills. Unlike oxygen, molecular CO_2 undergoes hydration reactions and is in chemical equilibrium primarily with bicarbonate ions and carbonate ions. At physiological pH, approximately 98% of the total CO_2 in the hemolymph exists as bicarbonate ions. The hydration of CO_2 to carbonic acid (H_2CO_3) is very slow (eqn 2) and is rate limiting in regard to both CO_2 excretion and ion regulation (Burnett et al. 1981; Henry and Cameron 1982).

$$CO_2 + H_2O \xrightarrow{slow} H_2CO_3 \longrightarrow HCO_3^- + H^+$$
$$HCO_3^- \longrightarrow CO_3^{=} + H^+ \quad (2)$$

The enzyme carbonic anhydrase, which greatly accelerates interconversion between CO_2 and carbonic acid, is not present in blue crab hemolymph (Henry and Cameron 1982) but has long been known to occur in crab gills (Ferguson et al. 1937). As described in a previous section, intracellular carbonic anhydrase in gill tissues functions primarily in the hydration of intracellular CO_2 to form HCO_3^- and H^+ that can be exchanged at the apical surface of the gill for Cl^- (via chloride/bicarbonate exchange) and Na^+ (via sodium/hydrogen ion exchange) (Fig. 11). A separate carbonic anhydrase, located on the basolateral surfaces of the gills, dehydrates the relatively large hemolymph pool of bicarbonate ions to form molecular CO_2, which diffuses easily across the gills to the ambient water (Burnett 1984; Burnett and McMahon 1985; Henry 1987, 1988b).

Carbonic anhydrase has also been implicated in acid-base balance in the blue crab (Henry and Cameron 1983). In crabs acclimated to brackish water, the carbonic anhydrase inhibitor acetazolamide causes sodium and chloride ion concentrations in the hemolymph to be lowered, with chloride ions being lowered to a greater extent (Fig. 12). The difference in the hemolymph sodium and chloride ion concentrations contributes to a change in the strong ion difference, a major factor in acid-base balance, resulting in a metabolic alkalosis, i.e., elevated hemolymph pH and bicarbonate ion concentration. These results suggest that acid-base status is controlled, at least in part, by mechanisms in the gills for translocating ions. It is known that the antennal gland in freshwater-acclimated blue crabs does not function in acid-base balance (Cameron and Batterton 1978). Furthermore, compensation occurs for an acidosis induced by hypercapnia (elevated CO_2) in the water and changes in sodium and chloride ion fluxes have been implicated as a mechanism of compensation (Cameron 1978b).

Respiration During Swimming

Blue crabs are known to swim great distances (Wolcott 1995). Much of their movement occurs when they take advantage of tidal currents. However, blue crabs actively swim using the paddle-shaped fifth pereiopods in a sculling motion (Spirito 1972). The influences of activity on oxygen transport have been investigated in a number of crustaceans (Herreid et al. 1979; McDonald et al. 1979; McMahon et al. 1979; Wood and Randall 1981a,b; Booth et al. 1982, 1984).

Booth et al. (1982) and Booth and McMahon (1985) have assembled a rather complete picture of the respiratory adaptations of *C. sapidus* related to swimming. Blue crabs swimming for at least 25 min increase the demand for oxygen from 2.97 μmol g^{-1} h^{-1} to 7.72 μmol g^{-1} h^{-1}, a 2.6-fold increase over resting values (Table 2). The crabs accomplish this demand by increasing gill ventilation rates and cardiac output within minutes of initiating swimming. In addition, they are able to make efficient use of hemocyanin in transporting oxygen (Fig. 16).

The ventilation rate during sustained swimming increases and remains elevated for the duration of exercise. The increase in ventilation is brought about by an increase in the frequency of the movements of both scaphognathites, although the stroke volumes of the scaphognathites decrease at the higher frequencies. Oxygen extraction from the ventilatory current decreases slightly as the ventilation rate increases during swimming.

Table 2. Ventilation and perfusion in the blue crab in response to swimming (data from Booth et al. 1982).

Variable	Rest	Swimming	Ratio
$f_{scaphognathite}$ (beats min^{-1})	94	312	3.32
Ventilation rate (mL kg^{-1} min^{-1})	490	1400	2.86
Oxygen uptake (μmol g^{-1} h^{-1})	2.97	7.72	2.6
f_{heart} (beats min^{-1})	89	143	1.61
Cardiac output (mL kg^{-1} min^{-1})	151	345	2.3
Cardiac stroke volume (ml)	1.7	2.4	1.4

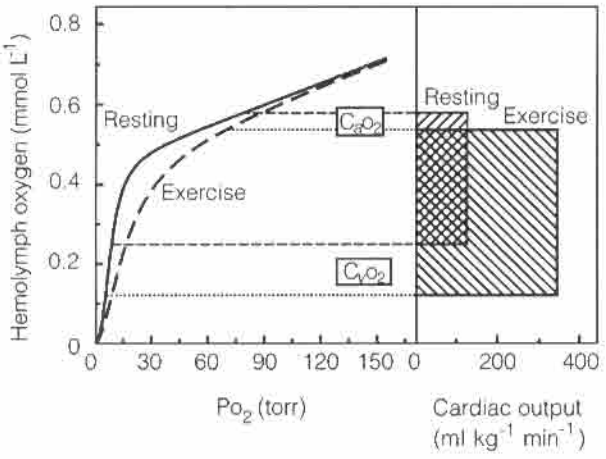

Figure 16. The respiratory responses of *C. sapidus* to exercise. Oxygen equilibrium curves are shown with the cumulative effects of changes in oxygen affinity. Oxygen uptake is represented as the product of the postbranchial (Ca_{O_2}) and prebranchial (Cv_{O_2}) oxygen concentration difference (y-axis) and cardiac output (x-axis). Data from Booth et al. (1982).

Cardiac output, or the flow of hemolymph through the crab, also increases during swimming. This increase is brought about by an increase in the heart rate and an increase in the cardiac stroke volume. The control of cardiac output in crustaceans by adjustments in cardiac stroke volume is quite common (McMahon and Burnett 1990).

Thus, the mechanical pumps supplying water to the gills and hemolymph to the tissues respond to the increased oxygen demand during swimming by moving water and hemolymph at faster rates. There are a number of adaptive changes in oxygen transport that occur during this time that enable the hemocyanin to transport effectively larger amounts of oxygen. As blue crabs swim, a large amount of lactic acid is produced, indicating that swimming activity is not fully aerobic. Lactate appears in the hemolymph rapidly, rising from 0.7 mmol L^{-1} at rest to 2.4 mmol L^{-1} within 2 min and increasing to 9.8 mmol L^{-1} after 25 min. There is a large hemolymph acidosis associated with the increase in lactic acid. Because blue crab hemocyanin has a large normal Bohr effect, this decrease in pH dramatically decreases the affinity of the pigment for oxygen. However, the oxygen affinity of blue crab hemocyanin is modulated specifically by lactate ions (Johnson et al. 1984), such that an adaptive increase in oxygen affinity is brought about by lactate. Thus, the lactate effect directly opposes the decrease in oxygen affinity caused by the reduced pH, thereby stabilizing the function of hemocyanin (Booth et al. 1982).

Respiration During Hypoxia

Living in coastal waters and estuaries, the blue crab encounters low oxygen partial pressures, or hypoxia, frequently. Because blue crabs are quite mobile, one response to hypoxia might be to swim or walk toward more oxygenated water. Blue crabs have been shown in the laboratory to detect hypoxic water and to avoid it (Das and Stickle 1993, 1994). Similar results have been obtained with field

studies (Pihl et al. 1991). Hypoxia in coastal waters along the eastern and the Gulf coasts of the United States occurs commonly and has been documented in many studies (see Diaz and Rosenberg 1995 for review). Hypoxia may be of short duration as in tidal creeks (Cochran and Burnett 1996) or it may persist for long periods (Officer et al. 1984).

The responses to short-term exposure to hypoxia in blue crabs are similar to those found in other crabs. Many crabs respond to short-term hypoxia by increasing the ventilatory flow of water past the gills (Truchot 1975; Burnett 1979; Burnett and Johansen 1981; Lallier and Truchot 1989). When blue crabs are exposed to hypoxia (water Po_2 = 50 torr), they greatly increase the ventilation rate (Batterton and Cameron 1978). This hyperventilation persists for 5 d and then returns to the normoxic baseline (deFur and Pease 1988). The hyperventilation often produces a respiratory alkalosis in crabs (Burnett and Johansen 1981), elevating the hemolymph pH and thus producing an adaptive increase in the oxygen affinity of hemocyanins with normal Bohr shifts. This alkalosis associated with hypoxia has sometimes been observed in *C. sapidus* (Pease et al. 1986), though not always (Mangum and Weiland 1975). Heart rate increases by as much as 30% and, like ventilation, heart rate remains elevated for 5 d and returns to the normoxic baseline despite the persistence of hypoxia (deFur and Pease 1988). Although cardiac output increases during hypoxia in *H. americanus* (McMahon and Wilkens 1975) and the spider crab *Libinia emarginata* (Burnett 1979), it increases by only a small amount in blue crabs (Fig. 17) despite the increase in heart rate.

Long-term exposure to moderate hypoxia has been well studied in the adult blue crab (deFur et al. 1990). Blue crabs sustain hyperventilation for at least 5 d (deFur and Pease 1988). After 25 d of exposure to moderate hypoxia (Po_2 = 50 to 55 torr or 33% air saturation), oxygen uptake is no different from that of crabs living in well-oxygenated water (Fig. 17). Furthermore, cardiac output is only slightly elevated. In contrast, juvenile blue crabs are sensitive to hypoxia with 28-day LC_{50} values ranging from 74 to 123 torr O_2 at 10 to 30 salinity and 20° to 30°C (Stickle et al. 1989; Das and Stickle 1993).

Most of the adjustments that account for sustaining oxygen uptake during chronic hypoxia in adult blue crabs occur with the hemocyanin molecule itself (Table 3). Small increases in oxygen affinity (i.e., decreases in P_{50}) occur as a result of changes in extrinsic factors such as hemolymph lactate, urate, and ion concentrations including hydrogen, calcium, magnesium, and sodium ions. Intrinsic factors are changes in the structure of the hemocyanin molecule itself. Interestingly, elevated water CO_2 that accompanies hypoxia (Cochran and Burnett 1996) results in elevated hemolymph CO_2 in blue crabs (Cameron 1978b) that in turn increases oxygen affinity of hemocyanin independently of pH (Mangum and Burnett 1986), contributing to the adaptive response (Fig. 17). Taken together, extrinsic factors result in an increase in oxygen affinity of approximately 3 torr.

More profound changes occur in the hemocyanin molecules themselves (Mangum 1997). Hemocyanin concentration increases by about 40%, enhancing the capacity of the hemolymph to carry oxygen (Table 3). Hemocyanin concentra-

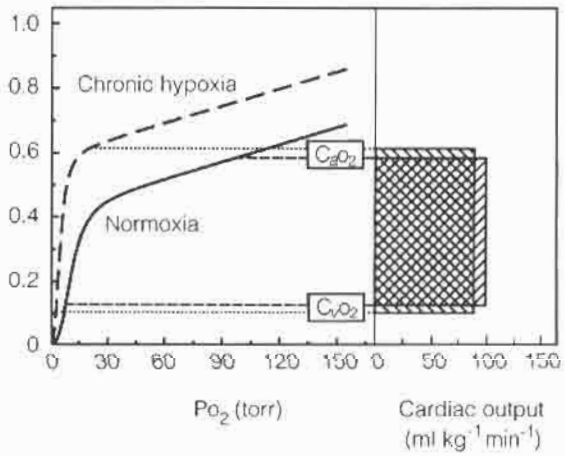

Fig. 17. The respiratory responses of *C. sapidus* to chronic (25 d) exposure to moderate hypoxia. Oxygen equilibrium curves are shown with the cumulative effects of changes in hemocyanin concentration and in oxygen affinity due to both intrinsic and extrinsic factors. Oxygen uptake is represented as the product of the postbranchial (Cao_2) and prebranchial (Cvo_2) oxygen concentration difference (y-axis) and cardiac output (x-axis). Data from deFur et al. (1990).

Table 3. Response of blue crabs exposed to hypoxia for 25 d (deFur et al. 1990).

Variable	Normoxia (ambient Po_2 = 140–155 torr)	Hypoxia (ambient Po_2 = 50–55 torr)	Results
Lactate (mmol L^{-1})	0.01	0.94	Decrease in P_{50} < 1 torr each
Urate (mmol L^{-1})	0.05	0.14	
Ca^{++} (mmol L^{-1})	6.73	10.1	
Hemocyanin (g 100 ml^{-1})	3.11	4.40	Increase O_2 carrying capacity
Subunit composition (6 monomers)		Increase in subunits 3, 5, 6	Decrease in P_{50} of 5 torr

tions have also been shown to increase in the shrimp *Crangon crangon* during prolonged exposure to hypoxia (Hagerman 1986). The most interesting result of the blue crab study, however, was the intrinsic adaptation of hemocyanin oxygen affinity to low oxygen. deFur et al. (1990) hypothesized that the net synthesis of hemocyanin during hypoxia can produce replacement molecules that differ from those in normoxic crabs. This possibility becomes even greater given that net synthesis of hemocyanin occurs during long-term hypoxia.

Intrinsic changes in hemocyanin oxygen affinity respond to chronic changes in environmental variables in crayfish (Rutledge 1981) and blue crabs (Mauro and Mangum 1982a; Mason et al. 1983; Mangum and Rainer 1988). The six different kinds of subunits that make up the large hemocyanin molecule are distinguishable electrophoretically. Three of the six subunits are known to be variable in different populations of blue crabs (Mangum and Rainer 1988) (see below). Subunits 3, 5, and 6, the variable subunits, decrease in concentration in relation to the other subunits in response to chronic hypoxia. The net result of the changes in subunit composition of hemocyanin is an overall decrease in P_{50} of 5 torr. The mechanisms behind these changes were elucidated by Charlotte Mangum and her students. It appears that the electrophoretic patterns observed in both the field and the laboratory bring about a higher hemocyanin oxygen affinity by favoring the more primitive 1 H 6-mer multiple of hemocyanin at the expense of the 2 H 6-mer, both of which are present in blue crab hemolymph (see Mangum 1997 for overview).

Respiration During Salinity Change

The influence of salinity on respiration has been studied from the perspective of total oxygen uptake, oxygen transport, and changes in the respiratory pigment hemocyanin. King (1965) and Findley et al. (1978) found that oxygen uptake in both immature and adult crabs was higher at low salinity (e.g., 7 to 10) than high salinity (30 to 35) (Table 1). Engel and Eggert (1974) obtained similar results using excised gill tissues. Laird and Haefner (1976) reported a similar pattern in crabs at 10°C, but salinity had no effect on oxygen uptake at 24°C (Table 1). Sabourin (1984) found no difference in oxygen uptake at 10 and 30 salinity (Table 1).

Oxygen uptake varies inversely with salinity when salinity is cycled at 10-5-10, 20-10-20, 30-10-30, or 10-30-10 salinity over 12-h cycles (Findley et al. 1978). Sabourin (1984) found no such clear patterns during salinity cycling, but found that heart rate peaks during low salinity exposure. Oxygen extraction also declines with time during salinity cycling (Sabourin 1984). It is unclear whether or not any of the observed changes during salinity cycling repre-

sent a true response to the cycling of salinity or a response to an acute change in ambient salinity.

Hemocyanin properties also change as a function of salinity. Sabourin (1984) reported that hemolymph protein and copper concentrations were higher in crabs acclimated to 10 salinity (34 mg protein ml^{-1} in winter crabs, 28 mg protein ml^{-1} in autumn crabs; 62 μg copper ml^{-1} in winter crabs, 54 μg ml in autumn crabs) compared with those at 30 salinity (41 mg protein ml^{-1} in winter crabs, 43 mg protein ml^{-1} in autumn crabs; 81 μg copper ml^{-1} in winter crabs, 83 μg ml^{-1} in autumn crabs). These findings suggest an increase in the concentration of hemocyanin. Sabourin (1984) also documented a significantly higher hemolymph pH in low salinity crabs compared with high salinity crabs and this would result in an increase in hemocyanin oxygen affinity. However, Sabourin (1984) independently measured a lower hemocyanin oxygen affinity at 10 salinity (P_{50} = 14.2 torr) than at 30 salinity (P_{50} = 11.8 torr), suggesting that other factors influence oxygen affinity.

As with hypoxia, the changes in the properties of hemocyanin as a function of salinity can be divided into changes due to extrinsic factors and those due to intrinsic structural alterations of the molecule itself. The oxygen affinity of *C. sapidus* hemocyanin rises when salinity increases within the physiological range (Mason et al. 1983). Sodium ions increase oxygen affinity at concentrations below 90 mmol L^{-1}, but have no effect at higher concentrations. Calcium and magnesium ions raise hemocyanin oxygen affinity throughout the physiological range, and the effect of calcium is nearly six times greater than that of magnesium. Chloride ions do not appear to affect oxygen affinity. Variation in inorganic ions or pH does not affect the cooperativity of oxygen binding.

Although the largest factor affecting oxygen affinity during salinity change is calcium ion concentration, this factor explains only a minor fraction of the changes that have been observed (Mason et al. 1983). Mangum and Rainer (1988) found that hemocyanin oxygen affinity differs in estuarine and seaside populations of blue crabs. The adaptive difference was accompanied by different proportions of the six subunits making up the native hemocyanin polymers. Three of the six subunits appear to be invariant. Mangum and Rainer (1988) noted that two of the six subunits were either present in low concentrations or absent from most seaside blue crabs. A third subunit was also variable, but the variation was not clearly correlated with locality. Thus, the two populations have different intrinsic hemocyanin oxygen affinities due to changes in the subunit composition of hemocyanin.

Respiration During Temperature Change

Oxygen uptake, like other functions, is sensitive to temperature. Leffler (1972) reported an increase in oxygen uptake between 13° and 34°C in juvenile male and female crabs (Table 1). Laird and Haefner (1976) documented similar results in adult male crabs; however, oxygen uptake at 10°C was much higher than that measured by Leffler (1972) at 13°C (Table 1). Leffler (1972) reported that crabs acclimated to 13°C were extremely sluggish. The findings in these two studies are typical of those for other crab species (Mauro and Mangum 1982b). However, the metabolism of blue crabs is extremely sensitive to low temperature. It is well known that crabs in the Chesapeake Bay burrow into the sediments, reducing all activities when water temperature falls below 10°C with the approach of winter (Warner 1976). Mauro and Mangum (1982a) observed that winter animals warmed above 10°C began to move about while summer animals cooled below 10°C became quiescent. Temperature coefficients for oxygen uptake are typically around 2 (i.e., Q_{10} = 2) between 15° and 25°C (Laird and Haefner 1976; Mauro and Mangum 1982a), but much greater between 5° and 15°C (Q_{10} = 4.92) (Mauro and Mangum 1982a).

A number of factors appear to act to bring about the winter "hibernation." Some of the metabolic responses to low temperature can be explained by the biochemical changes of two major tissues, hepatopancreas and muscle. The metabolic activity of muscle tissue, which comprises greater than two-thirds of the crab's biomass, is depressed to a much greater extent than that of the hepatopancreas (19%

of the biomass) (Mauro and Mangum 1982a). The difference is due to the relatively greater importance of the temperature-independent hexose monophosphate shunt in hepatopancreas compared with muscle (Robert and Gray 1972; Mauro and Mangum 1982a). Mauro and Mangum (1982a) suggest that this biochemical difference between hepatopancreas and muscle is important in allowing the hepatopancreas to remain active in synthesizing materials used in gametogenesis and in the burst of growth in the spring.

The other factor responsible for the high sensitivity of blue crabs to low temperature is the difference in the properties of the hemocyanin between summer and winter animals (Mauro and Mangum 1982a). At summer temperatures, hemocyanin oxygen affinity is relatively low and considerable amounts of oxygen are delivered to the tissues. At winter temperatures, the hemocyanin oxygen affinity is so high that little oxygen can be extracted by the tissue and oxygen is transported in its free form. Furthermore, the properties of hemocyanin change as a function of season. Hemocyanin from summer crabs has a greater oxygen affinity at 25°C than hemocyanin from winter crabs at the same temperature. Thus, as winter crabs warm up during the spring, the hemocyanin is pre-adapted (i.e., has a lower oxygen affinity) to deliver oxygen to the tissues. As summer crabs are cooled in the autumn, the hemocyanin with a relatively higher oxygen affinity does a poorer job of transporting oxygen to the tissues. Mauro and Mangum (1982a) suggest that the differences between "summer hemocyanin" and "winter hemocyanin" facilitate the entry of the crab into "hibernation" in the fall and the emergence of activity of crabs in the spring.

Respiration During Molting

Molting in blue crabs and other crustaceans involves many complex and interesting changes in the gas exchange system. These processes have been studied in isolation in a variety of species, but the mechanisms in blue crabs have been particularly well elucidated in a comprehensive examination of gas exchange and acid-base balance by Mangum et al. (1985).

During the pre-molt stages, oxygen uptake does not change significantly, even though the data in Table 1 suggest a slight decline. Oxygen uptake declines during molting (Lewis and Haefner 1976), increasing immediately after exuviation and gradually returning to premolt levels. The metabolism of epidermal tissue rises during the premolt, remains high during the postmolt, and declines late in stage B (Mangum et al. 1985). These results are similar to those found in *Gecarcinus lateralis* (Skinner 1962). The oxygen uptake of the claw closer muscle and the swimming muscle increases during the postmolt period in the blue crab (Mangum et al. 1985). Mangum et al. (1985) have suggested that the changes in oxygen uptake in muscles are correlated with dissolution (during premolt) and the reformation of the contractile elements.

Prior to molting, hemolymph Po_2 declines, suggesting an interruption of gas exchange. Postbranchial hemolymph Po_2 drops from 83 torr to 8.7 torr during the premolt stages (through D_4), and finally to 6.8 torr during exuviation. At the same time, hemolymph Pco_2 increases nearly ten fold (from 2.8 to 20 torr) and total carbon dioxide (i.e., all forms of CO_2 including bicarbonate and carbonate) is also elevated. As in the decline of oxygen, the increase in CO_2 is consistent with an interruption of normal gas exchange during the molt. The most likely explanation for these results is an increase in the diffusion distance between the hemolymph and the ambient medium at the gills as the gill epithelium prepares to separate from the thin exoskeleton. During this time the branchial chambers continue to be well ventilated and heart rate shows no significant change (deFur et al. 1985).

Hemocyanin concentration at the molt is one-third of the premolt values (Mangum et al. 1985). The oxygen affinity of the hemocyanin remains virtually unchanged through the molt (P_{50} = 11 torr). Interestingly, the rise in hemolymph Pco_2, which begins in stage D_3, is accompanied by an alkalosis that offsets the acidification of hemolymph occurrence due to CO_2. The mechanisms that bring about this alkalosis remain unclear.

Immediately after a molt, the soft shell of the blue crab, previously impermeable to oxygen,

becomes highly gas permeable (Mangum et al. 1985), perhaps temporarily ensuring an oxygen supply to superficial tissues, the site of the formation of the new exoskeleton. Hemolymph Po_2 rises sharply in the postmolt stages and Pco_2 declines. These changes are consistent with the restoration of gas exchange at the gill. The ventilation of the gill chamber is depressed after the molt, but gradually increases as the scaphognathite, softened during molting, becomes fully functional (deFur et al. 1985). Heart rate after the molt is significantly depressed but remains high (>145 beats min^{-1}) (deFur et al. 1985). A pulse of lactate (2.3 mmol L^{-1}) appears in the hemolymph immediately following the molt, suggesting the presence of anaerobic metabolism. Hemocyanin concentration begins to rise, but remains low (1.89 g 100 ml^{-1}) through stage B_2. Oxygen affinity rises slightly in stage B crabs (P_{50} = 14 torr) while cooperativity is unchanged.

Respiration During Air Exposure

Callinectes sapidus does not normally venture into air although we have observed small (100 g) individuals of *C. bellicosus* chasing fiddler crabs out of water into the air in Baja California. *Callinectes sapidus* is known to be able to survive exposure to air for hours or even days if kept cool (deFur et al. 1988), which is important to retail suppliers. A number of studies on the effects of air exposure in other crustacean species has revealed many important morphological and physiological adaptations to emersion (for example Gray 1957; Taylor and Butler 1978; deFur and McMahon 1984; Burnett 1988).

The gill lamellae of blue crabs are not adapted for breathing air. The distance between the lamellae in water is reported to be 0.33 to 0.6 μm and is only slightly larger (0.45 to 0.9 μm) in air (deFur et al. 1988). In the air-exposed gill there is no evidence that air or water moves into or out of the interlamellar spaces. After 2 h of emersion, gills clump together, exposing in places the lateral surface of a lamella. It is possible that some gas exchange occurs along these surfaces. It is also possible that gas exchange occurs along the marginal channels that run along the edges of the lamellae.

Crabs continue to ventilate the branchial chambers during emersion (O'Mahoney and Full 1984; deFur et al. 1988). Small blue crabs (<70 g) reduced their scaphognathite beat frequency while larger (<118 g) crabs increased the frequency. Heart rate patterns were identical to those of the scaphognathites, decreasing in small crabs and increasing in large crabs. Postbranchial hemolymph Po_2 decreased dramatically from 100 to 24 torr during air exposure, indicating a reduction in gas exchange at the gills and resultant internal hypoxia (deFur et al. 1988). An acidosis occurred in the tissues that was partially metabolic because hemolymph lactate increased to nearly 2 mmol L^{-1}. Hemocyanin can function for at least 4 h because the acidosis, which would decrease hemocyanin oxygen affinity, is opposed by the effects of lactate and CO_2, both of which increase oxygen affinity (Burnett 1992). Thus, the position of the oxygen equilibrium curve is stabilized and postbranchial hemolymph is approximately 90% saturated with oxygen. All of these changes are enough to provide oxygen to blue crabs at a rate 35% of that found in resting submerged animals (O'Mahoney and Full 1984).

INTERACTING AND COUNTERBALANCING PHYSIOLOGICAL SYSTEMS IN THE BLUE CRAB

A review of physiological adaptations in *Callinectes sapidus* is not complete without recognizing that systems interact and often counterbalance each other. Although studies of isolated organs, cells, and molecules reveal much about their individual functions, only in the context of the whole organism can we understand their contributions to adaptive success. Remarkably, the sum total of these individual contributions does not lead to homeostasis, a constancy of internal conditions, in most lower vertebrates and invertebrates. From an earlier review of blue crab physiology, here are the words of Charlotte Mangum:

> The limited phylogenetic distribution of

homeostasis has an implicit corollary: most animals must either tolerate wide fluctuations in their life processes or else they must live in stable environments. There is undoubtedly a great deal of truth in this generalization, but it exaggerates the difficulties of life without homeostasis.

Another form of physiological regulation also permits freedom from the simple consequences of environmental change. We propose to designate this kind of adaptation as *enantiostasis*, a word composed of three Greek elements meaning a counterbalanced state of opposing conditions. An enantiostatic regulation occurs when the effect of change in one chemical or physical property of the internal milieu is opposed by a change in another. Thus the internal milieu is unstable, but the net effect of the flux on a particular physiological system is stability, or a tendency toward it (Mangum and Towle 1977).

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