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The Role of Branchial Ventilation in Hemolymph Acid-Base Changes in the Shore Crab *Carcinus maenas* During Hypoxia

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Summary. 1. When exposed to hypoxia in the ambient medium, the crab *Carcinus maenas* increased and sustained high levels of branchial ventilation for up to 70 h (Fig. 1).

2. The role of hyperventilation in establishing changes in hemolymph acid-base status during hypoxia was investigated. Hemolymph pH and P_{co_2} changes in hypoxic crabs (Figs. 2 and 4) were compared with crabs whose branchial chambers were artificially hyperventilated in normoxia by siphoning water through a mask attached to the carapace (Fig. 3).

3. Hyperventilation alone does not account for the observed alkalosis during hypoxia. It is suggested that changes in both CO_2 production and ventilation may be responsible for altering hemolymph acid-base status.

4. Artificial hyperventilation in normoxic crabs resulted in a respiratory alkalosis which is fully compensated after 16 hours by a metabolic acidosis (Fig. 3).

5. Reduction of the hemolymph bicarbonate pool during hypoxia did not interfere with the ability of crabs acclimated to low salinity to regulate hemolymph chloride ion concentration (Fig. 5).

Introduction

Analysis of CO_2 transport across gills is complicated by the fact that the gills are also the sites of ion exchange, presumably involving bicarbonate ions, between the internal fluids of the animal and the external medium. The mechanism of CO_2 excretion across gills is not well known. However, CO_2 excretion is thought to be sensitive to changes in ventilation in a pattern similar to that exhibited by air-breathers. Changes in ventilation, however, are due primarily to variability in both the supply and the demand for oxygen and have not been shown to be sensitive to elevated CO_2 levels of the blood in water-breathers (Dejours 1973; Batterton and Cameron 1978). Ventilation rates in water-breathers are set by the O_2 requirements and are much higher than rates needed to excrete CO_2 (cf. review by Reeves 1977). This imposes serious limitations on the use of ventilation as a mechanism to adjust blood acid-base status.

Blood CO_2 content, and thus its acid-base status, is responsive not only to increases or decreases in CO_2 elimination but also to changes in metabolic CO_2 production by the tissues. Many studies describing correlations between ventilation and blood acid-base status in water-breathers have been carried out by exposing animals to hypoxia (Dejours 1973; McMahon et al. 1978a; Burnett 1979), hyperoxia (Dejours and Beekenkamp 1977) and changing temperature (Randall and Cameron 1973; Truchot 1978; McMahon et al. 1978b), all of which may affect metabolic CO_2 production. In these studies the contribution of ventilation changes in establishing a different blood acid-base status cannot be clearly distinguished from the contribution of CO_2 production changes.

The present study was undertaken to determine the response of the hemolymph acid-base status to hypoxia in the shore crab, *Carcinus maenas* (Linnaeus), and to test the extent to which this response results from compensatory adjustments in branchial water flow. The problem has been studied by measuring hemolymph acid-base status and branchial ventilation at a level of hypoxia well below the critical oxygen pressure for oxygen uptake. The influence of ventilation changes on hemolymph pH was evaluated by monitoring hemolymph pH and P_{co_2} at artificially elevated branchial ventilation rates.

We also monitored changes in both the hemolymph acid-base status and hemolymph chloride ion

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concentrations during hypoxia in crabs acclimated to a reduced ambient salinity. This experiment was designed to test the hypothesis that the chloride/bicarbonate exchange mechanism in the gill epithelium, initially postulated by Krogh (1938), is sensitive to hypoxia-induced fluctuations in the concentration of bicarbonate ions in the hemolymph. We chose to test this hypothesis by determining if there were any differences between hypoxic and normoxic crabs in their ability to maintain hemolymph Cl^- at a concentration well above that of the ambient medium.

Materials and Methods

Freshly collected specimens of *Carcinus maenas* were obtained from the Fisheries Museum in Esbjerg, Denmark, and transported to Aarhus where they were held for at least five days in recirculating sea water at 10 °C and 35‰ salinity. Crabs were fed mussels at least three times a week.

Ventilation During Hypoxia

For the direct measurement of ventilation volume, exhaled water was made to exit through a mask. Ventilation was measured by placing the mask over the mouthparts and exhalant openings of the gill chamber of the crab and monitoring the flow of water through the mask aperture with an electromagnetic flow probe (Micron Instruments) (Johansen et al. 1970; Batterton and Cameron 1978). The mask was constructed from a flexible polymer called Drufosoft (Dreve Dentamid, Unna, FRG) and fitted tightly to the contours of the carapace using a rubber base impression material ('Coe Flex', Coe Laboratories, Inc., Chicago, USA). Masks were positioned so that the anterior appendages could move freely. Crabs fitted with masks were placed in a small container (about 10 liters) through which sea water was flowing. The container was partially filled with pebbles to provide a natural substrate for the animals. Masks were placed on crabs at least 24 h prior to ventilation measurements. Water P_{o_2} was controlled by bubbling nitrogen or air into the container.

Hemolymph Acid-Base Status and Chloride Ion Concentration

In order to determine the effect of hypoxia on hemolymph acid-base status, crabs were first maintained in the flow-through container for at least 24 h in well aerated sea water of 35‰ salinity. The ambient water P_{o_2} was then lowered gradually for one hour to a final P_{o_2} of 20–25 Torr. Hypoxia was maintained at 20–25 Torr during the remainder of the experimental period. Although the ambient P_{co_2} of the recirculating water was not measured, vigorous aeration of the water prior to flowing into the flow-through container followed by vigorous bubbling in the flow-through container with combinations of air and N_2 preclude the existence of P_{co_2}'s much higher than that of air.

Hemolymph samples were taken from crabs at various times of exposure to hypoxia. Only one sample was taken from each crab. Hemolymph was sampled from the infrabranchial sinus at the base of the third or fourth walking leg using a 1 ml glass syringe and a 26 gauge needle. pH was measured by transferring the hemolymph anaerobically to the glass electrode of a Radiometer acid-base analyzer (BMS-2). P_{co_2} was estimated using the Astrup equilibration method. Hemolymph bicarbonate and carbonate concentrations were calculated according to the Henderson-Hasselbalch equation using constants determined by Truchot (1976) for *C. maenas* at the appropriate temperature and salinity.

Chloride ion concentrations of hemolymph samples were measured using an amperometric chloride titrator (Radiometer CMT10).

A second set of experiments was carried out on animals held for at least one week at 10 °C and 17‰ salinity. These crabs were exposed to hypoxia and hemolymph was sampled as described above.

Artificial Hyperventilation at Normoxic Conditions

Alteration of the ventilation volume of the crabs was accomplished by connecting a length of water-filled tubing to the exhalant opening of the mask. When the distal end of the tubing was placed below the water level outside the tank, a siphon effect occurred and the ventilation was artificially increased. The siphon effect was used to simulate the hyperventilation response to hypoxia (1,000–1,500 $ml \cdot kg^{-1} \cdot min^{-1}$) without reduction of the water P_{o_2} (>140 Torr). During the experiments hemolymph was sampled 4, 8 and 16 h after siphoning was begun. Hemolymph was also sampled from a control group of crabs which had masks but no siphons. The acid-base status of each sample was determined as previously described.

A similar experiment was also performed on a much larger crab species, *Cancer anthonyi* (170–250 g), fitted with masks and whose hemolymph was repetitively sampled at different times during artificial hyperventilation at 21 °C and 33‰ salinity. Hemolymph samples were analyzed for pH and total CO_2 content using the method of Cameron (1971).

Results

Ventilation and Hemolymph Acid-Base Responses to Hypoxia

After 4–8 h of hypoxia branchial ventilation increased to approximately 300% of the initial normoxic level and remained high showing only a slight decline after 66–70 h (Fig. 1). After 2 h of hypoxic exposure hemolymph pH increased significantly ($P<0.001$; using Student's t test and converting pH to H^+ concentration) while $HCO_3^- + CO_3^{--}$ concentration decreased slightly but not significantly ($P>0.10$); thereafter, $HCO_3^- + CO_3^{--}$ concentration declined at constant pH (Fig. 2).

Artificial Hyperventilation in Normoxic Sea Water

When ventilation of the branchial chambers under normoxic conditions was increased artificially by si-

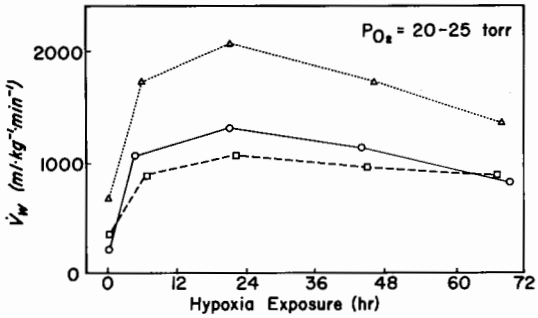

Fig. 1. Branchial ventilation in three individuals of *Carcinus maenas* as a function of hypoxia exposure time when ambient water P_{o_2} is reduced to 20–25 Torr

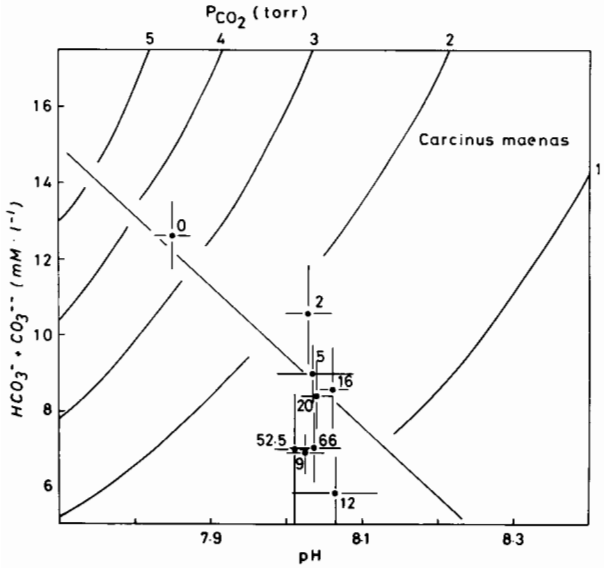

Fig. 2. Hemolymph acid-base changes in *Carcinus maenas* at 10 °C and 35‰ salinity when ambient water P_{o_2} is reduced to 20–25 Torr. Data show means ± S.E.. In each case $N=8$ except at time = 0 where $N=16$. Exposure time is indicated in hours. The diagonal in vitro buffer line was determined by equilibrating hemolymph at various partial pressures of CO_2

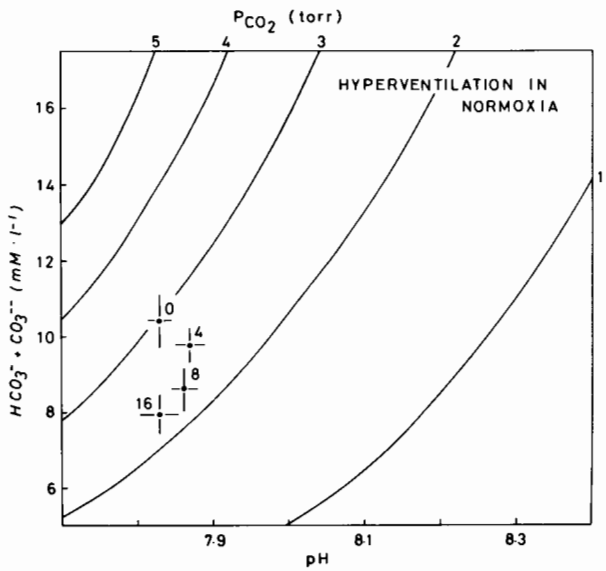

Fig. 3. The acid-base status of hemolymph in *Carcinus maenas* when the branchial chambers are artificially hyperventilated in well aerated water. Data show means ± S.E.. The numbers next to the data points represent hyperventilation time in hours. $N=7$, 14, 13 and 9 at times 0, 4, 8 and 16 h, respectively

phoning, hemolymph pH did not change significantly ($P>0.05$) from initial values throughout 16 h of measurement. $HCO_3^- + CO_3^{--}$ concentration remained unchanged after 4 h but decreased significantly ($P<0.025$) after 8 and 16 h (Fig. 3).

Cancer anthonyi responds to hyperventilation in a manner similar to that of *C. maenas* (Table 1). Normal branchial ventilation rates in these animals were assumed to be around 600 ml·kg^{-1}·min^{-1} based on findings in other crabs in the same size and temperature range (Batterton and Cameron 1978; Burnett 1979). When the branchial chambers are artificially hyperventilated at rates three times normal for 16 h, hemolymph pH remains the same while total CO_2 content declines to 70% of the initial value.

Responses to Hypoxia at Reduced Salinity

Crabs acclimated to 17‰ salinity responded to hypoxia in a manner similar to crabs in 35‰ salinity water (Fig. 4). As described previously (Truchot 1973), pH was significantly higher ($P<0.001$) than in crabs in 35‰ sea water. Hemolymph bicarbonate and carbonate concentrations were also significantly greater ($P<0.025$) in normoxic crabs acclimated to 17‰ salinity than in crabs at 35‰ salinity. After 2 h of exposure to hypoxia, hemolymph pH was significantly higher ($P<0.001$) than that in normoxic crabs while $HCO_3^- + CO_3^{--}$ concentration remained unchanged; thereafter, $HCO_3^- + CO_3^{--}$ concentration declined at constant pH.

Table 1. Hemolymph pH and total CO_2 content (mM·l^{-1}) in 4 individual *Cancer anthonyi* (21 °C and 33‰ salinity) at different durations of artificial hyperventilation. Data collected by T.B. Warren, University of San Diego

Duration of hyperventilation (h)	0		4		8		12		16	
Ventilation rates (ml/kg·min)	pH	Co_2tot	pH	CO_2tot	pH	CO_2tot	pH	CO_2tot	pH	CO_2tot
2200	7.786	11.67	7.816	10.85	7.847	8.41	7.740	7.89	7.817	6.97
1890	7.868	9.50	7.871	9.59	7.801	8.56	7.840	7.82	7.824	7.34
1850	7.741	9.31	7.749	9.16	7.742	8.55	7.758	7.34	–	–
2010	7.812	10.02	7.789	9.97	7.779	8.93	7.776	8.03	7.787	6.83
$\bar{x}$	7.799	10.13	7.804*	9.89*	7.791*	8.61**	7.777*	7.77**	7.809*	7.05**

* Not significantly less than values at time = 0

** Significantly less than values at time = 0 ($P<0.05$; according to a paired t test)

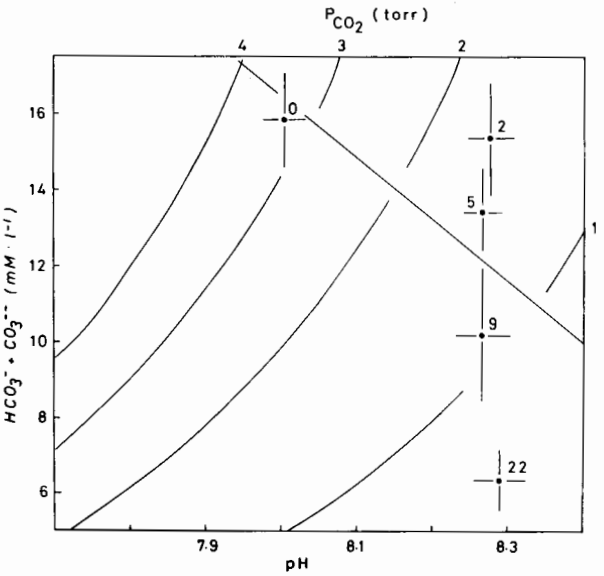

Fig. 4. Hemolymph acid-base changes in *Carcinus maenas* at 10 °C and 17‰ salinity when ambient water P_{o_2} is reduced to 20–25 Torr. Data show means ± S.E.; in each case $N=8$. Exposure time is indicated in hours. The diagonal in vitro buffer line was determined by equilibrating hemolymph at various partial pressures of CO_2

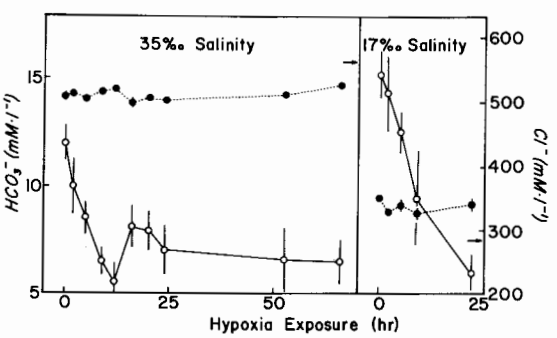

Fig. 5. Hemolymph chloride ion concentrations (closed circles) and bicarbonate ion concentrations (open circles) in *Carcinus maenas* as a function of exposure time to hypoxia ($P_{o_2}=20$–25 Torr) at two different salinities. Arrows indicate the chloride ion concentration of the medium at each salinity. Data show means ± S.E.. In some cases the symbol was larger than S.E.; $N=8$ in all cases except for 0 and 12 h at 35‰ salinity where $N=16$ and 6, respectively, and 9 h at 17‰ salinity where $N=7$

Hemolymph chloride ion concentration is strongly regulated in crabs acclimated to 17‰ salinity (Fig. 5). Chloride regulation is not significantly affected when crabs are exposed to hypoxia during which time the hemolymph HCO_3^- ion concentration is significantly ($P<0.001$) reduced.

Discussion

Hypoxia-induced alkalosis is important in animals such as the crustaceans that have a hemolymph O_2 carrier with a large and normal Bohr shift. A pH-induced increase in hemocyanin oxygen affinity can be very adaptive to crustaceans in enabling a moderately hypoxic hemolymph to be highly oxygenated at the gills (McMahon et al. 1978a; Burnett 1979). Truchot (1975) observed a significant hemolymph alkalosis in *Carcinus maenas* exposed to various low ambient oxygen pressures for two hours. Similar results have been obtained for the lobster, *Homarus vulgaris* (McMahon et al. 1978a), the spider crab, *Libinia emarginata,* and the mud crab, *Panopeus herbstii* (Burnett 1979).

Our data indicate that hemolymph P_{co_2} as well as bicarbonate and carbonate levels in *C. maenas* decrease when the branchial chambers of the crab are artificially hyperventilated with normoxic water (Fig. 3). The induced increase in branchial ventilation above normal rates must be more effective in increasing CO_2 partial pressure gradients at the surfaces of the gill lamellae, allowing more CO_2 to move out of the hemolymph and through the gill. The ventilatory 'washout' of CO_2 results in a decrease in hemolymph P_{co_2} accompanied by a decline in bicarbonate and carbonate ion stores.

It has been pointed out by Stewart (1978) that hydrogen ion concentrations in biological solutions are determined by three independent variables: the strong ion difference (S.I.D.), the partial pressure of CO_2, and the total weak acid present. The respiratory alkalosis resulting from artificial hyperventilation is a consequence of the decline in one of these variables, the hemolymph P_{co_2}. With time the alkalosis is fully compensated by a metabolic acidosis resulting in a decline in hemolymph HCO_3^- and CO_3^{--} at constant P_{co_2}. The metabolic acidosis must, therefore, be due to a decrease in the S.I.D. or an increase in the total weak acid present. In this case, compensation by changing S.I.D. may result from ionic exchanges between the ambient medium and the hemolymph involving Cl^-/HCO_3^- and/or Na^+/H^+.

Performing a similar experiment on a much larger species, *C. anthonyi,* allowed us to observe changes in hemolymph acid-base status in individual organisms throughout 16 h of artificial hyperventilation. Although repetitive hemolymph sampling may have induced a small sampling acidosis (McMahon et al. 1978a) the pattern of response was remarkably similar to *C. maenas*(Table 1).

The major difference between artificially hyperventilated crabs in normoxia and hypoxic crabs is the presence of a much larger respiratory alkalosis in hypoxic crabs at 35‰ salinity. A compensatory metabolic acidosis results in base deficits of similar magnitude in both groups (2.5 mM·l^{-1}). The metabolic acidosis may again be attributed to a decrease

in the S.I.D. or, in the case of hypoxic crabs, the production of metabolic acids by anaerobic pathways. Bridges and Brand (1980) have demonstrated a hemolymph lactic acid buildup in *C. maenas* held at a $P_{o_2} <$ 20 Torr at 10 °C, similar to the level of hypoxia used in this study. However, compensation for the hypoxia-induced alkalosis is incomplete.

The larger respiratory alkalosis occurring in the hypoxic crabs in comparison with crabs artificially hyperventilated in normoxia is due primarily to a larger decline in hemolymph P_{co_2}. In crabs at both salinities there is a small metabolic component to the alkalosis which occurs during the first two hours of hypoxia. The causes for the metabolic alkalosis are unknown, however, the decline in hemolymph P_{co_2} apparently involves both a decrease in metabolic CO_2 production due to reduced O_2 availability and an increase in CO_2 excretion due to hyperventilation. When the ambient P_{o_2} falls below the critical level to 20–25 Torr, oxygen uptake is significantly reduced (Taylor 1976). Although the quantity of CO_2 produced by anaerobic metabolic pathways under these conditions is unknown, it is unlikely that it is greater than the quantity of CO_2 produced by aerobic metabolic pathways. The situation is more easily analyzed by an evaluation of the volume of water which passes through the gill chambers per unit of CO_2 produced (l water · mMol CO_2^{-1}), a quantity known as the convection requirement (Dejours 1975). Assuming only aerobic production of CO_2 and a respiratory quotient of 0.9, the quantities of CO_2 produced in normoxic conditions can be calculated from O_2 uptake measurements at the two ambient oxygen levels using data from Taylor (1976) adjusted to 10 °C by using $Q_{10} =$ 1.65 (Newell et al. 1972).

In normoxic conditions, 10 l of water pass through the branchial chambers per mMol CO_2 produced. When ambient P_{o_2} is lowered to 20–25 Torr, the value increases to 251 l · mMol^{-1} due to both an increase in ventilation and a decrease in CO_2 production. Artificial hyperventilation of the branchial chambers mimics the ventilatory response to hypoxia. Assuming that O_2 uptake, and thus CO_2 production, remains the same as that in normoxic animals, 82 l of water pass through the branchial chambers per mMol CO_2 produced, a value about three times higher than that observed in normoxic crabs but only one third the value in hypoxic crabs. Thus, any increase in the CO_2 convection requirement results in a decline in P_{co_2}.

The treatment of data obtained from crabs artificially hyperventilated in normoxia involves several assumptions. We assume that water passing through the branchial chambers follows a path similar to that in hypoxic crabs hyperventilating at the same rates. Another assumption is that O_2 uptake during artificial hyperventilation is similar to that in normoxic crabs. The most obvious difference between the two groups is the role of the scaphognathites in moving water through the branchial chambers. We have some data (unpublished) from *C. anthonyi* indicating that the scaphognathites remain active during artificial hyperventilation. It may be argued, however, that artificial hyperventilation reduces the work done by the scaphognathites, reducing the metabolic demand of the muscles operating these pumps. Therefore, small differences in O_2 uptake due to different scaphognathite workloads may result in an overestimate of the convection requirement during artificial hyperventilation. Finally, we also assume that the pattern of hemolymph flow through the gills is the same in both hypoxic and normoxic crabs. There is currently no evidence suggesting the existence of hemolymph shunting mechanisms within crab gills. However, there is evidence that in severely hypoxic crabs cardiac output, and thus hemolymph flow through the gills, is lowered (Taylor 1976; Burnett 1979).

The hemolymph alkalosis observed in normoxic crabs acclimated to 17‰ salinity compared with normoxic crabs acclimated to 35‰ salinity is similar to that noted in *C. maenas* by Truchot (1973) and in *Callinectes sapidus* by Weiland and Mangum (1975). Weiland and Mangum (1975) attributed the increase in hemolymph pH to elevated ammonium ion levels, an effect equivalent to the reduction of total weak acids. In the present study the hemolymph P_{co_2} at both salinities are very similar, ruling out changes in this independent variable to account for pH changes. This leaves two possibilities. First, a base excess imposed by the addition of base to the hemolymph (e.g. ammonia) or excretion of a weak acid from the hemolymph would result in a hemolymph alkalosis. Second, a change in S.I.D. could achieve a similar effect. Available data on *C. maenas* and *C. sapidus*, both of which demonstrate a hemolymph alkalosis at reduced ambient salinities, indicate that the strong ions Na^+, K^+, Mg^{++}, Ca^{++}, Cl^- and SO_4^{--} are each regulated to varying degrees (Gifford 1962; Lockwood and Riegel 1969; Zanders 1980). A comparison of strong ion differences in these two species at high and low salinities suggests that part of the observed alkalosis at reduced salinities may be accounted for by increases in S.I.D..

Finally, the reduction of hemolymph bicarbonate ion concentration during hypoxia does not appear to affect the ability of this crab to regulate hemolymph Cl^- concentration at 17‰ salinity. This result indicates that either the inward transport of chloride from the ambient medium does not operate on a simple type of counterion exchange mechanism with

bicarbonate, or if Cl^-/HCO_3^- exchange occurs, it can operate at low circulating bicarbonate concentrations.

It is clear that the water flow rate across respiratory surfaces of *C. maenas* has the ability to influence hemolymph P_{co_2} and thus the acid-base status. But the hypoxia-induced hyperventilation does not fully account for the observed hemolymph alkalosis. Our results suggest that the hemolymph acid-base status in crabs depends upon both the rate of water flow over the gills and the metabolic production of CO_2.

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