GAS EXCHANGE, HEMOLYMPH ACID-BASE STATUS, AND THE ROLE OF BRANCHIAL WATER STORES DURING AIR EXPOSURE IN THREE LITTORAL CRAB SPECIES[1]

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O_2 uptake and hemolymph acid-base status, together with branchial water volume, CO_2 content, and titratable alkalinity, were measured in three species of intertidal crabs. In *Pachygrapsus crassipes,* a grapsid crab that actively moves between air and water, O_2 uptake increased on emersion. In *Eurytium albidigitum,* a mud-burrowing xanthid crab that is air exposed by tidal action, O_2 uptake declines dramatically on emersion. There is no significant lactate production by either crab following emersion. An emersion-induced respiratory acidosis was fully compensated in *P. crassipes* and another grapsid, *Hemigrapsus nudus,* but uncompensated in *E. albidigitum.* Branchial water volume 10 min after emersion was 0.013 ml/g crab weight in *P. crassipes* and 0.072 ml/g in *E. albidigitum.* The CO_2 content of branchial water in *P. crassipes* increased rapidly during air exposure and was accompanied by an increase in titratable alkalinity (TA). The CO_2 content of branchial water in *E. albidigitum* remained constant for at least 4 h and increased slightly after 8 h. TA remained unchanged for up to 8 h. We suggest that the ability of the crabs to compensate for the respiratory acidosis and to increase branchial water TA is correlated with osmoregulation in *P. crassipes* and *H. nudus.* On the other hand, *E. albidigitum* is an osmoconformer and neither compensates for the respiratory acidosis nor changes its TA during air exposure. Possible adaptive advantages of the two different strategies may be related to the relatively short duration of emersion and active habits of *P. crassipes* and the longer periods of air exposure and inactivity of *E. albidigitum.*

INTRODUCTION

The transition between an aquatic and a terrestrial mode of existence is nowhere more apparent than in the intertidal zone along almost any shoreline. While all organisms of the intertidal zone can tolerate air exposure, few are as active and mobile as representatives of the decapod crustaceans. The adaptations which enable these decapods to move readily and rapidly into and out of water have been well studied from a morphological perspective (Pearse 1929; Gray 1957; Edney 1960; Bliss 1968; Diaz and Rodriguez 1977). Recent physiological studies have centered largely on the exchange of gases between the medium (air or water) and the hemolymph (Truchot 1979; Wheatly and Taylor 1979; Taylor and Wheatly 1980, 1981; Burggren and McMahon 1981; Randall and Wood 1981; Wood and Randall 1981*a,* 1981*b;* deFur and McMahon 1984*a,* 1984*b*). One feature common to many of these active littoral crabs is the retention of water in the branchial chambers even when the crab is active in air (Carter 1931; Gross 1955; Flemister 1958; Wheatly and Taylor 1979; Wood and Randall 1981*a;* Wolcott 1984). While the water is obviously important in keeping the lining of the branchial chamber and the gills moist, its role in gas exchange and in the regulation of hemolymph ion and acid-base status is unknown.

Representatives of the intertidal decapod crustaceans exhibit a wide range of activity when naturally air exposed. The most sluggish forms are those which are air exposed only by tidal action. Crabs such as the eastern mud crabs of the family Xanthidae and

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the much larger crabs of the family Cancridae by and large remain inactive, burrowing in the substratum or sheltered in clumps of oyster shells. At the other extreme, the family Grapsidae includes representatives that move voluntarily from water to land and vice versa (Gross 1957). We were interested in examining representatives of both groups to determine the extent to which the observed activity levels could be correlated with differences in the efficacy of mechanisms of physiological compensation to aerial exposure. We were particularly interested in the role of the branchial water stores.

Initially, we measured O_2 uptake of crabs in both air and water as a means of comparing the resting metabolism of the two groups. We then investigated hemolymph acid-base status, branchial water volume, and branchial water CO_2 content to determine whether the active crabs were better able to excrete CO_2 in air. The animals chosen for the comparison were *Eurytium albidigitum* (Rathbun) a relatively inactive burrowing mud crab (family: Xanthidae) air exposed by tidal action and found in the Gulf of California, Mexico; and *Pachygrapsus crassipes* (Randall) and *Hemigrapsus nudus* (Dana), members of the family Grapsidae that are relatively active and readily move in and out of water along most of the western coast of North America.

MATERIAL AND METHODS

ANIMAL COLLECTION AND MAINTENANCE

Eurytium albidigitum (males; 22–33 g) were collected from the mud flats at Laguna Percebu, Mexico, in the upper part of the Gulf of California. *Pachygrapsus crassipes* (males; 16–26 g) were collected in Mission Bay, San Diego. *Hemigrapsus nudus* (males; 12–22 g) were collected in and adjacent to Grappler Inlet near the Bamfield Marine Station, British Columbia. *Eurytium albidigitum* and *P. crassipes* were held in 10-gal aquaria at 23 ± 1 C at the University of San Diego. *Hemigrapsus nudus* were held in running seawater at the Bamfield Marine Station (11 ± 0.5 C). A temperature of 23 C was chosen for *E. albidigitum* and *P. crassipes* since it reflects a temperature that each of the crabs encounters during much of the year, although *E. albidigitum* experiences temperatures much higher (36 C) than *P. crassipes* (about 25 C). *Hemigrapsus nudus* was held at 11 C, the temperature of its habitat during the time these experiments were carried out. Crabs were fed fish twice each week but starved at least 24 h prior to experimentation.

AIR EXPOSURE PROCEDURE

Crabs were removed from water and placed in dry opaque plastic containers with dampened rocks. Care was taken to assure that there were no pools of water anywhere in the container. A damp paper towel was placed over the rocks, and a sheet of plastic covered the entire container to maintain high humidity.

O_2 UPTAKE

O_2 uptake was measured on individual *P. crassipes* and *E. albidigitum* during both immersion (crab totally submerged in water) and emersion (crab air exposed). O_2 uptake on immersed animals was measured by monitoring the rate of oxygen depletion from a closed container. Crabs were placed in glass respirometers (volume = 1 liter) that were held in a water bath thermostated to 23 ± 0.1 C during measurements. In all cases, individual crabs were held in the respirometer for at least 2 h, during which the respirometer was periodically flushed to prevent the P_{O_2} from falling below 100 torr. Since both *P. crassipes* and *E. albidigitum* are strong oxygen regulators, O_2 uptake is insensitive to ambient P_{O_2} over this range (unpublished observations). Therefore, O_2 uptake is reported here for ambient P_{O_2} greater than 100 torr.

We were concerned, however, that CO_2 accumulation in the closed respirometer during the period of measurement might lead to an increase in hemolymph P_{CO_2}, i.e., a respiratory acidosis. Since the effects of a respiratory acidosis on oxygen uptake are unknown, we measured the water P_{CO_2} (Radiometer P_{CO_2} electrode E5036/0) within the respirometer during several experiments to determine whether there was, in fact, a reason to be concerned. Our results indicate that the P_{CO_2} increase per torr P_{O_2} decrease in the closed respirometer is small ($\bar{X} \pm SEM = 0.011 \pm 0.004$ torr P_{CO_2}/torr P_{O_2}, $N = 3$). In our experiments, a

maximum P_{CO_2} increase of 0.6 torr occurred when P_{O_2} declined from air saturation (about 155 torr) to 100 torr within the respirometer. We are assuming, therefore, there is no effect in this experiment of P_{CO_2} on oxygen uptake, although this assumption needs to be verified experimentally. In general, P_{CO_2} increases in closed respirometers can be minimized by assuring that the water in the respirometer is free or nearly free of CO_2. This is true because the capacitance coefficient for CO_2 in water (i.e., how much CO_2, HCO_3^-, and $CO_3^=$ water can hold per torr of P_{CO_2}) is nonlinear at low P_{CO_2} (see Truchot 1984). In water, where P_{CO_2} is less than 0.5 torr, the capacitance coefficient is 5–21 times greater than in water where P_{CO_2} is greater than 0.5 torr (Truchot 1984).

During the first hour of emersion, aerial O_2 uptake was measured by monitoring the O_2 levels of air entering and leaving a respirometer at a known flow rate. O_2 levels were measured using an Applied Electrochemical Oxygen Analyzer and displayed on a strip chart recorder. The aerial oxygen uptake of quiescent *E. albidigitum* was so small that O_2 differences in air entering and leaving the respirometer were not detectable even at very low airflow rates. Therefore, the flow of air through the respirometer was interrupted for 20–40 min. When the flow of air through the respirometer was resumed, a measurable change in O_2 was recorded, reflecting a depletion of oxygen in the closed container. Measured oxygen levels exiting the respirometer gradually returned to baseline (the oxygen level of air entering the vessel). Integration of the resulting curve on the recorder allowed us to calculate O_2 uptake.

BRANCHIAL WATER VOLUME DURING EMERSION

The volume of water remaining in the branchial cavity following 10 min of air exposure was determined for both *P. crassipes* and *E. albidigitum.* Crabs were placed in large glass beakers containing 200 ml filtered (Millipore 0.8 μm) seawater, 10 mg/liter tetracycline, and ^{3}H-inulin (New England Nuclear). After 10 min the activity of ^{3}H-inulin was measured on samples of the water bathing the crabs using a liquid scintillation counter (=^{3}H activity [init] below). Crabs were then air exposed by removing the water by suction to minimize disturbance to the animal. Care was taken to remove all traces of the water from the container. The crab was air exposed for 10 min and then reimmersed in exactly 200 ml of seawater as above, but containing no ^{3}H-inulin. The branchial water containing the ^{3}H-inulin was now diluted by the water containing no inulin. Ten minutes after reimmersion a sample of the well-mixed seawater was removed and the quantity of ^{3}H-inulin present was assayed as before (=^{3}H activity [dil] below).

The quantity of water in the branchial chamber was calculated using the formula

$$\text{Branchial water (ml)} = 1 - \frac{^3\text{H activity (init)} - {}^3\text{H activity (dil)}}{^3\text{H activity (init)}} \times 200\ \text{ml}.$$

Branchial water volume is expressed per gram wet animal weight. To correct for water (and thus ^{3}H-inulin) adhering to the outside of the crab in the above procedure, measurements were made on each species, using the carcass of a dead crab and with the large openings to the branchial chamber at the base of the chelipeds sealed with wax. The volume of water adhering to the outside of the crab was approximately 70% of the water present in the branchial chambers plus that adhering to the crab in *P. crassipes* and 40% in *E. albidigitum.*

BRANCHIAL WATER AND HEMOLYMPH SAMPLING AND ANALYSIS

To sample water from the branchial chamber, small-diameter (0.9 mm) Tygon tubing was inserted into the branchial chamber via a small hole drilled in the branchiostegite just above and between the first and second walking legs. The tubing was attached to the crab's carapace with cyanoacrylate glue (Krazy glue) and dental dam. The dead space of the cannula was filled with air. Branchial water samples were drawn through the cannula into a 1-ml glass syringe and stored on ice for later analysis. The maximum possible volume of branchial water obtained in this manner was variable from animal to animal within a species and ranged from 50 to 100 μl. Fur-

thermore, the longer the period of emersion, the more difficult it was to obtain samples of branchial water. Branchial water was sampled from immersed crabs prior to air exposure and then at different times during emersion. Water samples were analyzed for total CO_2 content using either a Capnicon CO_2 Analyzer (Cameron Instruments Ltd.) or the method described by Cameron (1971).

Titratable alkalinity was measured on branchial water samples taken from a separate group of animals. Exactly 100 µl of branchial water was added to a small open vial into which a micro combination pH electrode (Model MI-710, Microelectrodes, Inc.) was inserted. Nitrogen was bubbled into the sample to provide mixing and to remove CO_2. The sample was titrated to an end point of pH = 3.0 with 6 mM HCl. The number of acid equivalents needed for the titration is termed the titratable alkalinity. After the titration, 100 µl of the branchial water and acid mixture was then assayed for ammonia using the method of Solorzano (1969) and accounting for the dilution of the branchial water by the acid.

Prebranchial hemolymph (0.4 ml) was sampled from the infrabranchial sinus, penetrated via a hypodermic needle inserted into the base of the third or fourth walking leg. The hemolymph sample was drawn into a 1-ml glass syringe, placed on ice, and analyzed for pH and total CO_2 content. The pH was measured with a Radiometer microelectrode thermostated to 23 ± 0.1 C for *P. crassipes* and *E. albidigitum* and 11 ± 0.1 C for *H. nudus*. Total CO_2 content was measured as above.

To determine the presence and extent of the role of anaerobic metabolism during the period of emersion, hemolymph lactate concentrations were determined. Hemolymph lactate concentration was measured in *P. crassipes* and *E. albidigitum* immersed and then emersed for 1 h. Lactate was measured according to Sigma Technical Bulletin No. 826-UV with the modifications suggested by Graham et al. (1983).

Buffer curves were determined in vitro on samples of hemolymph taken from each of four individuals of *P. crassipes* and *E. albidigitum* during immersion. Hemolymph samples were incubated at constant P_{CO_2} for at least 1 h at 23 C and at a range of P_{CO_2} (7.4, 14.8, 22.2, and 29.4 torr) delivered by Wosthoff gas-mixing pumps. The pH and total CO_2 were determined on each hemolymph sample at each equilibration stage using the methods described above.

Chloride ion concentration of hemolymph was measured using a chloride titrator (Radiometer CMT10). Osmolality was determined using a vapor pressure osmometer (Wescor Model 5100C).

STATISTICAL ANALYSIS

Sample means were compared and analyzed for differences using a Student's *t*-test.

RESULTS

O_2 UPTAKE

When compared under similar conditions, i.e. quiescent, O_2 uptake is significantly greater in *Pachygrapsus crassipes* than *Eurytium albidigitum* when the crabs are in water (table 1). On emersion, O_2 uptake decreases significantly in *P. crassipes* and decreases significantly in *E. albidigitum*.

BRANCHIAL WATER DURING EMERSION

The amount of water stored in the branchial chambers of *E. albidigitum* and *P. crassipes* was compared after 10 min emersion (table 1). When compared on a weight-specific basis, *E. albidigitum* retained 5.5 times as much water as did *P. crassipes*. Thus, animals weighing 20 g (a weight close to the average for species used in this study) started with water stores of 0.26 ml for *P. crassipes* and 1.44 ml for *E. albidigitum*. During the course of a prolonged period of emersion in all three species, the amount of water recoverable by the sampling methods used gradually decreased, until at 4 h for *Hemigrapsus nudus*, 8 h for *E. albidigitum*, and 12 h for *P. crassipes* it was rarely possible to collect more than 20 µl of branchial water, suggesting that the amount of water stored was almost totally depleted by this time.

Water samples removed from the branchial chambers of *E. albidigitum, P. crassipes,* and *H. nudus* during immersion contained 1.86 ± 0.53 SEM (N = 51), 1.84 ± 0.31 SEM (N = 21), and 1.88 ± 0.22 SEM (N = 6) mM total CO_2, respectively.

TABLE 1

OXYGEN UPTAKE ($\dot{M}O_2$), BRANCHIAL WATER VOLUME, AND HEMOLYMPH LACTATE LEVELS DURING EMERSION IN *Pachygrapsus crassipes* AND *Eurytium albidigitum*

	$\dot{M}O_2$ (µmol/g-min)				Hemolymph Lactate (mM)		
	Immersed	Emersed (0–1 h)	*P* Value between Treatments	Branchial Water Volume (ml/g) Emersed (10 min)	Immersed	Emersed (1 h)	*P* Value between Treatments
P. crassipes:							
Mean	.031	.014	$P < .05$	.013	.98	.50	$.3 > P > .2$
SEM	.002	.002	df = 3	.007	.57	.12	df = 5
N	4	7		5	5	5	
E. albidigitum:							
Mean	.019	.0009	$P < .005$	.072	.37	.64	$.2 > P > .1$
SEM	.003	.0002	df = 5	.010	.02	.21	df = 4
N	5	4		5	5	5	
P value between crabs	$P < .005$	$P < .025$		$P < .005$	$.2 > P > .1$	$.3 > P > .2$	...

NOTE.—*P* values are shown for differences between means.

The mean values exceeded that measured in ambient seawater by 0.2–0.4 mM total CO_2; however, variability between animals and the limited resolution of the techniques available precluded demonstration of significant CO_2 excretion into the branchial water.

During emersion, the CO_2 content of water stored in the branchial cavities of both *P. crassipes* and *H. nudus* increased rapidly (significantly at 15 and 30 min, respectively) and remained elevated for as long as branchial water could be collected: 4 h for *H. nudus* and 12 h for *P. crassipes* (fig. 2). A completely different pattern was seen in *E. albidigitum,* where branchial water CO_2 levels were unaltered before 8 h and a significant ($P < .005$) elevation was observed.

Carbon dioxide accumulation in the branchial water is accompanied in *P. crassipes* by an increase in the titratable alkalinity (fig. 2). Titratable alkalinity (TA) remains unchanged in *E. albidigitum.* Ammonium ion levels in the branchial water were variable and showed no significant increase throughout an 8-h period of air exposure in either *P. crassipes* or *E. albidigitum.*

HEMOLYMPH ACID-BASE STATUS

Hemolymph acid-base status when compared for quiescent immersed animals was similar for the three species. On emersion, the hemolymph of all species showed a marked and progressive elevation of P_{CO_2}. In *E. albidigitum* this was associated with a marked hemolymph acidosis, but in both *P. crassipes* and *H. nudus* this respiratory acidosis was almost fully compensated (fig. 1).

Hemolymph lactate levels during immersion were not significantly different between *E. albidigitum* and *P. crassipes* (table 1).

REGULATION OF HEMOLYMPH IONIC STATUS

To determine whether there is a correlation between CO_2 accumulation in the branchial water and the ability of the crabs to regulate hemolymph ion and osmotic concentration, we measured hemolymph Cl^- levels and osmotic concentration in *E. albidigitum* (table 2). These variables are already known for *H. nudus* (Dehnel 1966) and *P. crassipes* (Prosser, Green, and Chow 1955; Burnett et al. 1981). As indicated by hemolymph Cl^- levels, *H. nudus* is a strong hyperionic regulator, and *P. crassipes* is both a hyper- and hypoionic regulator. In *E. albidigitum,* Cl^- levels clearly "conform" both above and below normal seawater levels (table 2), indicating little or no ability to regulate the hemolymph levels of this ion.

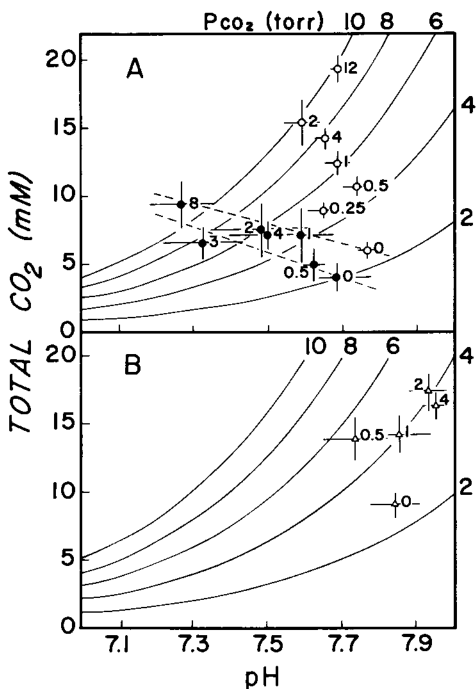

FIG. 1.—The pH-total CO_2 diagrams for *Pachygrapsus crassipes* (open circles) and *Eurytium albidigitum* (closed circles) at 23 C (*A*) and *Hemigrapsus nudus* (open triangles) at 11 C (*B*). In vitro buffer lines are also included in *A*. Values are mean ± SEM. The numbers adjacent to the data points represent the duration of air exposure in hours.

DISCUSSION

Field and laboratory observations reveal that *Pachygrapsus crassipes* is a more active species during air exposure than *Eurytium albidigitum* (Shoemaker and Burnett 1983; Burnett, unpublished observations). These differences are reflected in the changes that occur in oxygen uptake during emersion. *Pachygrapsus crassipes* is clearly able to utilize air breathing to maintain fairly high levels of O_2 uptake while in air and thus frequently moves voluntarily into air to fully exploit the resources available. *Eurytium albidigitum,* on the other hand, exhibits a larger decrease in O_2 uptake, perhaps associated with the collapse of the gills in air (deFur and McMahon 1984*a*), and passively survives air exposure by taking refuge in the burrow. Some *E. albidigitum* make their burrows near the waterline in the small creeks running along the edge of the mud flat and therefore remain immersed for the duration of the low tide. However, a large number (at least half) are found higher in the intertidal zone, where their burrows are totally devoid of free water for part of a tidal cycle. During this period of air exposure the animals remain quiescent.

Unlike *Cancer productus,* another intertidal crab species in which reduced oxygen uptake during air exposure was associated with marked increase in hemolymph lactate (deFur and McMahon 1984*a;* McMahon, Burnett, and deFur 1984), *E. albidigitum* shows no increase in lactate, suggesting that overall metabolism is reduced rather than diverted to anaerobic metabolism. Different patterns of oxygen uptake are seen in other transitional breathers among the decapod crustaceans. *Callinectes sapidus,* a subtidal species, shows a depression in oxygen uptake when air exposed (Batterton and Cameron 1978; O'Mahoney and Full 1984). Species that are found naturally in aerial environments often show little difference in oxygen uptake in air or water (Taylor and Butler 1978; Taylor and Wheatly 1980; O'Mahoney and Full 1984). Furthermore, some of the more terrestrial species appear to have a reduced oxygen uptake when immersed in water (O'Mahoney and Full 1984) and are unable to survive for very long (Bliss 1968). The reasons for these differences in oxygen uptake are unknown but possibly reflect, in part, structural modifications of the lamellae to prevent gill collapse during air exposure (Cameron 1981).

Despite the differences in the ability to utilize aerial O_2, CO_2 builds up in the hemolymph of each of the three animals used in this study (fig. 1). This is a common finding in air-exposed animals (Truchot 1975; Taylor and Wheatly 1981), but the present study reveals important differences both in the mechanisms involved and in the consequences in the two groups of crabs. An increase in hemolymph P_{CO_2} (and a reduction in oxygen uptake) in *E. albidigitum* presumably results in part from an inability of the gill to carry out gas exchange with air. In *P. crassipes,* additional CO_2 buildup may be expected to result from increased aerobic metabolism. Since the increase of hemolymph P_{CO_2} is similar for the three

TABLE 2

HEMOLYMPH CHLORIDE ION AND OSMOTIC CONCENTRATION IN *Eurytium albidigitum* AS A FUNCTION OF SALINITY

	Salinity (ppt)	Chloride Ion Concentration (mM)		Osmotic Concentration (mOsm/kg)	
		Ambient Medium	Hemolymph	Ambient Medium	Hemolymph
Mean ...	19.0	296	264.4	676	713.8
SEM			9		12
N			5		5
Mean ...	25.1	392	333.6	904	867.2
SEM			2		3
N			6		6
Mean ...	28.7	448	391.8	1,000	960.2
SEM			3		19
N			5		5
Mean ...	32.6	508	483.8	1,180	1,189.2
SEM			12		13
N			5		5
Mean ...	38.5	600	530.2	1,392	1,340.2
SEM			5		15
N			6		6
Mean ...	43.1	672	582.4	1,480	1,453.2
SEM			2		2
N			5		5

NOTE.—The salinity of the ambient medium was calculated from the measured chlorinity using the equation S (ppt) = Cl^- (ppt) × 1.80655.

species, it seems clear that CO_2 loss, as well as O_2 uptake, across the gill is also more efficient in *P. crassipes.*

In *E. albidigitum,* the consequence of the CO_2 buildup is a marked respiratory acidosis that is not compensated. Although the build up of CO_2 in the hemolymph of both *H. nudus* and *P. crassipes* appears equivalent, the expression of the acidosis is almost completely masked by compensatory mechanisms. As Truchot (1983) points out, the mechanisms of acid-base compensation in decapod crustaceans are not clearly understood. However, two major mechanisms may be postulated for immersed crabs, both involving a change in the hemolymph strong ion difference (SID) (Stewart 1981): (*a*) ionic exchange between the hemolymph and nonhemolymph body compartments and (*b*) ionic exchange between the hemolymph and the ambient medium (Truchot 1979, 1981, 1983). The first mechanism includes increasing SID by dissolving the calcium carbonates (calcium is a strong ion, carbonate is a weak electrolyte; see Stewart [1981]) of the exoskeleton, releasing calcium and carbonate ions into the hemolymph (deFur, Wilkes, and McMahon 1980; Henry et al. 1981). Although this mechanism is available to immersed crabs, they may not use it to any great extent (Cameron 1985).

Complete isolation of the gills from the aqueous environment during air exposure interferes with regulation of acid-base status both directly, since branchial exchange between the gills and the aquatic medium cannot occur (*a* above) and indirectly, since CO_2 elimination, on which effective utilization of the exoskeletal buffer stores depends (*b* above), may be reduced in air. Regulation of hemolymph acid-base status by either route may thus be compromised. In the crabs used in the present study, however, retention of water in the branchial chambers may allow some continued branchial involvement via either route.

Support for this hypothesis is provided by the present results. All of the animals retain branchial water stores. *Eurytium al-*

bidigitum, which has no ability to regulate hemolymph chloride levels, is also unable to regulate hemolymph acid-base status. Both *P. crassipes* and *H. nudus,* which are both good ion regulators (Prosser et al. 1955; Dehnel 1966) can also regulate hemolymph acid-base status (fig. 1). In both species compensation for the acidosis in air is correlated with a buildup of HCO_3^- in the branchial water—as would be expected if the SID were altered by ionic exchange occurring at the gills.

The accumulation of base in the branchial water of *P. crassipes* may also aid in the removal of molecular CO_2 from the branchial water and thus facilitate branchial CO_2 elimination, at least during the short periods of voluntary emersion that are common for this crab. Shoemaker and Burnett (1983) have determined that *P. crassipes* spends 20% of its time in air during the day and 51% of its time in air during the night when humidity is high (90%–97% relative humidity at 23 C) and seawater is 35 ppt salinity. The periods of emersion, however, are of short duration, most lasting less than 10 min (Shoemaker and Burnett 1983). The rapid accumulation of base observed in branchial water would tend to reduce branchial water P_{CO_2} levels and thus maximize the outward gradient for CO_2 elimination.

The mechanisms used by *E. albidigitum* correlate well with its habit of long air-exposure periods. Based on our field observations of the animal on the mud flats, *E. albidigitum* is usually air exposed much longer (hours) than *P. crassipes.* However, the problem of longer air-exposure time with the associated desiccation may be mitigated in *E. albidigitum* by its much larger branchial water capacity relative to *P. crassipes* (table 1).

Compensation for an air-exposure-induced acidosis is thought to involve mobilization of calcium carbonate derived from the shell (deFur et al. 1980; Henry et al. 1981). This mechanism may explain the apparently paradoxical increase in base in both the hemolymph compartment and the branchial water compartment in *P. crassipes.* Base (e.g., Na^+, K^+, Ca^{++}, Mg^{++}) excreted from the hemolymph compartment into the branchial water stores would have little effect on the quantity of base in the hemolymph since the size of the branchial water stores is relatively small. For instance, in *P. crassipes* weighing 20 g, the size of the hemolymph compartment is 6 ml (30% of body mass, based on Gleeson and Zubkoff [1977]), and the size of the branchial water stores is 0.26 ml. An increase in the titratable alkalinity of the branchial water by 5 meq/liter (fig. 2) would decrease the amount of titratable base in the hemolymph compartment by only 0.2 meq/liter. This minor depletion of base from within the hemolymph compartment could easily be

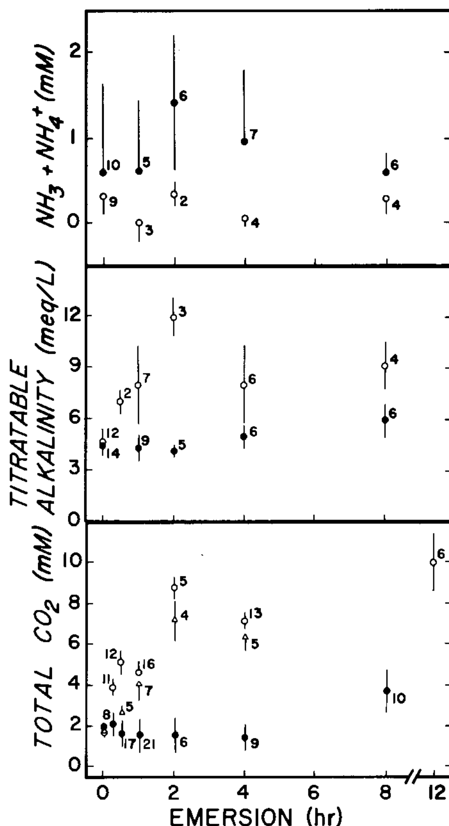

FIG. 2.—Branchial water ammonia, titratable alkalinity, and total CO_2 in *Pachygrapsus crassipes* (open circles) and *Eurytium albidigitum* (closed circles) as a function of the duration of air exposure. Branchial water total CO_2 is also shown for *Hemigrapsus nudus* (open triangles). Values are mean ± SEM. The number of observations appears beside each data point, except at time = 0 for total CO_2 where N = 21, 6, and 51 for *P. crassipes, H. nudus,* and *E. albidigitum,* respectively. Significant differences ($P < .05$) from values at time = zero occur at all times of air exposure in *P. crassipes* for both titratable alkalinity and total CO_2, at all times for *H. nudus,* and at 8 h in *E. albidigitum* for total CO_2. All other values are not statistically different.

made up with the addition of calcium from the exoskeleton. Therefore, the appearance of base in both the hemolymph and the branchial water during air exposure is accounted for by the much smaller volume of the branchial water stores compared with that of the hemolymph.

Since the size of the branchial water stores is small, it has a limited ability for absorbing CO_2 from the hemolymph. At this point, it is unclear whether the accumulation of titratable base imparts any adaptive advantage to an intertidal crab during the first few minutes of air exposure. Nonetheless, the results of this study raise many interesting questions. We know that *P. crassipes* and *H. nudus* regulate hemolymph ionic concentrations over a wide salinity range, and both maintain hemolymph acid-base status and retain branchial water when air exposed. *Eurytium albidigitum* is an ion conformer and cannot maintain acid-base balance when air exposed, even with branchial water. This suggests that gill ion pumps are involved in the maintenance of acid-base balance. Furthermore, there is the additional suggestion that organisms with branchial ion pumps are better able to more actively exploit the aerial environment than forms without this capability because they can maintain hemolymph acid-base balance. Thus, although the animals which are more active in air use more O_2 and produce more CO_2, they are better equipped to handle the CO_2 load.

The hypothesis that an acidosis induced by air exposure is compensated using gill ion exchange mechanisms needs to be tested further, using other intertidal species which are also osmotic and ionic conformers. The role of the branchial water in the CO_2 excretion process during air exposure will undoubtedly be elucidated by comparing animals from different habitats and with different air-exposure habits.

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