O₂ uptake and hemolymph acid-base status, together with branchial water volume, CO₂ content, and titratable alkalinity, were measured in three species of intertidal crabs. In Pachygrapsus crassipes, a crab species that actively moves between air and water, O₂ uptake increased on emersion. In Euryalus albidigitum, a mud-burrowing xanthid crab that is air exposed by tidal action, O₂ 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 species, Hesperograpsus nuda, 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 CO₂ content of branchial water in P. crassipes increased rapidly during air exposure and was accompanied by an increase in titratable alkalinity (TA). The CO₂ 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. nuda. 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 relative 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).

1 We are grateful to T. N. Dunn and C. Guastieri for their technical assistance. L. E. Burnett was supported by a Faculty Research Grant from the College of Arts and Sciences at the University of San Diego and a grant from the Research Corporation. B. R. McMahan was supported by NSERC grant 5762.

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3 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; Weathley and Taylor 1979; Taylor and Wheatley 1980; 1981; Burggren and Mc Mahon 1981; Randall and Wood 1981; Wood and Randall 1981a, 1981b; Defour and McMahan 1984a, 1984b). 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 (Cartier 1931; Gross 1955; Flemister 1958; Wheatley and Taylor 1979; Wood and Randall 1981a, W Dolcott 1984c). 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
The much larger crabs of the family Cancridae by and large remain inactive, bur-
rowing in the substratum or sheltered in clumps of oyster shells. At the other ex-
treme, the family Grapsidae includes re-
representatives that move voluntarily from
water to land and vice versa (Gross 1957).
We were interested in examining represen-
tatives of both groups to determine the ex-
tent to which the observed activity levels
should be correlated with differences in the
efficacy of mechanisms of physiological compensatio-
ne to aerial exposure. We were peculiarly interested in the role of the
branchial water stores.
Initially, we measured O₂ uptake of crabs
in both air and water as a means of com-
paring the resting metabolism of the two
groups. We then investigated hemolymph
acid-base status, branchial water volume,
and branchial water CO₂ content to deter-
mine whether the active crabs were better able to
excrete CO₂ in air. The animals chosen for the comparison were Eu-
ristum albidigum (Rathbun) a relatively inactive
burrowing mud crab (family: Xanthidae)
air exposed by tidal action and found in the
Gulf of California, Mexico; and Pachy-
grapsus crassipes (Randall) and Hemigrap-
sus 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 albidigum (males; 22-33 g)
were collected from the mud flats at Laguna
Percebo, 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. Eu-
ristum albidigum and P. crassipes were held
in 10-gal aquaria at 23 ± 1°C at the Uni-
versity of San Diego. Hemigrapsus nudus
were held in running seawater at the Bam-
field Marine Station (1 ± 0.5°C). A tem-
perature of 23°C was chosen for E. albi-
digum and P. crassipes since it reflects a
temperature that of the crabs encoun-
tered during much of the year, although
E. albidigum experiences temperatures
much higher (36°C) that P. crassipes (about
25°C). Hemigrapsus nudus was held at 11°C.
the temperature of its habitat during this
time these experiments were carried out.
Crabs were fed fish twice each week but
starved at least 24 h prior to experimenta-

tion.

AIR EXPOSURE PROCEDURE
Crabs were removed from water
and placed in dry opaque plastic containers with
dampered 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.

O₂ UPTAKE
O₂ uptake was measured os individual
P. crassipes and E. albidigum during both
immersion (crab totally submerged in wa-
ter) and emersion (crab air exposed). O₂ 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 res-
pirimenter for at least 2 h, during which the
respirimeter was periodically flushed to
prevent the PO₂ from falling below 80 torr.
Since both P. crassipes and E. albidigum
are strong oxygen regulators, O₂ uptake is
insensitive to ambient PO₂ over this range
(unpublished observations). Therefore, O₂ uptake is reported here for ambient PO₂
greater than 100 torr.

We were concerned, however, that CO₂
accumulation in the closed respirimeter during the period of measurement might
lead to an increase in hemolymph pH, i.e., a respiratory acidosis. Since the effects of
a respiratory acidosis on oxygen uptake
are unknown, we measured the water PCO₂
(Radiometer PCO₂ electrode E5036/8)
within the respirimeter during several
experiments to determine whether there was
in fact, a reason to be concerned. Our re-
sults indicate that the PCO₂ increase per torr
PO₂ decrease in the closed respirimeter is
small (K = 1/7 SEM = 0.011 ± 0.004 torr PCO₂/ torr PO₂, N = 3). In our experiments, a
maximum PCO$_2$ increase of 0.6 torr occurred when P CO$_2$ declined from air saturation (about 155 torr) to 100 torr within the respirometer. We are assuming, therefore, that there is no effect in this experiment of PCO$_2$ on oxygen uptake, although this assumption needs to be verified experimentally. In general, PCO$_2$ 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$_3^{2-}$ can hold per torr of PCO$_2$) is nonlinear at low PCO$_2$ (see Truoch 1984). In water, where PCO$_2$ is less than 0.3 torr, the capacitance coefficient is 5–21 times greater than in water where PCO$_2$ is greater than 0.3 torr (Truoch 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. albidaigillum 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 EMERGENCE**

The volume of water remaining in the branchial cavity following 10 min of air exposure was determined for both P. crustifer and E. albidaigillum. Crabs were placed in large glass beakers containing 200 ml filtered (Millipore 0.8 μm) seawater, 10 mg/liter tetracycline, and H-imulin (New England Nuclear). After 16 min the activity of H-imulin was measured on samples of the water bathing the crabs using a liquid scintillation counter ("H activity [m] below). Crabs were then air exposed by re-moving 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 containing no H-imulin. The branchial water containing the H-imulin was now diluted by the water containing no imulin. Ten minutes after reimmersion a sample of the mixed-seawater was removed and the quantity of H-imulin present was assayed as before ("H activity [m] below). The quantity of water in the branchial chamber was calculated using the formula:

\[
\text{Branchial water (ml)} = \frac{1}{1 - \text{H activity (m)}} \times 200 \text{ ml}
\]

Branchial water volume is expressed per gram wet animal weight. To correct for water (and thus H-imulin) adhering to the outside of the crab in the above procedure, measurements were made on each species, using the carcasses 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. crustifer and 40% in E. albidaigillum.

**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-mI glass syringe and stored on ice for later analyses. The maximum possible volume of branchial water obtained is the 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 ana-
falyzed for total CO₂ content using either a Capnograph CO₂ Analyzer (Cameron Instru-
ment Co., Ltd.) or the method described by Cameron (1971).

Titratable alkalinity was measured on branchial water samples taken from a sep-
arate group of animals. Exactly 100 μl of branchial water was added to a small open vial into which a micro combination pH electrode (Model M-710, Microelectrodes, Inc.) was inserted. Nitrogen was bubbled into the sample to provide mixing and to remove CO₂. 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 alkal-
inity. 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 with a hypodermic needle in-
serted 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 CO₂ content. The pH was measured with a Ra-
diometer microelectrode thermostated to 23 ± 0.1 C for P. crassipes and E. albi-
digitum and 11 ± 0.1 C for H. nudus. Total CO₂ 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. Hemo-
lymph lactate concentration was measured in P. crassipes and E. albidigitum immersed and then emersed for 1 h. Lactate was mea-
sured according to Sigma Technical Bul-
letin 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 emersion. Hemo-
lymph samples were incubated at constant PCO₂ for at least 1 h at 23 C and at a range of PCO₂ (7.4, 14.8, 22.2, and 29.4 torr) de-
livered by Wothoff gas-mixing pumps. The pH and total CO₂ were determined on each hemolymph sample at each equilibration stage using the methods described above.

Chloride ion concentration of hemo-
lymph was measured using a chloride titer-
ator (Radiometer CMT 10). Osmolarity was determined using a vapor pressure osmome-
ter (Wescor Model 5100C).

STATISTICAL ANALYSIS
Sample means were compared and an-
falyzed for differences using a Student's t-
test.

RESULTS
O₂ UPTAKE
When compared under similar condi-
tions, i.e. quiescent, O₂ uptake is signifi-
cantly greater in Pachygrapsus crassipes than Eusipho albidigitum when the crabs are in water (table 1). On emersion, O₂ uptake decreases significantly in P. crassipes and decreases significantly in E. albi-
digitum.

BRANCHIAL WATER DURING EMERSION
The amount of water stored in the branchi-
al chambers of E. albidigitum and P. crassipes was compared after 10 min emer-
sion (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 meth-
ods used gradually decreased, until at 4 h for Hemigrapsus nudus, 8 h for E. albi-
digitum, and 12 h for P. crassipes it was barely 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 branchi-
al chambers of E. albidigitum, P. cras-
ipes, and H. nudus during immersion contained:

| Species | SEM (N = 5) | 1.88 ± 0.22 SEM (N = 6) | mEq total CO₂ respectively. |
### Table 1

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<td>10</td>
<td>0.8</td>
<td>0.5</td>
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</tbody>
</table>

### Notes

- *P* values are shown for different treatments.

The mean values exceeded that measured in ambient seawater by 0.2-0.4 mEq/l total CO2; however, variability between animals and the limited resolution of the techniques available precluded demonstration of significant CO2 excretion into the branchial water.

During emersion, the CO2 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. albida* where branchial water CO2 levels were unaltered before 8 h and a significant (*P < 0.001*) 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. albida*. 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. albida*.

### Hemolymph Acid-Base Status

Hemolymph acid-base status when compared for quiescent immersed animals was similar for the three species. On emergence, the hemolymph of all species showed a marked and progressive elevation of PCO2. In *E. albida* this was associated with a marked hemolymph acidity, 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. albida* and *P. crassipes* (table 1).

### Regulation of Hemolymph Ionic Status

To determine whether there is a correlation between CO2 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. albida* (table 2). These variables are already known for *H. nudus* (Delude 1966) and *P. crassipes* (Prouser, 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. albida*, 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.
**DISCUSSION**

Field and laboratory observations reveal that *Pachygrapsus crassipes* is a more active species during air exposure than *Eurytmus albispinosus* (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. *Eurytmus albispinosus*, on the other hand, exhibits a larger decrease in $O_2$ uptake, perhaps associated with the collapse of the gills in air (de Fur and McMahon 1984a), and passively survives air exposure by taking refuge in the burrow. Some *E. albispinosus* 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 (de Fur and McMahon 1984a; McMahon, Burnett, and de Fur 1984), *E. albispinosus* 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 (Batterson 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 (Blos 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 PCO$_2$ (and a reduction in oxygen uptake) in *E. albispinosus* 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 PCO$_2$ is similar for the three
<table>
<thead>
<tr>
<th>SALINITY (ppt)</th>
<th>CHLORIDE ION CONCENTRATION (mM)</th>
<th>ORbicatal CONCENTRATION (mOM/kg)</th>
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</table>

NOTE.—The salinity of the ambient medium was calculated from the measured chloride using the equation $S_{ppt} = C_{ppt} × 1.80635$.

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. albidaugtum_, 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 evident, the expression of the acidosis is almost completely masked by compensatory mechanisms. As Truschet (1983) points out, the mechanisms of acid-base compensation in hemolymph of 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) exchange between the hemolymph and nonhemolymph body compartments and (b) exchange between the hemolymph and the ambient medium (Truschet 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 (deFar, 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 1983). Complete isolation of the crabs from the aequorine 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 depend (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 albi_
bidigium, 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 HCO₃⁻ 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 CO₂ from the branchial water and thus facilitate branchial CO₂ 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 seawater branchial water would tend to reduce branchial water PCO₂ levels and thus maximize the outward gradient for CO₂ elimination.

The mechanisms used by *E. albidigium* correlate well with its habit of long air-exposure periods. Based on our field observations of the animal on the mud flats, *E. albidigium* 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. albidigium* 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⁺, Ca²⁺, Mg²⁺) extricated 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 Zulkoff [1977]), and the size of the branchial water stores is 0.26 ml. An increase in the titratable alkalinity of the branchial water by 0.2 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

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**FIG. 2.—** Branchial water ammonia, titratable alkalinity, and total CO₂ in *Pachygrapsus crassipes* (open circles) and *Euryalina albidigium* (closed circles) as a function of the duration of air exposure. Branchial water total CO₂ is also shown for *Hemigrapsus nudus* (open triangles). Values are mean ± SEM. The number of *P. crassipes* N = 21, b, and 51 for *P. crassipes*, *H. nudus*, and *E. albidigium*, respectively. Significant differences (P < .05) from values at time 0 occur at all times of air exposure in *P. crassipes* for both titratable alkalinity and total CO₂, at all times for *H. nudus*, and at 8 h in *E. albidigium* for total CO₂. 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 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 impairs 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. cassipes and H. nudus regulate hemolymph ionic concentrations over a wide salinity range, and both maintain hemolymph acid-base status and retain branchial water when exposed. Exuvium albidum is an anion conformer and cannot maintain acid-base balance when air exposure is even with branchial water. This suggests that gill putrescine ions 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 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 ionic and tonic 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.

LITERATURE CITED


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