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The Burrow Microhabitat of the Land Crab *Cardisoma guanhumi:* Respiratory/Ionic Conditions and Physiological Responses of Crabs to Hypercapnia

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Abstract

Cardisoma guanhumi *in Puerto Rico spend much of the dry season in burrows that descend to groundwater. Burrows are sometimes 3–4 m deep and may be capped with dried mud for over 3 mo. Samples of gas and water from burrows at three locations in Puerto Rico were analyzed for* O_2, CO_2, *and ions. Osmolality and ion composition varied widely with location, from about 10% to 130% of seawater. Both gas and water* P_{O_2}*'s were generally 90–120 mmHg, although some water samples were extremely hypoxic (*$P_{O_2} < 20$ *mmHg). Most burrows were extremely hypercapnic, with gas and water* P_{CO_2}*'s up to 60 and 90 mmHg, respectively. Whether the burrow was plugged or even occupied by a crab made little difference to ion or gas concentrations. Radiotelemetry of the vertical position of crabs in their burrows indicated that they did not avoid hypercapnia and hypoxia by staying close to the entrance.* Cardisoma guanhumi *are thus exposed to extreme changes in* P_{CO_2} *when they descend deep in their burrows after foraging at dawn and dusk. Impedance measurements of branchial ventilation and heart rate in resting crabs exposed to environmental hypercapnia in artificial burrows revealed two distinct types of ventilatory responses based on the degree and duration of hypercapnia: hyperventilation in response to gradual, chronic exposure to elevated burrow* CO_2 *and apnea in response to rapid elevations of burrow* CO_2 *above 4%. These physiological responses suggest the presence of both "external" and "internal" receptors of hypercapnia that may allow* Cardisoma *to buffer large acid-base disturbances when moving between extremes in gaseous microenvironments.*

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Introduction

Cardisoma guanhumi is a semiterrestrial brachyuran crab that is widely distributed throughout the Caribbean in low-lying areas close to the sea. Although it may be found up to 5 km from the ocean in areas without surface water, *Cardisoma* is considered to be semiterrestrial because it burrows down to the water table and thus always has a pool of water available. A typical burrow is almost vertical, with an "entry" at a shallower angle, and has only a single entrance (Feliciano 1962; Herreid and Gifford 1963). Burrows are dug by the crabs themselves and are only slightly larger in diameter than the width of the carapace. Although activity patterns depend on season, local climate, and the environment surrounding the burrow (Gifford 1962), in dry areas the crabs spend the majority of their time in their burrows, coming out to forage in the early morning and late afternoon (Feliciano 1962; Gifford 1962; Henning 1975).

Ion concentrations in the water in burrows vary widely (Herreid and Gifford 1963; Wood and Boutilier 1985), but respiratory conditions in burrows are less well known. Because burrows are long, narrow, blind-ending tubes and thus poorly ventilated, hypoxia and hypercapnia are likely. Burrows of the closely related species *Cardisoma carnifex* were moderately hypoxic and hypercapnic (Wood and Boutilier 1985). Because the burrows of *C. guanhumi* at the locations chosen in Puerto Rico can be much deeper and narrower than those sampled by Wood and Boutilier (Feliciano 1962), the gas composition might be more dramatically altered from ambient air. Burrows are often capped with dried mud during much of the dry season, which potentially decreases ventilation even further and potentially produces an even more hypercapnic and hypoxic atmosphere. The crabs moult while sealed in their burrows, potentially depleting the limited pool of Ca^{2+} available for hardening the carapace. Because acid-base regulation is likely to involve branchial ion exchange, ion concentrations in burrow water may also be important during compensation of hypercapnic acidosis.

The relationship between burrow shape and respiratory gases is unknown, as is whether capping the burrow changes the interior atmosphere, or whether hypercapnia and hypoxia are due to respiration of the crabs themselves or to other organisms cohabiting the burrows. The vertical distribution and movement patterns of crabs in their burrows, and thus their exposure to hypercapnia and hypoxia, are unknown. Finally, the cardiorespiratory responses of *Cardisoma* to hypercapnia have been little studied, especially in burrows, the natural environment of resting crabs, wherein they are exposed to hypercapnia.

The purpose of this investigation was (1) to measure ions, P_{O_2}, and, especially, P_{CO_2} in the burrow microhabitat of *C. guanhumi* at several locations in Puerto Rico, (2) to monitor activity and position of the crabs to estimate their exposure to ambient air and burrow atmosphere, and (3) to test whether the crab modifies conditions within its own burrow by respiration and ion exchange, especially in plugged burrows. Finally, ventilatory and cardiac responses of captive crabs in artificial burrows to hypercapnia similar to that encountered in the field were investigated.

Material and Methods

Field Measurements

Three locations in Puerto Rico were chosen on the basis of distance from the ocean (thus, depth of the water table and length of the burrows) and size of crabs (thus, diameter of burrows). One site was on Cabo Rojo National Wildlife Refuge, approximately 300 m from the ocean, and contained deep (180–330 cm), small-diameter (<7 cm) burrows. Both plugged and open burrows were sampled. Two sites were on the island of Culebra (about 5 miles east of Puerto Rico); one was 1–10 m from the ocean and had very shallow burrows (60–150 cm), the other was about 50 m from the ocean and had deeper burrows (170–210 cm) of up to 16 cm in diameter. Water and ion samples were taken during March, at which time little or no rain had fallen in over 2 mo. Crab activity and position in burrow were monitored at Cabo Rojo in September of the same year, again during the dry season, when crab activity patterns were similar to those in March.

Gas and water samples were taken through PE-205 tubing cabled together with an electronic temperature probe to a plumber's snake. Enough gas and water (10–20 mL) was drawn through the tubing to flush the dead space. Gas samples were drawn into glass 5-mL syringes and sealed with metal caps until measured. Water samples were drawn into syringes, then stored without gas bubbles in 1.5-mL microcentrifuge tubes on ice until gases were measured (1–2 h). Other water samples were frozen in microcentrifuge tubes for later measurement of osmolality and ionic composition. Although freezing samples with high ionic content may have caused subsequent measurements of Ca^{2+} and Mg^{2+} to be low (because of formation of insoluble complexes during freezing), this would not cause systematic differences between groups of samples.

Water pH and temperature (air and water) were measured at the sampling site with a portable Fisher pH meter (precision .05 unit) and a Cole-Parmer electronic thermometer, respectively. Gases (P_{O_2} and P_{CO_2}) were measured

within about 2 h of sampling with an Instrumentation Laboratory (IL) μ13 blood gas analyzer and IL electrodes maintained at the temperature measured at the bottom of the burrows (27°–29°C) and calibrated with a Fisher standard gas mixture (5.0% CO_2, 95.0% N_2) and air. Osmolality was measured with a Wescor model 5000 vapor pressure osmometer; Na^+ and K^+ were measured with an IL model 143 flame photometer; Cl^- was measured with a Radiometer CMT 10 chloride titrator; Mg^{2+} and Ca^{2+} were measured with a Perkin-Elmer model 280 atomic absorption spectrophotometer.

Positions of three crabs inside their burrows were determined by radiotelemetry. A short-range (25 cm) FM radio transmitter (a cylinder approximately 10 mm long, 4 mm in diameter, weighing 1.2 g) was attached to the dorsal posterior edge of the crab's carapace with cyanoacrylate glue and three or four layers of latex rubber sheeting. A four-section antenna with receiving sections spaced 50 cm apart was pushed down the burrow (see diagram at right of fig. 5*B* below). An FM radio was used to receive the signal; each of the four antenna stations could be attached with a telephone jack to the antenna input of the radio, which was stationed 1 m from the burrow entrance. Crab position was determined by finding the section of the antenna receiving the strongest signal from the radio transmitter (usually only one antenna section picked up any signal, never more than two). Crabs to be studied by telemetry were trapped overnight. The transmitters were attached in the field the next morning, and the crabs were released back into their own burrows. Position recording commenced the following day. Because of the small size and weight of the transmitter compared to the size of the crab (80–120-mm carapace width, 150–250 g), and the placement of the transmitter at the edge of the carapace behind the most posterior walking leg, the crabs were able to move freely up and down their burrows.

Laboratory Measurements

Cardisoma guanhumi collected near the study area were airlifted to the University of Massachusetts and maintained in aquaria with shallow water (2–3 cm) and sections of polyvinyl chloride (PVC) pipe for refuge. The crabs were fed vegetables ad lib., and room temperature was maintained at 27°–30°C.

Respiratory and cardiac responses of six crabs (body mass 160 ± 15 g, ±SE) to hypercapnia were measured while crabs resided in artificial burrows (fig. 1). A curved section of PVC pipe (7.5 cm in diameter, 0.75 m long) placed inside an aquarium was plumbed to allow controlled delivery of water to the bottom 5 cm and gas mixtures to the air space above. Gravel adhered to the inside lining of the pipe with silicon cement allowed the

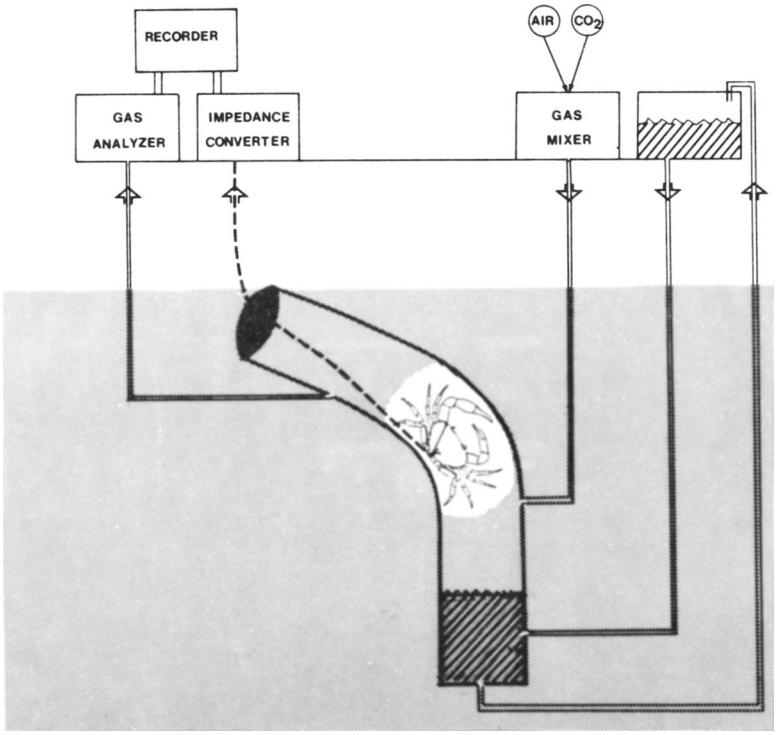

Fig. 1. Artificial burrow used to measure f_b *and ventilation rates in response to environmental* CO_2*. Components are explained in text.*

crabs to easily move up and down the burrow. Water (50%-strength artificial seawater) was circulated between the burrow and an aerated aquarium by a peristaltic pump (10 mL/min). Gas mixtures of air and increasing levels of CO_2 were delivered through a Wösthoff pump (250 mL/min) into the burrow just above water level and were allowed to exit the large opening at the top. Gas concentrations within the burrow were continuously sampled by aspirating 25 mL/min from a midburrow location into Beckman OM11 oxygen and LB-2 carbon dioxide analyzers.

Respiratory effort (left scaphognathite movements) and heart rates were measured by changes in impedance between pairs of fine copper electrodes (40 gauge) placed between the carapace and the hypodermis over the left scaphognathite channel and the heart. The electrodes and the carapace at these sites were covered with small pieces of sheet latex that were cemented with cyanoacrylate glue. The twisted electrode pairs, protected inside polyethylene tubing (PE-120, Intramedic) and anchored at the dorsolateral edge of the carapace, were led out the top of the artificial burrow and connected to impedance converters (UFI model 2991). The amplified impedance from

left scaphognathite beating and heart contraction was recorded on a Narco Bio-Systems Physiograph (Mk IV).

Crabs implanted with impedance electrodes were placed in the artificial burrow singly and were allowed 24–48 h to acclimate. The acclimation time was determined by occasional sampling of respiratory and cardiac rates to determine when they had decreased to minimum values. Physiological responses were observed as crabs were presented with environmental hypercapnia administered as either gradual increases to fixed CO_2 levels or as acute, rapid changes to CO_2 levels. In the first case, the crabs were presented with alternating, 30-min periods of air interspersed between air + CO_2 (2%–10%), in which the gas concentration was metered to cause a slow (10–15 min) turnover within the burrow. Scaphognathite movements and heart rates were measured during the last 5 min of the 30-min treatment. In order to simulate rapid changes in ambient CO_2, as crabs might experience by moving into deep burrow locations in the field, crabs in the artificial burrows were also exposed to large and rapid CO_2 changes (e.g., 4% change in <2 min). The measured variables in these hypercapnic trials were forward scaphognathite beating frequency (f_{sc} in beats per minute [bpm]), the duration of time out of 5 min that the scaphognathite was beating (d_{sc} in %), and heart frequency (f_h in bpm). All measurements were made in a dimly lit and sound-insulated room at 23°–25°C.

Results

Field Measurements

The gas in most burrows was moderately hypoxic and the water was significantly more hypoxic than the gas (fig. 2); the lowest recorded P_{O_2} in water was 1 mmHg. Even more interesting is the fact that most burrows were extremely hypercapnic. The highest P_{CO_2}'s measured in gas were approximately 60 mmHg, while the highest P_{CO_2} in water was 90 mmHg. It is surprising that P_{O_2}'s and P_{CO_2}'s in plugged burrows were not significantly different from those in open burrows. Despite the very high P_{CO_2} in water, pH was neutral (mean pH 6.9, range 6.3–7.6). Burrow depth reflected the depth of the water table at the four locations. The temperature in all burrows was 28°C (range 27.0°–29.5° ± 0.1°C [SE]).

Depth was the major factor determining P_{O_2} and P_{CO_2} in burrow gas (fig. 3); shallow burrows (<140 cm) showed little hypoxia or hypercapnia, while burrows deeper than 140 cm rapidly became more hypoxic and hypercapnic with increasing depth. Burrow width was a minor but significant factor (including width with depth in a multiple regression against P_{O_2} increased r^2

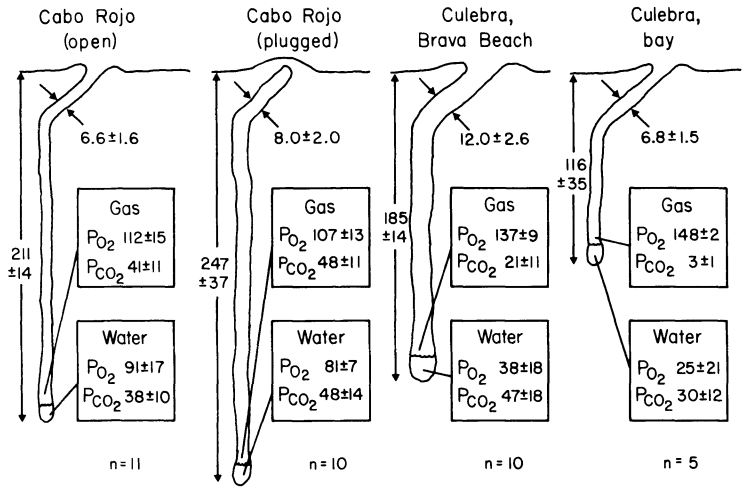

Fig. 2. The P_{O_2} and P_{CO_2} partial pressures in water and gas at the bottom of burrows and scale diagrams of the burrows from the four sampling locations. Burrow dimensions are in centimeters; gas partial pressures are in millimeters of mercury.

to 0.62 from 0.58, $P < 0.05$). Burrow gas P_{O_2} and P_{CO_2} were closely correlated, with a slope of −0.93; $r^2 = 0.95$. There was no correlation of water P_{O_2} or P_{CO_2} with depth of the burrow or with each other.

To test the influence of the resident crab on the burrow atmosphere, we compared burrows occupied by crabs to burrows from which the crabs had been removed. Burrows were flushed with fresh air immediately after the crabs were removed to ensure that gas partial pressures measured 24 h later were established after the removal and were not merely the partial pressures of stagnant gas remaining from before the crabs were removed. Both P_{O_2} and P_{CO_2} 24 h after flushing were very close to the values measured before flushing, whether there was a crab in the burrow or not (table 1).

There were P_{O_2} and P_{CO_2} gradients between ambient air and gas at the bottoms of burrows (fig. 4). Exposure of crabs to hypoxia and hypercapnia thus depends on the amount of time the crabs spend at various positions within the burrow as well as time spent outside the burrow foraging. A rough estimate of the surface activity of the crabs was made by counting the number of crabs visible in a 360° sweep from a single position. The crabs were active and foraging on our arrival shortly after dawn, but almost all disappeared from the surface between 0830 and 0930 hours (fig. 5*A*). Only one or two could be seen most of the day, then large numbers reappeared starting at about 1630 hours. Spot checks at night with a flashlight showed few crabs on the surface.

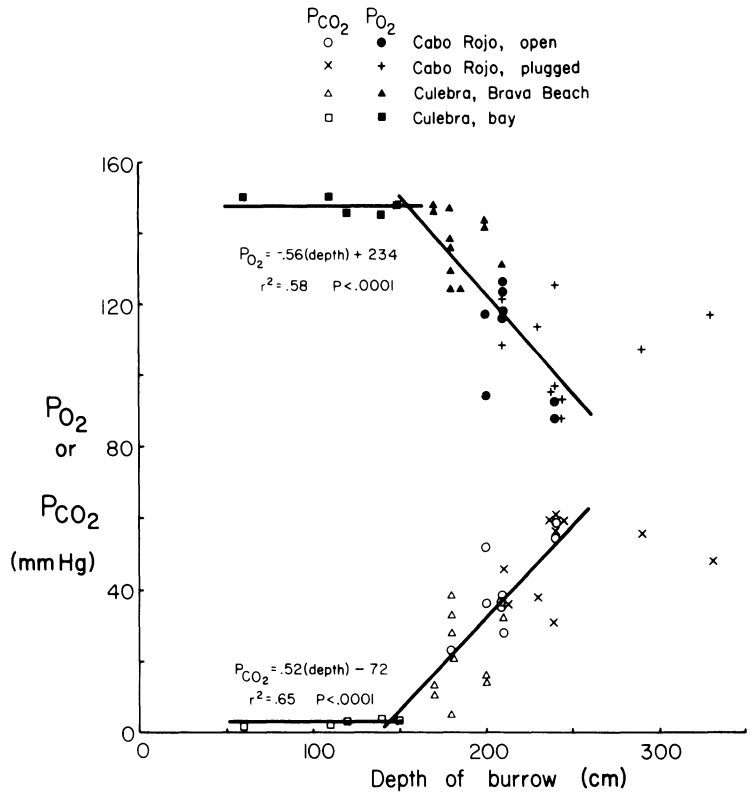

Fig. 3. The P_{O_2} and P_{CO_2} of gas at the bottom of burrows as a function of the depth of the burrow. Regression equations were derived from data from all sites; only burrows over 140 cm deep were included in the regression.

The movements of three crabs were monitored in more detail by radiotelemetry (fig. 5*B*). Once the crabs disappeared from the surface they went relatively deep into their burrows. They did not remain near the surface and make short forays down to the water; they remained at or near the bottom for several hours. It was not possible to tell from these records whether the crabs were immersed in the water at the bottom or not.

Ion concentrations were highly variable between locations and even between burrows at a single location, ranging from a little over 10% of the osmolality of seawater up to over 130% seawater strength in two burrows within 2 m of the ocean (table 2). The Ca^{2+} concentrations were high in all samples (>5 mM, even for the samples with the lowest osmolalities), perhaps because of underlying limestone in the areas tested, and were significantly

TABLE 1
Effect of crab respiration on burrow gases in three burrows from which the resident crabs were removed compared to three burrows in which the crabs were left in place

	Before Flush		Immediately after Flush		After 24 h	
Burrow	P_{O_2}	P_{CO_2}	P_{O_2}	P_{CO_2}	P_{O_2}	P_{CO_2}
Crabs removed:						
Burrow 1 ...	...	...	151	<3	114	51
Burrow 2 ...	128	30	152	<3	138	27
Burrow 3 ...	103	55	150	<3	128	42
Crabs left in place:						
Burrow 4 ...	...	...	152	<3	132	27
Burrow 5 ...	105	58	151	<3	103	57
Burrow 6 ...	93	63	151	<3	110	56

lower in the samples from plugged burrows than from open burrows at Cabo Rojo ($P < 0.005$; t-test). Other ions and osmolality were not significantly different between open and plugged burrows.

Physiological Responses to Hypercapnia

Branchial chamber ventilation measured in six resting, unrestrained crabs during exposure to air within the artificial burrow was intermittent and occurred as infrequent bouts of scaphognathite movements. Forward left f_{sc} averaged 3.7 ± 2.2 bpm (range 0–14 bpm), and d_{sc} was only 6.5% ± 4.6%. Heart rates of crabs decreased from 100–120 bpm when the crabs were first placed in the burrows to 30.5 ± 3.9 bpm after 24–48 h of acclimation. Reverse scaphognathite movements, as noted by impedance traces of opposite polarity to forward movements, occurred primarily when crabs emerged from the water and were generally associated with a tachycardia. All subsequent scaphognathite beating in air was forward.

Thirty-minute exposure of crabs to incremental levels of ambient CO_2 resulted in relatively uniform and incremental increases in f_{sc} and d_{sc} (fig. 6). Each 2% increment of CO_2 was accompanied by an approximately 20-

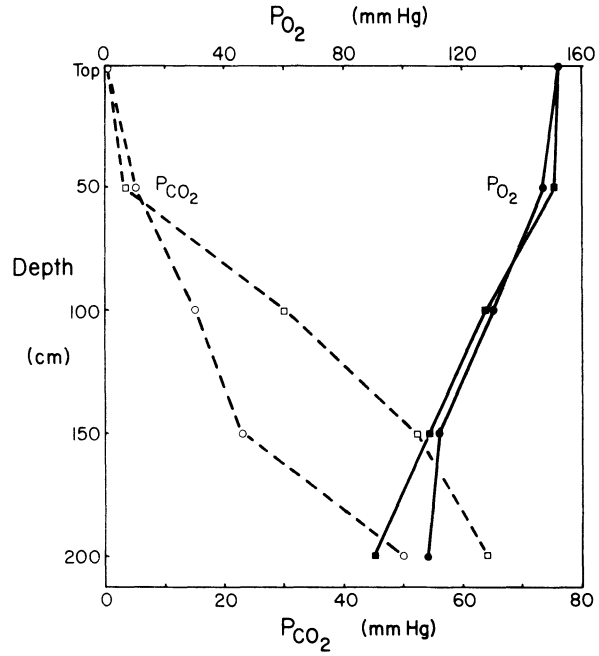

Fig. 4. Vertical stratification of gas P_{O_2} and P_{CO_2} in two burrows. The P_{O_2} (solid symbols, solid lines) and P_{CO_2} (open symbols, dashed lines) were measured at 50-cm intervals. Differently shaped symbols (circles and squares) are used for each burrow. The gradients are fairly linear, although there is little change in gases in the top 50 cm.

bpm increase in f_{sc}, due largely to an increase in d_{sc}, until scaphognathite beating at 10% CO_2 was nearly continuous at 91 bpm. Although d_{sc} began to plateau above 6% CO_2 as the scaphognathite approached continuous activity, f_{sc} showed little sign of plateauing up to 10% CO_2, which suggests that the duration of individual scaphognathite beats was decreasing. Heart rates of crabs during these hypercapnic exposures were extremely stable (fig. 6) and did not differ significantly from control values (0% CO_2; ANOVA, $P > 0.5$).

Rapid changes in burrow CO_2 concentrations between 0% and 3% resulted in f_{sc} changes similar to those described above. In all crabs ($n = 5$) exposed to rapid, intraburrow CO_2 concentration elevations above 4%, scaphognathite beating promptly ceased and remained inactive until ambient CO_2 was again decreased below 4% (fig. 7). Heart rate during these ventilatory apneas was not altered (fig. 7) suggesting that these apneas were not associated with movement or disturbance. Apneas also appeared to be initiated and terminated without the need for the crab to ventilate the branchial chamber with the hypercapnic gas.

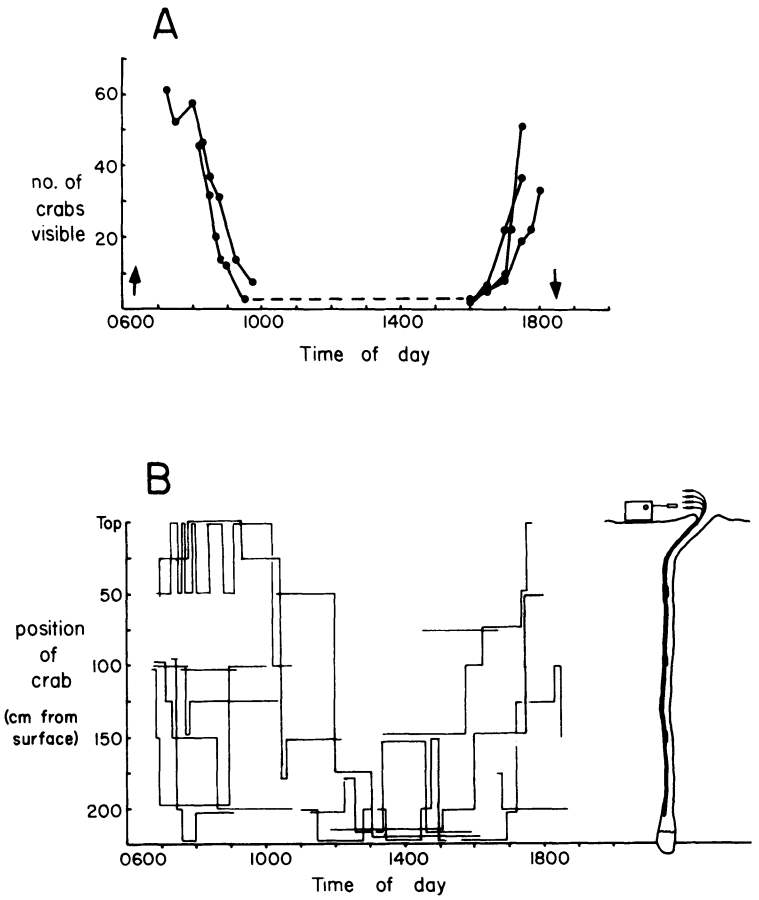

Fig. 5. A, *The number of crabs visible on the surface from a single observation post, a measure of surface activity. The arrows indicate sunrise and sunset.* B, *Positions of individual crabs within their burrows during the day at Cabo Rojo. Each line represents the position of one of the three crabs with an attached radio transmitter during an approximately 4-h period. Each crab was followed for a different part of the day for several days, covering the entire period between sunrise and sunset. The diagram to the right of* B *shows the arrangement of the four-section aerial in the burrow (the four receiving sections are represented by thickened sections in the line representing the aerial) and the radio receiver.*

Discussion

Gas Concentrations

Burrow geometry, particularly depth, strongly influences gas concentrations at the bottom of the burrow. Burrows up to 140 cm deep are apparently

TABLE 2

Ion concentrations in burrows from various locations around Puerto Rico

Location	Ca^{2+} (mmol/L)	Mg^{2+} (mmol/L)	Na^{+} (mmol/L)	K^{+} (mmol/L)	Cl^{-} (mmol/L)	Osmolality (mosm/kg)
Cabo Rojo, unplugged (n = 11)	30 ± 7.2 (21–42)	56 ± 7.7 (45–71)	358 ± 44 (316–442)	2.7 ± .45 (2.1–3.6)	482 ± 62 (417–616)	843 ± 113 (720–1,080)
Cabo Rojo, plugged (n = 10)	18 ± 2.3 (14–20)	48 ± 5.9 (38–54)	311 ± 30 (254–334)	2.2 ± .15 (2.0–2.4)	404 ± 44 (325–440)	710 ± 75 (570–775)
Culebra, Brava Beach (n = 10)	7 ± 2.3 (5–11)	10 ± 2.5 (7–15)	84 ± 25 (64–143)	1.8 ± 1.1 (.8–4.4)	101 ± 33 (65–176)	181 ± 50 (138–300)
Culebra, bay (n = 5)	28 ± 11 (18–46)	33 ± 12 (21–46)	496 ± 175 (226–640)	8.8 ± 3.6 (4.9–12.8)	618 ± 191 (352–812)	1,120 ± 393 (650–1,543)

Note. Data are presented as mean ± SD, with range in parentheses below.

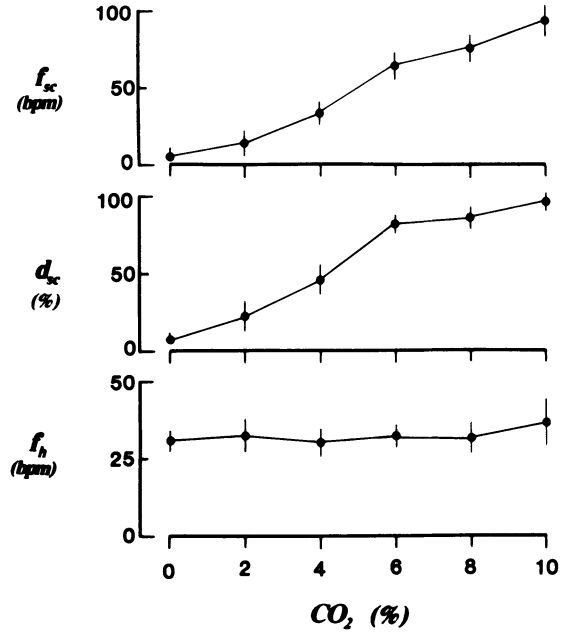

Fig. 6. Average (±SD) f_{sc}, f_h, *and* d_{sc}, *measured in response to a 30-min exposure to increasing* CO_2 *in the artificial burrow* (n = *6 crabs).*

well ventilated and have only slightly altered P_{O_2}'s and P_{CO_2}'s from normal atmospheric levels. Deeper burrows have progressively higher P_{CO_2}'s and lower P_{O_2}'s, with maximum gaseous P_{CO_2}'s of over 60 mmHg and P_{O_2}'s under 100 mmHg. Burrows in this study were much more hypoxic and hypercapnic than those sampled by Wood and Boutilier (1985), probably because we sampled deeper burrows; burrows of similar depth had similar gas pressures. That the crab is not the only, or perhaps even a major, component of total burrow respiration is inferred from the observation that unoccupied burrows had the same gas concentrations as occupied burrows (table 1). The crabs share their burrows with various insects, centipedes, and other inhabitants, and the burrow walls are damp for much of their length, providing a large surface area for microbial growth; the slope of the correlation between P_{O_2} and P_{CO_2} suggests an aggregate respiratory quotient of 0.93. It is surprising that there were no differences between plugged and open burrows; it was expected that ventilation would be reduced by the plug, and therefore the burrow gas would be more hypoxic and hypercapnic. Excavated plugs had large cracks through them and through the surrounding soil, which suggests that much of the ventilation of the burrow is actually exchange through the surrounding soil.

Gas partial pressures in the water at the bottoms of burrows were not closely related to partial pressures in burrow gas, although P_{O_2} of water was

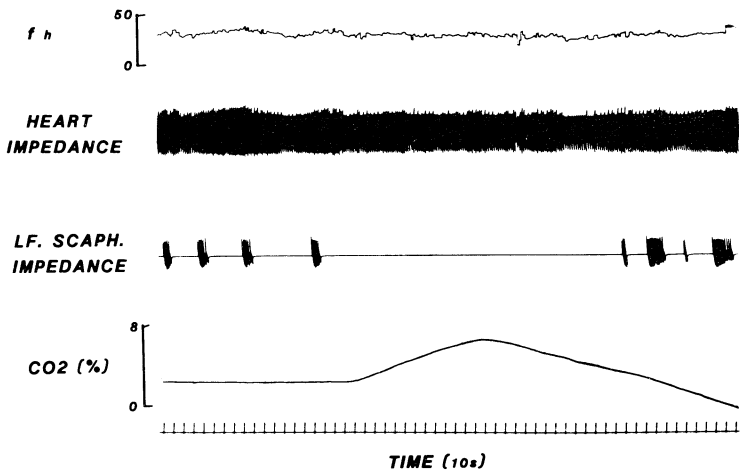

Fig. 7. Physiograph recording of heart and left scaphognathite impedance and instantaneous f_b *(bpm) of an individual crab in response to rapidly altered levels of* CO_2 *within the artificial burrow. Note ventilatory apnea is sustained for more than 4 min of elevated* CO_2*;* f_b *is unchanged during the experimental hypercapnia.*

almost always lower and P_{CO_2} generally higher than the gas immediately above it. The two sites nearest the ocean, which had almost normal atmospheric P_{O_2}'s and P_{CO_2}'s in burrow gas, had extremely hypoxic and hypercapnic water; P_{O_2} ranged from 1 to 62 mmHg, and P_{CO_2} ranged from 19 to 90 mmHg. Water P_{O_2} and P_{CO_2} were not closely correlated with each other. Gas partial pressures in the water thus seem to be a complex function of organismal respiration, equilibration with burrow gas, and soil and water chemistry. Mismatching of water and air gas tensions may also have been influenced by the water sampling technique, in which some degassing of the sample may have occurred because of the subambient pressures necessary to pull samples up from the bottoms of deep burrows. Although *Cardisoma guanhumi* and *Cardisoma carnifex* ventilate water through their branchial cavity when a shallow pool is available (Shah and Herreid 1978; Wood and Randall 1981*a;* O'Mahoney and Full 1984; Burggren et al. 1985), it is unlikely that the water in the burrow is used as a respiratory medium, because it is so hypoxic and hypercapnic.

In spite of the extremely high P_{CO_2} of some samples, pH was always close to neutral. To have a pH of 6.5 with a P_{CO_2} of 60–90 (the high end of the range we measured in burrows) the water would have to have a bicarbonate concentration of 6–8 mM. Although this is higher than seawater (approximately 2 mM), it may have been provided from dissolution of underlying limestone, along with the high Ca^{2+} measured.

Exposure to Deep Burrow Conditions

As an amphibious land crab, *Cardisoma* does not require water immersion to respire; in fact the gills and "lung" (lining of the branchial chamber) appear to work equally well in air and water for O_2 extraction (O'Mahoney 1977). However, water taken into and held in the comparatively large branchial chambers is essential for ion regulation, nitrogen excretion, and acid-base regulation (Wood and Boutilier 1985). Wood and Randall (1981*a*) noted that up to 65% of total CO_2 excretion in *C. carnifex* was by way of the branchial water. Unlike more terrestrial crabs, *Cardisoma* is not dehydration resistant (McMahon and Burggren 1988) and therefore must frequently replenish its branchial water supply, and, in light of burrow gas conditions, expose itself to hypoxia and severe hypercapnia.

Thus, two possible scenarios of interaction between the crab and its hypoxic and hypercapnic burrow exist. The first is that crabs make brief forays to the burrow bottom to replenish branchial water and that they minimize the respiratory consequences of the burrow conditions by "holding their breath" while near the bottom of the burrow. Physiological support for this scenario resides in the observation that ventilatory apnea was invariably seen in crabs exposed to large step changes in CO_2, particularly above 4%, the average CO_2 concentration halfway down natural burrows at Cabo Rojo. It is evident from figure 7 that crabs initiate apnea prior to scaphognathite movement, which suggests that this response is mediated by "external" chemo- or irritant receptors on the crab's external integument. The stability of f_h during the onset of apnea and the typical duration of sustained apnea (>10 min at 8% CO_2) indicate that this is not a disturbance apnea and is sufficiently long to support a brief journey to the burrow bottom. Crabs make short forays to the bottoms of their burrows when they are deepening them; they carry mud up from the bottom and dump it around the entrance. They use the same mud to cap the burrow. Crabs deepen their burrows quite frequently, once or twice a week during the dry season, presumably in response to a dropping groundwater level (Feliciano 1962; A. W. Pinder and A. W. Smits, personal observations). Field measurements of scaphognathite activity during excavating will be necessary to determine whether crabs ventilate while gathering mud at the bottom of the burrow.

In contrast, chronic exposure of crabs to gradual increases in environmental CO_2 up to 10% causes ventilatory hyperventilation. This ventilatory response is consistent with previous studies on *Cardisoma* (Cameron 1975; O'Mahoney and Full 1984) and other land crabs (Cameron and Mecklenberg 1973; Cameron 1975; Smatresk and Cameron 1981; Greenaway, Taylor, and Bonaventura 1983) that support the hypothesis that ventilation in the more

terrestrial crabs is driven primarily by hypercapnia and less by hypoxia (McMahon and Burggren 1988).

Resting f_{sc} and d_{sc} measured in this study in normocapnia were much lower than those reported by Cameron (1975) (f_{sc}, 4 bpm compared to 45 bpm), presumably because our crabs were less disturbed in our "burrows." It is difficult to compare our results directly with those of O'Mahoney and Full (1984) because their ventilatory measurements were of ventilatory volume, whereas we measured f_{sc}; however, the "resting" ventilation volume reported is higher than that reported by Cameron (1975), suggesting that their animals were also much more disturbed than ours. We also allowed more time (up to 48 h, compared with 1–2 h or overnight) for the animals to settle down before starting experiments. "Resting" f_h in this study (30 bpm) is much lower than those reported by Shah and Herreid (1978) or Herreid, O'Mahoney, and Shah (1979) (around 50–70 bpm), also suggesting that our animals were much more habituated prior to measurement. As a result of our much lower resting ventilation rates, the factorial increase in ventilation is much higher in our experiments than in those of Cameron (1975) or O'Mahoney and Full (1984). The increase in ventilation with hypercapnia in our experiments is probably not complicated by disturbance, since f_h did not change during hypercapnia.

Another difference in CO_2-induced hyperventilation in *Cardisoma* in the present study was that there was little sign of saturation of f_{sc}, even in 10% CO_2, as was observed in the same species by Cameron (1975) and O'Mahoney and Full (1984). It is possible that the crabs in those studies reached a maximum rate for scaphognathite pumping and could not respond further, while in our study, with less disturbed animals and much lower initial f_{sc}'s, the crabs may not have reached their maximum possible beat frequency.

Although the anatomical sites of CO_2 chemoreception in crustaceans are still unknown, our data indicate that *Cardisoma* may possess both externally oriented and internally oriented receptors for CO_2 or pH that have opposing effects on f_{sc} and function to reduce CO_2 loading through apnea in environmental hypercapnia (external) and rapid CO_2 elimination through hyperventilation when crabs are in air (internal). There is good evidence for both peripheral and central receptors of oxygen levels in other crustaceans, particularly in aquatic forms (McMahon and Wilkens 1983; Massabuau and Burtin 1984; Wilkens, Young, and DiCaprio 1989). In contrast, CO_2 receptors of terrestrial crabs remain largely undescribed.

Although moderate hypoxia exists in deep burrow sites, it is unlikely that hypoxia is as great a respiratory stimulus to *Cardisoma* as the elevated CO_2. *Cardisoma* show only modest increases in f_{sc} in response to hypoxia and appear capable of maintaining oxygen uptake at levels of P_{O_2} well below

the minimum P_{O_2} in gas found in deep burrow sites (Herreid et al. 1979). *Cardisoma* can also survive several hours of total anoxia (Cameron 1975).

If the crabs do not use the water for gas exchange, they have the option of avoiding hypercapnia and hypoxia at the bottom of the burrow by remaining close to the surface and making forays to the bottom of the burrow to draw water into the branchial cavity. *Cardisoma carnifex* is able to seal the branchial cavity well enough to carry a supply of water even during exercise (Wood and Randall 1981*a*); a supply of water could thus be maintained in the branchial cavity for ion exchange even while the crab was out of the water. Thus, a second possible scenario is that the crabs, which ventilate their branchial cavities intermittently (Cameron 1975; Wood and Randall 1981*a*; the present article), could ventilate only near the top of their burrows and "hold their breath" while near the bottom. The crabs, however, do not seem to avoid hypercapnia and hypoxia. When crabs were monitored in their burrows with radiotelemetry, they spent several hours each day at or near the bottom (fig. 5*B*). There was no evidence of a pattern of remaining close to the surface most of the time with short forays to the bottom to pick up water, or of making short forays to the top to replenish gas stores in the branchial chambers.

If the crabs return to their burrows at night during the dry season, as Henning (1975) and our night spot checks suggest, the crabs encounter remarkable step changes in inhalent P_{CO_2} twice daily for much of the year as they shuttle between foraging on the surface and sitting in their hypercapnic burrows. *Cardisoma* are also exposed to prolonged normocapnia during breeding migrations, when the crabs may be away from their burrows for several days or weeks, and to hypercapnia for up to 3 mo when they cap their burrows to moult (Feliciano 1962; Gifford 1962; Henning 1975; Canals 1982; A. W. Pinder and A. W. Smits, personal observations). Clearly, hypercapnia of the magnitude recorded at the bottoms of some of the deeper burrows, which approaches 10% CO_2, must cause considerable acid-base disturbance to *Cardisoma,* which in room air maintain a branchial P_{CO_2} of 2–12 mmHg (Wood and Randall 1981*a*) and a hemolymph P_{CO_2} of 9–15 mmHg (Cameron 1981; McMahon and Burggren 1981; Wood and Randall 1981*b;* Wood, Boutilier, and Randall 1986). Crabs in more moist habitats may be less exposed to hypercapnia since their burrows tend to be shallower—thus less hypercapnic—and they spend much more time on the surface (Gifford 1962).

Ion Concentrations

Differences in ion concentrations among sampling sites are undoubtedly due to differences in the ground water rather than due to the crabs them-

selves. The range of ion concentrations in which *Cardisoma* were found in this study, from 10% to 130% seawater, suggests that these animals are excellent osmoregulators. Herreid and Gifford (1963) also found *C. guanhumi* burrows with a wide range of salinity, including fresh water. Wood and Boutilier (1985) established that the closely related species *C. carnifex* was able to osmoregulate in salinities ranging from fresh water to seawater, although hemolymph osmolality and ion concentrations were somewhat higher in seawater. *Cardisoma hirtipes* is able to survive with access only to fresh water with 0.5 mM Na^+ (Greenaway 1989). Thus, *Cardisoma* does not appear to select burrowing sites on the basis of water quality; the only requirement is that water be close enough to the surface to be reached by burrowing. The deepest burrow we found was over 3 m, much deeper than the burrows measured by Wood and Boutilier (1985) and probably close to the maximum for these crabs (Feliciano 1962; Herreid and Gifford 1963). Our measurements were made in the dry season; thus, the water table was close to its minimum, and there were only a few centimeters of water in the burrows; during the rainy season, burrows may be almost filled with water (Feliciano 1962). Ion concentrations must also show large and rapid changes with rainfall.

The only evidence we found that the crabs influence water ion concentrations was that the Ca^{2+} concentration was significantly lower in plugged burrows than in open burrows. Since *Cardisoma* plug their burrows while they moult (Feliciano 1962; Henning 1975), this suggests that the Ca^{2+} concentration in plugged burrows was lowered by Ca^{2+} uptake by the crabs to help recalcify the carapace. It also implies that the turnover rate of water in the burrow was slow, or the difference in Ca^{2+} concentration would not be measurable. Nonetheless, even slow water turnover, along with Ca^{2+} salvaged from the moulted carapace, would probably provide sufficient Ca^{2+} for calcification. Although the Ca^{2+} concentration in plugged burrows was somewhat lower than in unplugged burrows, all burrows we sampled had high Ca^{2+} and total CO_2 concentrations; in fact, both Ca^{2+} and bicarbonate (calculated from pH and P_{CO_2}) were in higher concentrations than in seawater, which is consistent with dissolution of the limestone underlying the area. Thus, we have no evidence that recalcification of the carapace might be limited by the small pool of water available at the bottom of the burrow. It is still possible that Ca^{2+} is depleted more completely from the burrow pool during the first few days of carapace hardening, when Ca^{2+} uptake rates are highest (Cameron and Wood 1985), but we did not sample any burrows during this period, which is only a small percentage of the time the crabs keep their burrows plugged.

Summary

Cardisoma guanhumi in dry areas of Puerto Rico inhabit burrows that are increasingly hypercapnic and hypoxic with increasing depth and decreasing diameter because of restricted ventilation and the metabolism of soil microorganisms and other inhabitants of the burrow. The water at the bottom is often more hypoxic and hypercapnic than the gas, which suggests that the water is not used as a respiratory medium. The crabs do not avoid this hypercapnic environment; activity patterns suggest that some crabs are exposed to alternating normocapnia and 8%–10% CO_2 twice a day as they alternate between foraging on the surface and resting deep in their burrows. *Cardisoma* have two responses to hypercapnia: a short-term apnea in response to rapid changes in external P_{CO_2} and a long-term increase in ventilation in response to longer-term hypercapnia. The onset of short-term apnea between bouts of ventilation suggests that *Cardisoma* have external CO_2-sensitive chemoreceptors outside the branchial cavity.

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