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Metabolic Status and Respiratory Physiology of *Gecarcoidea natalis*, the Christmas Island Red Crab, During the Annual Breeding Migration

AGNIESZKA M. ADAMCZEWSKA[1,*] AND STEPHEN MORRIS[2]

[1]*Ocean Research Institute, University of Tokyo, 1-15-1 Minamidai, Nakano-ku, Tokyo 164-8639, Japan; and* [2]*Morlab, School of Biological Sciences, Woodland Road, University of Bristol, Bristol BS8 1UG, UK*

Abstract. With the arrival of the monsoonal rains and after months of inactivity during the dry season, the terrestrial crab *Gecarcoidea natalis* embarks on its annual breeding migration to the coast. The physiological demands of the migration were assessed by determining respiratory gases in the hemolymph, key metabolites, and energy stores in *G. natalis* during two migratory seasons. At the end of each day of migration the pulmonary hemolymph Po_2 decreased by 1-2.5 kPa, but the hemocyanin remained saturated with O_2 and the venous reserve was largely unchanged ($O_2 > 0.4$ mmol $\cdot$ l^{-1}). The breeding migration of red crabs was accomplished without recourse to anaerobiosis, even though at times walking speeds (up to 6.2 $\pm$ 0.5 m $\cdot$ min^{-1}) exceeded those that promoted anaerobiosis in non-migrating crabs and in crabs exercised in the laboratory. In contrast to all previous studies, at the end of each day of migrating, red crabs experienced an alkalosis (up to 0.1 pH units) rather than any acidosis. This alkalosis was removed overnight when the crabs were inactive.

Although there were seasonal fluctuations in the glycogen, glucose, and triglyceride stores, crabs engaging in the migration did not draw on these stores and must have fed along the way. In contrast, crabs returning from breeding activities on the shore terraces had significantly depleted glycogen stores. Additionally, in 1993, the male crabs returning from the breeding activities on the terraces were dehydrated and experienced a decrease in muscle tissue water of 11%. In contrast to the breeding migration *per se*, fighting for burrows in which breeding occurs produced severe anaerobiosis in males, especially the victors: after 135 s of combat, the maximum L-lactate concentration in the hemolymph was 35 mmol $\cdot$ l^{-1}. It appears that burrowing, courtship, and mating are more demanding than the migration itself. Furthermore, the data provide evidence that the metabolic responses of migrating individuals of *G. natalis* might be different from those at other times of the year.

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* To whom correspondence should be addressed. E-mail: ishka@ori.u.tokyo.ac.jp

Introduction

All terrestrial gecarcinid crabs must migrate to the coast to release their eggs into the ocean (Gibson-Hill, 1947; Gifford, 1962; Klaassen, 1975; Wolcott, 1988; Adamczewska and Morris, 2001). However, the annual breeding migration of *Gecarcoidea natalis*, the Christmas Island red crab, is unique among the gecarcinid crabs for a number of reasons. Red crabs live further inland than most other gecarcinids (*e.g.*, Bliss *et al.*, 1978; Wolcott and Wolcott, 1985) and must thus travel farther to reach the coast. Furthermore, the red crabs undertake their migration during daytime, immediately after the arrival of the monsoonal rains and following extended periods of inactivity during the dry season. Since the migration is closely synchronized with the lunar cycle, when the rains in some years arrive late, the red crabs may have to make their downward migration in a relatively short period of time if they are to complete their burrowing and courtship activities. The red crabs may have to travel more than 4–5 km in 5–6 days during "rushed migrations" (Adamczewska and Morris, 2001).

The severity of exercise is often determined by measurements of metabolic rate as well as acid-base status and key metabolites in the hemolymph and muscle tissue. An exten-

sive body of literature demonstrates that most crustaceans, including red crabs, tend to have low aerobic scope and rely on anaerobiosis to partially support locomotor activity (*e.g.*, review: McMahon, 1981; Booth and McMahon, 1985; Head and Baldwin, 1986; Herreid and Full, 1988; Greenaway *et al.*, 1988; Forster *et al.*, 1989; van Aardt, 1990; Henry *et al.*, 1994; Adamczewska and Morris, 1994b, 1998a). During their migration, red crabs must sustain locomotion for up to 6 consecutive days to reach the shore terraces where the males construct and defend burrows and engage in mating activities (Hicks *et al.*, 1990; Green, 1993, 1997; Adamczewska and Morris, 2001).

Laboratory studies of exhausting locomotion revealed information about the respiratory physiology of the crabs and indicated that they have no exceptional exercise ability (Adamczewska and Morris, 1994a, b). The gills and lungs of *G. natalis* are involved in gas exchange to different degrees, depending on the severity of exercise (Adamczewska and Morris, 1994a, 1998a; Farrelly and Greenaway, 1994). During exercise, *G. natalis* experienced the classical respiratory and metabolic perturbations found in other crustaceans during exercise; that is, hemolymph acidosis, increased ventilation and heart rate, and elevated glucose and L-lactate concentrations (Wood and Randall, 1981b; Booth *et al.*, 1984; Herreid and Full, 1988; van Aardt 1990; Henry *et al.*, 1994; Thorpe *et al.*, 1995). The aerobic scope of red crabs was surprisingly poor, implying little scope for increased locomotion; despite the fact that the hemocyanin remains saturated with O_2 when leaving the gas exchange surfaces, *G. natalis* accumulated very high levels of lactate during exercise in the laboratory (Adamczewska and Morris, 1994b).

Intermittent locomotion can increase the total distance walked before the onset of fatigue in ghost crabs (Weinstein and Full, 1992) and thus could present an important locomotor strategy for migrating crabs. However, red crabs in the laboratory incurred greater respiratory and metabolic perturbations during enforced intermittent locomotion than during continuous exercise (Adamczewska and Morris, 1998a). The crabs became anaerobic even though their walking speeds were within the range recorded for red crabs during the migration (Adamczewska and Morris, 2001).

On Christmas Island, seasonal differences in respiratory status and metabolite concentrations in free-ranging red crabs were related primarily to a higher level of basal activity in the crabs during the wet season (Adamczewska and Morris, 2000). When required to walk for 5 min, non-migrating crabs responded to exercise in essentially the same way during both the wet and the dry seasons. The responses of the crabs to non-exhausting exercise in the field during either season were comparable to those in the laboratory studies (Adamczewska and Morris, 1998a; 2000) and did not help explain how this species completes the annual breeding migration. The conditions and circumstances of the migration cannot be simulated in the laboratory nor replicated outside of the migration season. Thus, understanding the physiological processes involved and the consequences for migrating red crabs could only be addressed by making determinations *in situ* as the crabs journeyed to the ocean to breed.

The migration is the most intense period of activity during the year. Its physical demands immediately follow prolonged periods of inactivity during the dry season, when the crabs may spend up to 2 months in their burrows (Green, 1993). This abrupt change in activity levels demands a sudden change in energy metabolism and is compounded by the fact that the red crabs have reduced foraging opportunities because of the unfavorable environmental conditions during the dry season. Because the migratory activities are energetically demanding, they may require the use of stored metabolic fuels. Indeed, crustaceans are known to have substantial lipid and glycogen reserves in the midgut gland and in muscle tissues, which are utilized during starvation (*e.g.*, Parvathy, 1971; Pillay and Nair, 1973; Gibson and Barker, 1979; review: Chang and O'Connor, 1983; Nishida *et al.*, 1995).

G. natalis appears to have no superior locomotor abilities compared to other crabs, and this raises a number of questions. Primarily, is there a significant change in the respiratory physiology and metabolism at the onset of the migration, such that a different exercise regime can be supported? This could include, for example, tolerating an exceptional diurnal anaerobiosis and O_2 debt. Is prolonged locomotion facilitated by a reliance on stored fuels? Do the migrating crabs tolerate physiological perturbations such as dehydration and acidosis that limit their behavior at other times? These factors could circumscribe the potential migratory strategy of this species. Do the male crabs attempt to reach the terraces as fast as possible to obtain the best places for burrowing, arriving in less than peak condition? Alternatively, do they take more time to complete the downward migration—thus arriving at the shore terraces fit for combat, with ample energy stores—and then attempt to displace already resident crabs from their burrows?

The comprehensive description of the red crab migration (Adamczewska and Morris, 2001) provided a context for the first field investigation of the respiratory physiology and metabolism of migrating terrestrial crabs. The field investigations were carried out on Christmas Island during two migrations as well as in the inter-migratory period to assess annual as well as seasonal differences. Metabolites were measured in the hemolymph and muscle of migrating crabs during breeding activities and after the migration. The function and functioning of the respiratory and circulatory systems in gas exchange and transport were also determined. Stores of metabolic energy were quantified at various stages of the migration, and their importance in facilitating the migration and the associated breeding activities was as-

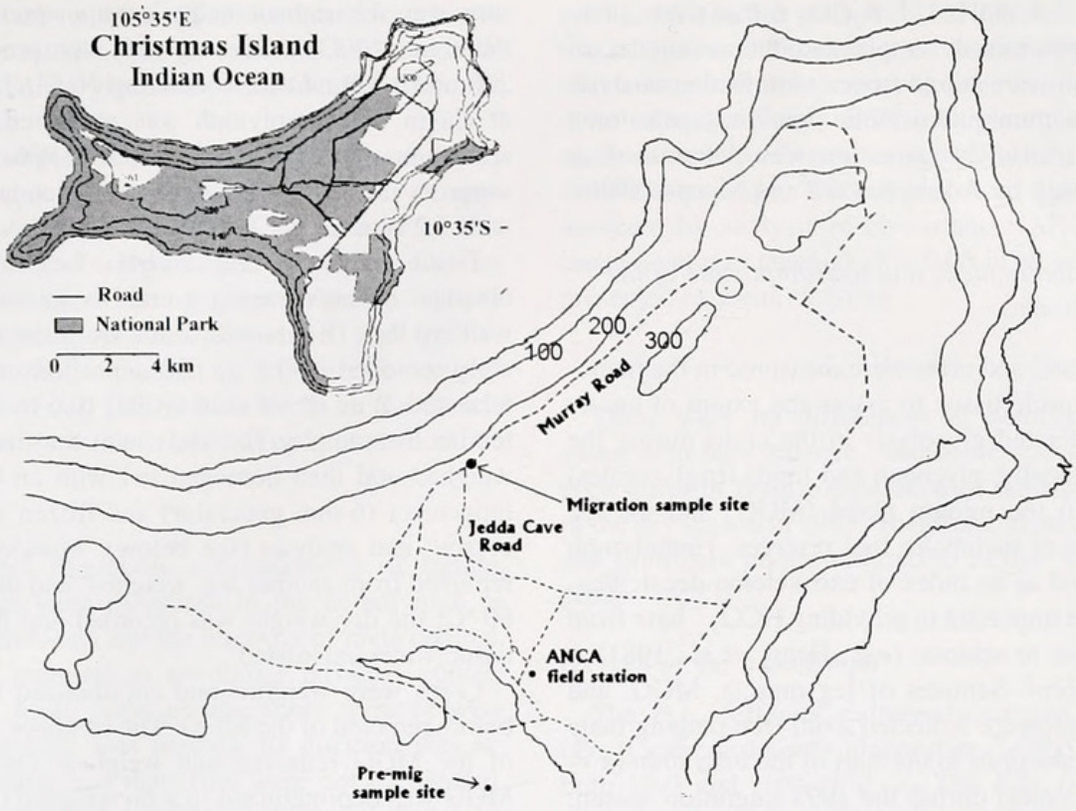

Figure 1. Map of Christmas Island showing the location of the ANCA (Parks Australia) field station and the locations of sampling sites prior to the start of the migration and during the migration.

sessed. The data were interpreted together with the behavioral and ecological information to provide a model for migrating individuals of *G. natalis*.

Materials and Methods

Respiratory gas and acid-base status in the hemolymph

Red crabs (*Gecarcoidea natalis*) were sampled in the field during two migration seasons (November and December) of 1993 and 1995. To assess the functioning of the respiratory surfaces and the circulatory system in gas exchange and transport to and from the tissues, hemolymph was sampled at various times and analyzed for respiratory gases and pH. Hemolymph was sampled from three groups of crabs in 1993 and from two groups in 1995. In 1993 the first group of crabs was sampled at the end of the dry season prior to the start of the migration, during the limited amount of time when they were found outside of their burrows (n = 12: 6 males and 6 females). The study site for this group was a 5-min drive from the ANCA (Parks Australia) Field Station ('Pre-mig study site': Fig. 1). In 1995 the migration of the red crabs commenced earlier than expected (Adamczewska and Morris, 2001), and thus it was not possible to obtain samples from resident red crabs in the immediately pre-migratory condition.

The remaining sample groups were composed of crabs migrating down to the coast. Two groups were sampled during both the 1993 and 1995 downward migration at the same site located on Murray Road near the junction of Jedda Cave Road (Fig. 1). Crabs sampled before sunrise, after "sleeping" the whole night, were designated as the morning, or "AM', group. Red crabs become immobile and unresponsive at night and "sleep" with their eyestalks folded down. The second group, referred to as the "PM" group, consisted of crabs sampled in the afternoon, at about 1630, after they had been walking for most of the day (Adamczewska and Morris, 2001) and had crossed Murray Road towards the shore terraces and the coast. In 1993 the AM and PM samples were taken on each of 5 days, and thus n = 5 for each group; in the slower migration of 1995, n = 9 for each of AM and PM sample groups. Hemolymph was sampled from large individuals of both sexes, but most often from male crabs (Adamczewska and Morris, 2001).

Three types of hemolymph were sampled from each crab: pulmonary (300 μl from the post-pulmonary vessel, hemolymph leaving the lungs); arterial (500 μl from the pericardial cavity, a mixture of post-pulmonary and post-branchial hemolymph); and venous hemolymph (700 μl from the venous sinus at the base of the last walking leg). The samples were analyzed for O_2 content ($[O_2]$) and partial pressure (Po_2), CO_2 content ($[CO_2]$), and pH at the ANCA Field Station as described previously by Adamczewska and Morris (1996). An aliquot of the remaining hemolymph was deproteinized with $HClO_4$ (0.6 mol $\cdot$ l^{-1}, ratio 1:1) and

neutralized with 2.5 mol · l^{-1} K_2CO_3 for analysis of L-lactate. The deproteinized sample and the remainder of whole hemolymph were stored frozen until further analysis of metabolites, calcium, and osmotic pressure (see below). The percentage of Hc-O_2 saturation was determined as described previously by Adamczewska and Morris (1996).

Metabolites, metabolic fuels, and hydration state of the hemolymph and tissues

Glucose, L-lactate, and urate were measured in the hemolymph and leg muscle tissue to assess the extent of anaerobiosis and accelerated glycolysis in the crabs during the migration. Additionally, glycogen and lipids (triglycerides) were measured in the midgut gland (MGG) and the leg muscle as indices of metabolic fuel reserves. Hemolymph [Ca] was measured as an index of exoskeleton decalcification, which can be important in providing HCO_3^- base from $CaCO_3$ in response to acidosis (*e.g.*, Henry *et al.*, 1981).

Sampling protocol. Samples of leg muscle, MGG, and venous hemolymph were collected from free-ranging male and female red crabs prior to the start of the migration (n = 6 males and 6 females) during the 1993 migration season. Leg muscle tissue and venous hemolymph were collected each day of the downward migration in both 1993 and 1995: in the morning before sunrise (AM, n = 4) and in the afternoon at about 1630 (PM, n = 4). To minimize the number of sacrificed animals, samples of the MGG were taken only from the PM group.

During the 1995 season, no samples could be taken from crabs prior to the start of the migration; however, to provide a comparison, samples were taken from non-migrating crabs on the 10th day of the downward migration (color-coded crabs that did not leave their home ranges; Adamczewska and Morris, 2001).

Muscle, MGG, and hemolymph samples were also taken from crabs that had arrived at the lowest shore terraces but had not yet begun breeding activities (terrace: n = 8). Male (n = 8) and female (n = 8) red crabs were sampled during their respective return migrations from the shore terraces to the plateau, during both migration seasons. The site of sampling for the crabs returning from the terraces was the same as for the downward migration (Fig. 1).

Samples of the MGG and leg muscle were also taken from crabs during the times of the year when the crabs were not involved in breeding activities: in June 1994 (the middle of the dry season) and in February 1995 (the wet season—2 months after breeding activities ended). These samples were analyzed for energy stores in the form of glycogen and triglycerides (see below).

Hemolymph analysis. In the laboratory, hemolymph samples were analyzed for L-lactate (Boehringer Mannheim test kit #138 084), glucose (Sigma Diagnostics test-kit #510), and urate (Sigma Diagnostics test kit #685). Osmotic pressure was determined using a vapor-pressure osmometer (Wescor 5100C) calibrated with two precision standards, 290 and 1000 mOsm, containing NaCl. The concentration of Ca in the hemolymph was measured with an atomic absorption spectrophotometer (GBC 906, Melbourne). To suppress interference, samples and standards were diluted with 7.2 mmol · l^{-1} $LaCl_3$.

Tissue extraction and analysis. Leg muscle tissue was obtained by encouraging a crab to autotomize the second walking leg. The muscle tissue from the leg was immediately removed (~0.3 g) and deposited into a preweighed tube with 2 ml of ice-cold $HClO_4$ (0.6 mol · l^{-1}) to deproteinize the sample. The vials with the muscle tissue were weighed and then homogenized with an OMNI 1000 homogenizer (6-mm generator) and frozen until further processing and analysis (see below). Muscle tissue was also removed from another leg, weighed, and dried in an oven at 60 °C; the dry weight was recorded and the percentage of tissue water calculated.

Crabs were weighed and anesthetized by cold narcosis before removal of the MGG. The carapace was lifted and all of the MGG removed and weighed. One portion of the MGG was deproteinized in a preweighed tube with 2 ml of ice-cold $HClO_4$ (0.6 mol · l^{-1}) and homogenized in the field for later analysis. A second portion of MGG was set aside for triglyceride analysis. The remainder of the MGG was weighed and dried in an oven at 60 °C; the dry weight was recorded and the percentage of tissue water calculated. All samples to be analyzed for metabolites, lipids, and ions were kept frozen and transported on dry ice.

In the laboratory, muscle and MGG samples previously deproteinized with $HClO_4$ were homogenized with a glass homogenizer (Wheaton type) to obtain a finer homogenate. The homogenates were centrifuged in a refrigerated centrifuge for 15 min at 5300 × g, the supernatant was removed, and the pellet was resuspended in 0.7 ml of 0.4 mol · l^{-1} $HClO_4$ and centrifuged again. The second supernatant was removed and pooled with the first; the combined supernatant was neutralized with 0.8 ml of K_2CO_3 (3.75 mol · l^{-1}) and kept in an ice bath for 1 h. After another centrifugation, the final supernatant was removed and used for analysis. The concentrations of metabolites in the muscle tissue were expressed as millimoles per kilogram of wet tissue mass.

The deproteinized and neutralized leg muscle samples were analyzed for L-lactate (Boehringer Mannheim test kit #138 084). Additionally, the samples from both leg muscle and MGG samples were analyzed for glucose and glycogen by using a modified method described in Bergmeyer (1985). Briefly, amyloglucosidase (Boehringer Mannheim Cat. #208 469) made up to ≥ 10 kU · l^{-1} in an acetic acid buffer at pH 4.8 was used to hydrolyze glycogen into glucose during a 2-h incubation, with shaking, at 40 °C. At the end of the incubation the enzyme was denatured by addition of 0.6 mol · l^{-1} $HClO_4$. The solution was centrifuged at

10,000 × *g* for 10 min and the supernatant used for glucose analysis. For each sample, free glucose was also measured and subtracted from the glucose content obtained from the glycogen digestion.

Frozen MGG samples were analyzed for triglycerides by first homogenizing a sample with an equal mass of gum arabic (Sigma Catalog #G-9752) and 15 parts of bovine serum albumin (6% w/v Boehringer Mannheim Catalog #100-030) in a fine glass homogenizer (Wheaton type) at 55 °C. This homogenate was then diluted 1:10 with 6% w/v bovine serum albumin and used for triglyceride analysis (Sigma test kit #334-A). Triglyceride levels in the muscle tissue of red crabs were not detectable by this method.

Terrace activities

During the 1995 migration, concentrations of lactate, glucose, and urate were measured in the hemolymph as indicators of anaerobiosis and the intensity of male combat. Only male-male interactions involving physical contact were considered. Male crabs on the shore terraces were observed and if a fight was noticed, its duration was recorded. Fights lasting less than 10 s were not considered. The fight was considered to have ended when the victor remained in possession of the burrow and the loser retreated or was chased away by the other opponent. At this point both crabs were promptly captured (within 5 s) and hemolymph was withdrawn from the venous sinus by puncturing the arthrodial membrane. The design of the experiment dictated that fights could be used only if they occurred near the observer and only if they were observed from start to finish. A total of 14 fighting pairs were sampled over 4 days of breeding activities on the terraces. Hemolymph samples were also taken from six male crabs resting at the entrance of their burrows; these were used to determine the basal metabolite status of male crabs on the terraces during the breeding activities. The hemolymph samples were treated and stored as described above and analyzed in Sydney for glucose, L-lactate, and urate.

Statistical analysis

The data were tested for homogeneity of variances using Bartlett's χ^2 test, and any heterogenous data were log- or square-root transformed. Respiratory gas data were analyzed using two-factor ANOVA for the three hemolymph types (pulmonary, arterial, and venous) and the different sampling groups in each season. Seasonal differences between the AM and PM groups were also examined by ANOVA.

Statistical analyses on tissue and hemolymph metabolites and on water and ion measurements between the AM and PM samples for the days of downward migration were carried out by two-factor ANOVA. If no difference between the AM and PM groups was detected, then the data were pooled for each day for further analysis by ANOVA. Analyses between tissue types and between the two sampling seasons were also carried out where appropriate. *Post hoc* testing was done using the Tukey HSD multiple comparison test. All analyses were performed using the Systat for Windows 5.0 statistical package. The samples from fighting males were analyzed by analysis of co-variance (ANCOVA). Significance level was taken as $P = 0.05$ in all cases. All data are presented as mean ± SEM.

Results

There were no differences in hemolymph respiratory gases, acid-base balance, metabolite concentrations, or energy stores of male compared with female crabs at the end of the dry season in 1993. Therefore, these data were pooled and from here on are referred to as the "resident pre-mig" group.

Oxygen in the hemolymph

The P_pO_2 [efferent pulmonary oxygen partial pressure (PO_2)] was consistently higher than the P_aO_2 (arterial PO_2) in both 1993 and 1995 (Fig. 2). The P_vO_2 (venous PO_2) was always lower than the P_aO_2 and the P_pO_2. During the 1995 migration, the hemolymph PO_2 of the PM group was statistically lower than in the AM group (Fig. 2). In 1993, although the PO_2 in the pulmonary hemolymph (8.26 ± 2.20 kPa) of the AM group was 3.5 kPa higher than in the PM group (Fig. 2), there was no overall statistical difference between the crabs in these two groups.

The O_2 content ($[O_2]$) of the hemolymph was similar in both migration seasons (Fig. 3). The O_2 content of pulmonary hemolymph ($[O_2]_p$ = 1.13 and 0.96 mmol · l^{-1} in 1993 and 1995 respectively) was very similar to the arterial values, but $[O_2]$ in venous hemolymph decreased significantly, to about 0.50 mmol · l^{-1}, in both seasons. The AM group sampled during the 1993 migration season was an exception because the arterial $[O_2]$ was significantly lower than the pulmonary hemolymph concentration. Additionally, in 1993, the $[O_2]_v$ in the AM group was significantly greater than in the pre-mig group (Fig. 3).

The saturation of hemocyanin (Hc) with O_2 varied between samples in a manner similar to that of the $[O_2]$. The O_2 saturation of the pulmonary hemolymph was high (mean = 102 ± 6%) and similar to the Hc saturation of arterial hemolymph except in the AM group of 1993, in which the arterial hemolymph was only 74% saturated. In comparison, the mean Hc-O_2 saturation of venous hemolymph decreased significantly in both seasons, to 43% in 1993 and 63% in 1995 ($P < 0.001$).

Carbon dioxide content and pH of the hemolymph

The pH of pulmonary, arterial, and venous hemolymph was similar within each group of red crabs sampled (Fig. 4);

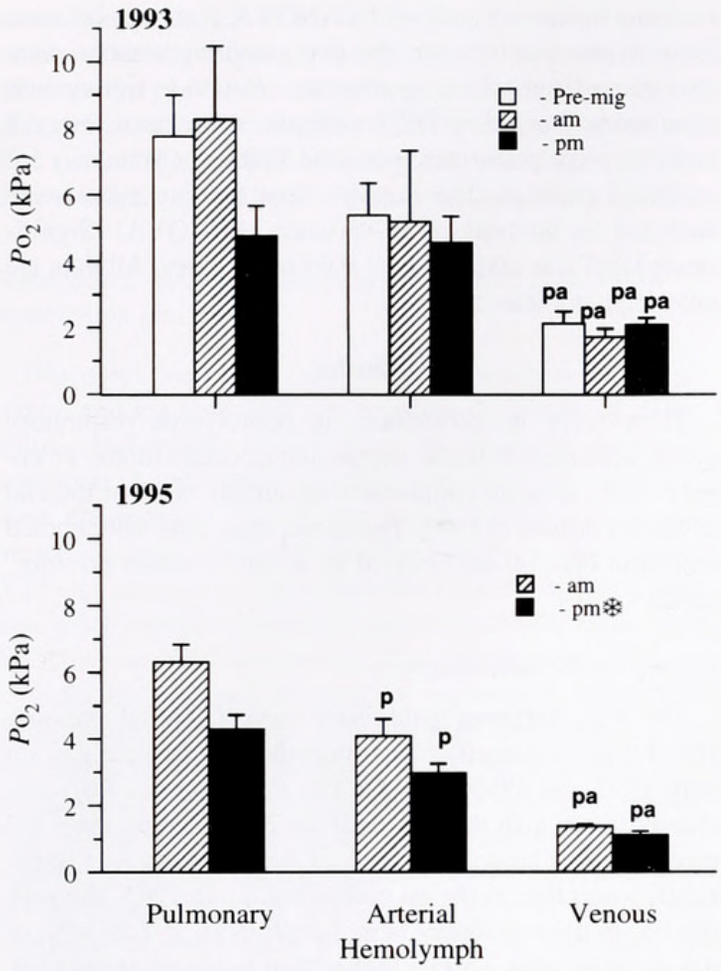

Figure 2. Oxygen partial pressure (Po_2, kPa) in the pulmonary, arterial, and venous hemolymph of *Gecarcoidea natalis* during two migration seasons, 1993 (top panel) and 1995 (bottom panel). Morning and afternoon groups were sampled during both migration seasons (n = 5 in 1993; n = 9 in 1995). Top panel (1993 season) includes measurements of Po_2 in crabs sampled 1 week prior to the start of the migration (pre-mig n = 12). The letters "p" and "a" indicate that the mean is significantly different from that of pulmonary or arterial hemolymph respectively, within a sample group. The ❄ symbol next to the legend indicates a significant difference between the AM and the PM groups.

thus, for further analysis between groups, the pH for the three hemolymph types was pooled for each sample group. The resident pre-mig red crabs in 1993 had a mean hemolymph pH of 7.61 ± 0.03, which decreased significantly after the onset of the migration to near 7.4 (Fig. 4). Most interestingly, the hemolymph was more acidic in crabs sampled in the morning (AM pH = 7.35 ± 0.07) than in crabs sampled in the afternoon (PM pH = 7.46 ± 0.06, $P <$ 0.001). A similar alkalosis at the end of the day of migration was seen in 1995 (AM-PM difference of 0.042 pH units) but was less significant than during 1993 (P = 0.068; Fig. 4).

There was no difference in hemolymph CO_2 content ($[CO_2]$) between pulmonary, arterial, and venous samples within any one group of red crabs sampled (Fig. 5). During 1993, the $[CO_2]$ in the hemolymph of migrating crabs was significantly greater than in the resident pre-mig crabs (mean $[CO_2]$= 9.8 ± 0.8 mmol · l^{-1}). In addition, the $[CO_2]$ in the hemolymph of the PM group was significantly greater than that of the AM group; the concentration was 3.3 mmol · l^{-1} higher in 1993 and 1.7 mmol · l^{-1} higher in 1995 (Fig. 5).

Hydration state of red crabs during migratory activities

There were no differences in the hydration state of crabs at the start and the end of a day walking; the AM and PM samples were thus pooled for each day. In the 1993 season the percentage of water in crab leg muscle was very stable during the entire downward migration (range 77.2%–78.3%) and, importantly, did not differ from that of the pre-migratory crabs (76.1% ± 0.4%). However, the male crabs returning from the shore terraces were dehydrated: the percentage of water in their muscle tissue was 11% lower

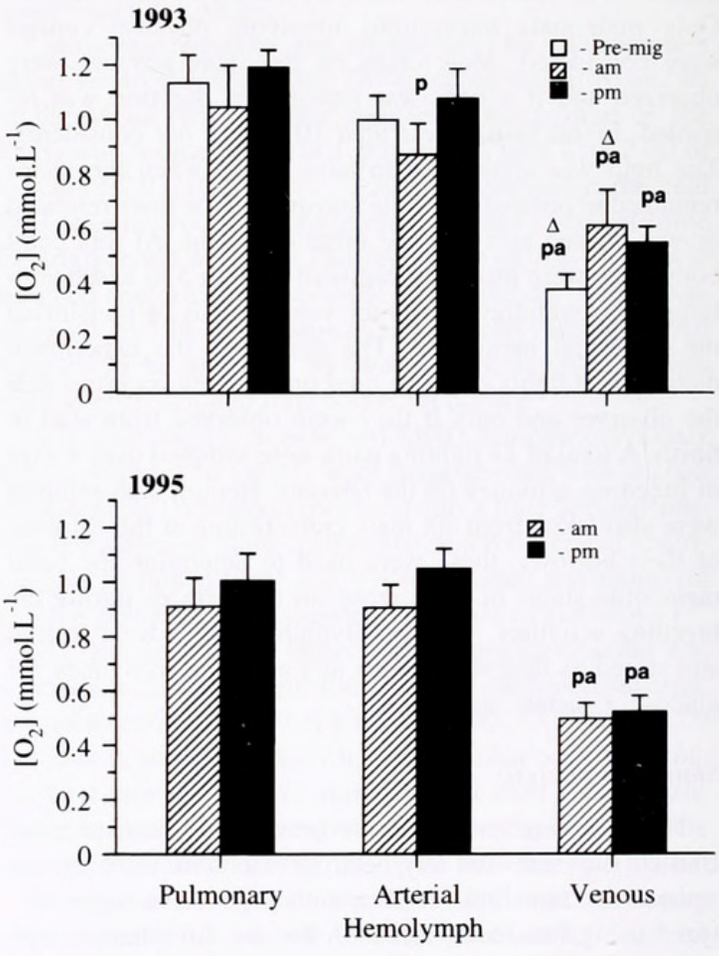

Figure 3. Oxygen content ($[O_2]$, mmol · l^{-1}) in the pulmonary, arterial, and venous hemolymph of *Gecarcoidea natalis* during two migration seasons, 1993 (top panel) and 1995 (bottom panel). Morning and afternoon groups were sampled during both migration seasons (n = 5 in 1993; n = 9 in 1995). Top panel (1993 season) also includes measurements of $[O_2]$ in crabs sampled 1 week prior to the start of the migration (pre-mig n = 12). The letters "p" and "a" indicate that the mean is significantly different from that of pulmonary or arterial hemolymph within a sample group. The pair of Δ symbols denote a significant difference in venous O_2 between the resident pre-mig and AM crabs.

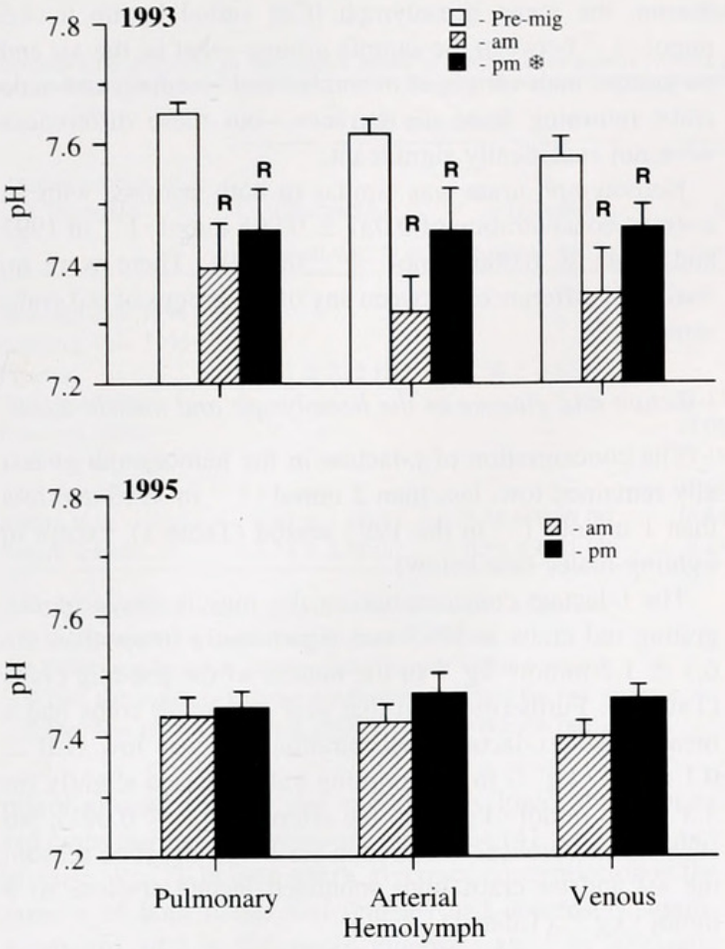

Figure 4. The hemolymph pH of pulmonary, arterial, and venous samples taken from *Gecarcoidea natalis* during two migration seasons, 1993 (top panel) and 1995 (bottom panel). Morning and afternoon groups were sampled during both migration seasons (n = 5 in 1993; n = 9 in 1995). Top panel (1993 season) also includes measurements of hemolymph pH in crabs sampled 1 week prior to the start of the migration (pre-mig n = 12). The ❄ symbol next to the legend indicates a significant difference between the AM and the PM groups. The "R" denotes a significant difference from the crabs at rest—that is, from the resident pre-mig crabs.

than in all the other groups sampled in that season. In contrast, during the 1995 migration season, both the returning male and female crabs had a significantly higher amount of water in muscle tissue (79.1%) than the crabs sampled during the downward migration (days 5–7; range 75.2%–76.9%).

The percentage of water in the MGG was more variable and significantly lower than in the muscle tissues, with a mean of 57.0% ± 1.5% during the 1993 migration and 61.7% ± 2.4% in 1995. Crabs that did not participate in the 1995 migration were unusual in that water constituted only 45.4% ± 1.8% of their midgut gland.

The osmotic pressure of the hemolymph was more variable than the percentage of water in the muscle tissue. The osmotic pressure in the hemolymph of the AM and PM groups on any one day was the same, and thus the data for each day were pooled for further analysis. The hemolymph osmotic pressure was largely constant during the downward migration of both the 1993 (771 ± 3 mOsm) and 1995 (803 ± 4) seasons; but in 1993 the osmotic pressure of crabs during the downward migration was significantly lower than that of the pre-mig crabs (815 ±7 mOsm) and the returning females (869 ± 14 mOsm). Additionally, in both 1993 and 1995 the returning males had lower osmotic pressure (mean 699 ± 16 mOsm) than did the crabs sampled during their downward migration.

Calcium and urate concentration in the hemolymph

The calcium concentration in hemolymph of resident pre-mig crabs (13.74 ± 0.42 mmol · l^{-1}) was significantly higher than in all the other groups sampled in 1993 (mean [Ca] = 9.29 ± 0.87 mmol · l^{-1}). During the 1995 migration

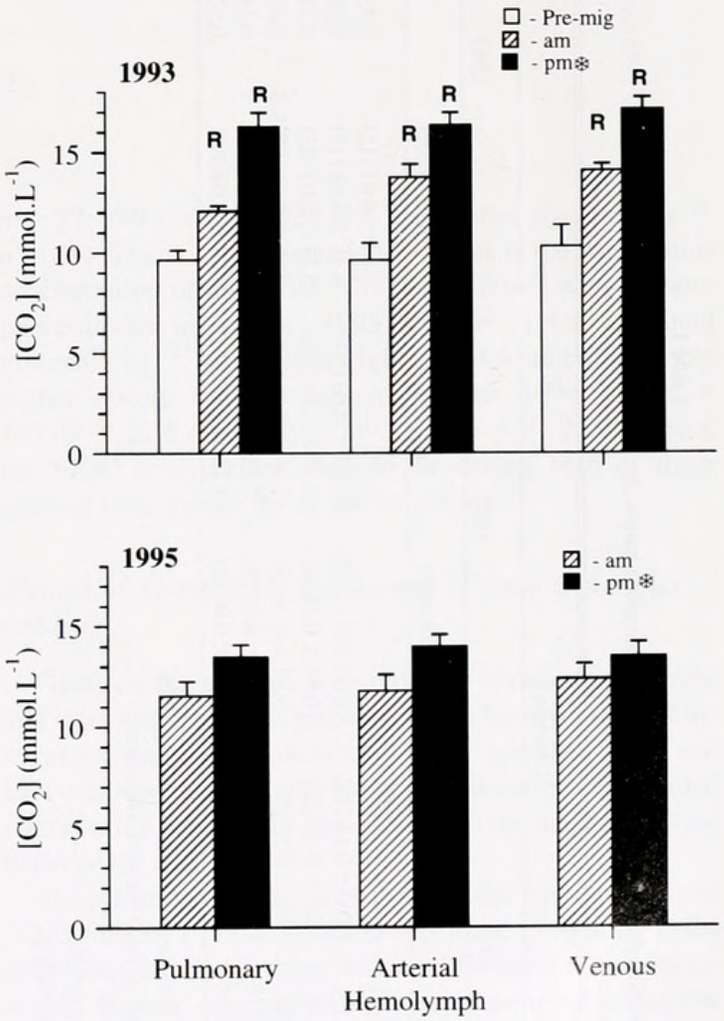

Figure 5. Carbon dioxide content ([CO_2], mmol · l^{-1}) in the pulmonary, arterial, and venous haemolymph of *Gecarcoidea natalis* during two migration seasons, 1993 (top panel) and 1995 (bottom panel). Morning and afternoon groups were sampled during both migration seasons (n = 5 in 1993; n = 9 in 1995). Top panel (1993 season) also includes measurements of [CO_2] in crabs 1 week prior to the start of the migration (pre-mig n = 12). The ❄ symbol next to the legend indicates a significant difference between the AM and the PM groups. The "R" denotes a significant difference of a sample group from the crabs at rest—that is, from the resident pre-mig crabs.

Table 1

L-lactate and glucose in the hemolymph and leg muscle tissue of Gecarcoidea natalis *during two migratory seasons, 1993 and 1995 (values given as mean ± SEM; sample size provided in parenthesis)*

	L-lactate				Glucose			
	1993		1995		1993		1995	
	Hemolymph	Leg muscle	Hemolymph	Leg muscle	Hemolymph	Leg muscle	Hemolymph	Leg muscle
pre-mig	1.8 ± 0.31 (12)	6.35 ± 1.22 (12)^{D}	—	—	0.44 ± 0.03 (12)	0.83 ± 0.09 (12)	—	—
AM	0.97 ± 0.34 (15)	1.00 ± 0.14 (15)	0.34 ± 0.17 (40)	2.83 ± 0.60 (20)	0.48 ± 0.04 (15)	0.26 ± 0.06 (15)	0.47 ± 0.08 (40)	0.24 ± 0.02 (20)
PM	1.10 ± 0.31 (25)	3.26^{I} ± 0.80 (25)	0.64 ± 0.18 (45)	3.11 ± 1.16 (25)	0.53 ± 0.07 (25)	0.28 ± 0.07 (25)	0.60 ± 0.11 (45)	0.35 ± 0.06 (25)
Terrace	0.91 ± 0.08 (8)	3.86 ± 0.55 (8)	0.14 ± 0.06 (8)	2.86 ± 0.45 (8)	0.85 ± 0.12 (8)^{R}♀♂	0.42 ± 0.06 (8)^{R}♀♂	0.42 ± 0.05 (8)	0.55 ± 0.20 (8)
Return male	1.26 ± 0.34 (8)♀	1.34 ± 0.56 (8)^{D}♀	0.35 ± 0.09 (8)	2.43 ± 0.88 (8)	0.38 ± 0.02 (8)	0.14 ± 0.03 (8)^{D}♀	0.35 ± 0.04 (8)	0.22 ± 0.04 (8)
Return female	1.69 ± 0.33 (8)♂	6.34 ± 1.30 (8)^{D}♂	0.30 ± 0.05 (8)	3.26 ± 0.88 (8)	0.48 ± 0.04 (8)	0.52 ± 0.12 (8)^{D}♂	0.32 ± 0.02 (8)	0.20 ± 0.04 (8)

^{R} Different from the crabs at rest—*i.e*, the resident pre-mig crabs.
^{D} Different from crabs engaged in the downward migration.
^{I} Increase in PM compared to AM.
♀ Different from the returning females.
♂ Different from the returning males.

season, the mean hemolymph [Ca] varied by up to 4.5 mmol · l^{-1} between the sample groups—that is, the AM and PM groups, males engaged in combat and breeding, and male crabs returning from the terraces—but these differences were not statistically significant.

Hemolymph urate was similar in both seasons, with an average concentration of 0.037 ± 0.005 mmol · l^{-1} in 1993 and 0.041 ± 0.006 mmol · l^{-1} in 1995. There were no statistical differences between any of the groups of red crabs sampled.

L-lactate and glucose in the hemolymph and muscle tissue

The concentration of L-lactate in the hemolymph generally remained low, less than 2 mmol · l^{-1} in 1993 and less than 1 mmol · l^{-1} in the 1995 season (Table 1), except in fighting males (see below).

The L-lactate concentration in the muscle tissue of migrating red crabs in 1993 was significantly lower than the 6.3 ± 1.2 mmol · kg^{-1} in the muscle of the pre-mig crabs (Table 1). Furthermore, in that year migrating crabs had a mean muscle L-lactate concentration that was low (1.0 ± 0.1 mmol · kg^{-1}) in the morning but increased slightly (to 3.3 ± 0.8 mmol · kg^{-1}) in the afternoon ($P < 0.001$). No such diel differences occurred in the 1995 migration season: the AM and PM crabs both contained lactate at close to 3 mmol · kg^{-1} (Table 1).

The glucose concentration in the hemolymph was below 1 mmol · l^{-1} in both seasons (Table 1). Red crabs descending to the terraces in 1993 had higher hemolymph glucose levels (0.85 ± 0.12 mmol · l^{-1}) than both the pre-migratory crabs (0.44 ± 0.04 mmol · l^{-1}) and the crabs sampled during their return migration (0.51 ± 0.06 mmol · l^{-1}, $P < 0.001$; Table 1). During the 1995 migration (mean [glucose] = 0.80 ± 0.09 mmol · l^{-1}) the variances of the data were severely heterogenous, and no significant differences could be detected between any of the sample groups (Table 1).

In the muscle tissue, the concentration of glucose was also less than 1 mmol · kg^{-1} during both seasons (Table 1). However, during 1993, the concentration in the leg muscle of the crabs engaged in the downward migration (0.29 ± 0.06 mmol · kg^{-1}) was lower ($P < 0.001$) than that in the resident pre-mig crabs (0.83 ± 0.09 mmol · kg^{-1}). The variances of the data collected during the 1995 migration were heterogenous, and it was not possible to detect any differences in glucose levels (Table 1).

Energy stores in the midgut gland and muscle tissues

Glycogen stores were measured as glucose units and expressed as concentration per wet weight of tissue. During the downward migration of 1995, leg muscle glycogen concentrations (69 mmol glucose · kg^{-1}) were about twice those of the crabs in 1993. The concentration of glycogen in the muscle of pre-migratory crabs in 1993 (31.6 ± 2.4

Table 2

Glucose and glycogen in the midgut gland of Gecarcoidea natalis *(values given as mean ± SEM; sample size provided in parenthesis)*

Day in relation to migration	1993 migration†		1995 migration		Non-migrating	
	Glucose†* (mmol · kg^{-1})	Glycogen† (mmol · kg^{-1})	Glucose* (mmol · kg^{-1})	Glycogen (mmol · kg^{-3})	Glucose (mmol · kg^{-1})	Glycogen (mmol · kg^{-1})
Non-mig Jun. 1994	—	—	—	—	9.65 ± 1.48 (8)#	0.95 ± 0.21 (8)‡
Non-mig Feb. 1995	—	—	—	—	3.83 ± 1.01 (8)#	12.14 ± 3.4 (8)‡
Pre-mig	17.81 ± 2.18 (12)	1.58 ± 0.35 (12)	—	—	—	—
5th day of mig	—	—	32.13 ± 7.11 (4)	18.36 ± 3.51 (4)	—	—
Non-mig (10th)	—	—	19.03 ± 1.82 (8)^{D}	4.31 ± 0.34 (8)	—	—
11th day of mig	—	—	29.18 ± 7.95 (4)	19.02 ± 5.78 (4)	—	—
12th day of mig	—	—	25.68 ± 1.64 (4)	14.81 ± 2.55 (4)	—	—
Return male	8.92 ± 1.14 (8)	3.55 ± 0.99 (8)	13.44 ± 2.53 (8)	5.49 ± 0.96 (8)^{D}	—	—
Return female	5.74 ± 0.88 (8)	0.55 ± 0.21 (8)	6.14 ± 0.84 (8)	8.22 ± 1.50 (8)^{D}	—	—

^{D} Different from crabs engaged in the downward migration.
† Difference between the two migration seasons.
* Difference between glucose and glycogen within a season.
# Two like symbols indicate a difference between the two groups.
‡ Two like symbols indicate a difference between the two groups.

mmol glucose · kg^{-1}) was significantly lower than that of red crabs during the downward migration (41.6 ± 5.7 mmol glucose · kg^{-1}). In both years, glycogen concentration in the muscle of both males and females had decreased significantly (to 14.2 ± 2.5 mmol glucose · kg^{-1} in 1993 and 24.9 ± 5.5 mmol glucose · kg^{-1} in 1995) by the time the crabs were sampled during their return migration.

The mean muscle glycogen concentration in crabs sampled during the dry season of June 1994 (17.6 ± mmol glucose · kg^{-1}) was just one-third of that measured in crabs during the wet season of February 1995.

The concentration of glycogen in the MGG was much lower than in the leg muscle, whereas the concentration of free glucose in the MGG was greater at all times (Table 2). The "total glucose" stores ([glucose] + [glycogen]) in the MGG during the 1995 migration season were consistently greater than in 1993 (Table 2).

The concentration of total glucose in the MGG of returning males (12.5 mmol · kg^{-1}) and returning females (5.7 ± 0.9 mmol · kg^{-1}) in 1993 was lower than that of crabs before the migration (19.4 mmol · kg^{-1}; Table 2). Similarly, in 1995 the returning males (19 ± 3 mmol · kg^{-1}) and females (14 ± 2 mmol · kg^{-1}) also had greatly reduced total glucose stores in the MGG compared with the crabs sampled during the downward migration (Table 2). There were otherwise no obvious seasonal trends in the total glucose stores in the MGG (Table 2).

The triglyceride content in the MGG of red crabs, measured as glycerol, did not change during the course of the migratory activities, and the mean concentration of triglycerides in the MGG was similar in both of the migration seasons (1993 mean = 247.3 ± 38.7 mmol glycerol · kg^{-1}, n = 52; 1995 mean = 234.8 ± 36.7 mmol glycerol · kg^{-1}, n = 44). There were seasonal differences in the triglyceride concentration of the MGG. The lowest levels were in samples collected in February 1995 (mean = 71.4 ± 6.6 mmol glycerol · kg^{-1}, n = 8), but triglycerides were considerably higher during the dry season in June 1994 (mean = 162.09 ± 22.6 mmol glycerol · kg^{-1}, n = 8). Nonetheless, the MGG triglycerides were lower during both of these seasons than during the actual migration.

Metabolite status in the hemolymph of male crabs after combat

Fights on the terraces occurred only between male crabs and were always for the possession of a burrow. Most of the fights on the terraces were very brief, less than 10 s, and thus were not used in this study. The duration of recorded fights varied between 18 and 135 s, with the majority lasting between 40 and 60 s.

The mean concentration of urate in the hemolymph was 0.043 mmol · l^{-1} and it did not increase in fighting crabs (Fig. 6A). Similarly, there was no difference in the hemolymph glucose concentration in crabs sampled at burrow entrances and immediately after a fight (Fig. 6B).

The mean concentration of L-lactate in the hemolymph of male crabs sampled at burrow entrances was high (7.4 ± 3.9 mmol · l^{-1}; Fig. 6C). Importantly, there was a significant increase in L-lactate in the hemolymph ($P < 0.01$) with increase in fight time, but only for the victorious crabs (Fig. 6C; ANCOVA and regression analysis). This increase can be described by the following equation [Lac] = time (s) × 0.25 − 3.20 (r^2 = 0.71). The maximum [L-lactate] in the

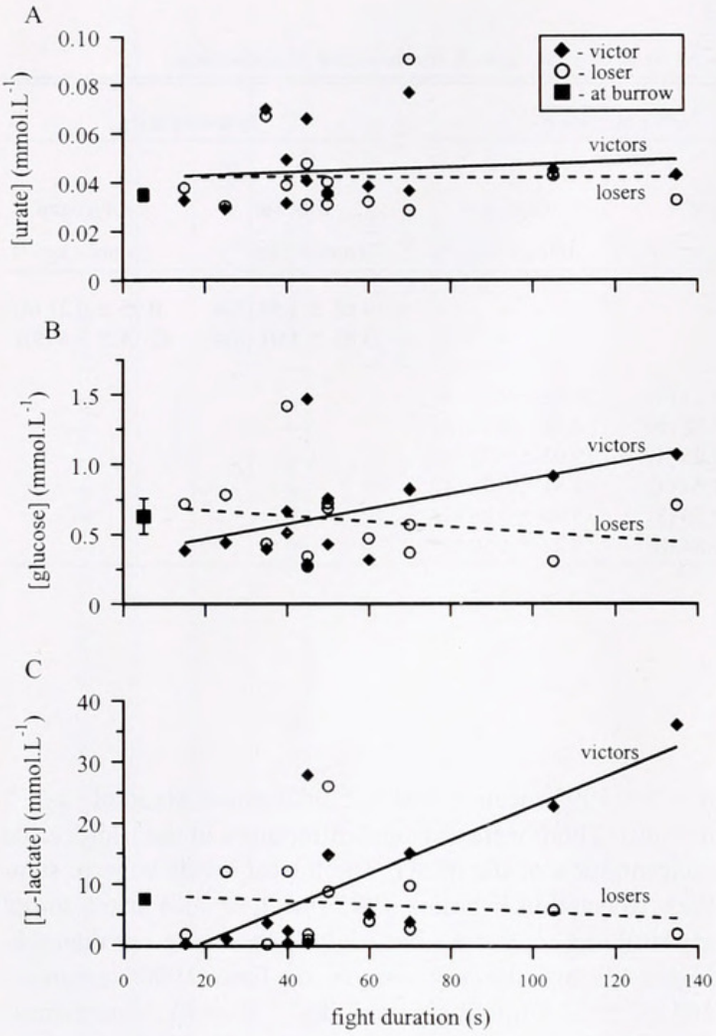

Figure 6. The concentration of (A) uric acid, (B) glucose, and (C) L-lactate (mmol · l^{-1}) in the hemolymph of male *Gecarcoidea natalis* guarding burrows during the breeding season (■) and immediately after combat, for losers (○) and victors (◆). Samples were taken from crabs engaged in physical fighting for possession of the burrows, and hemolymph metabolite concentration was plotted against the duration of the fight. The [L-lactate] was statistically dependent on fight duration in the victors but not the losers.

hemolymph of 35 mmol · l^{-1} was recorded after 135 s of combat for the crab that won.

Discussion

Christmas Island is composed largely of highly porous coral limestone with very little surface water outside of the wet season. Like other terrestrial crustaceans, the Christmas Island red crabs are prone to dessication and retreat into their burrows when humidity falls below 85% (Bliss *et al.*, 1978; Wolcott, 1992; Green, 1993, 1997). Dehydration has been shown to decrease locomotor capacity of some crustaceans (Weinstein *et al.*, 1994) and to interfere with respiratory gas transport in the hemolymph (Burggren and McMahon, 1981). The arrival of the monsoonal rains increases humidity, thereby creating more suitable conditions for activity above ground, and provides a cue for the start of the annual breeding migration of *Gecarcoidea natalis* (Hicks, 1985; Green, 1997; Adamczewska and Morris, 2001). During some years, when the monsoonal rains are late and an isolated shower triggers the migration, hemolymph osmolarity of 1100 mOsm has been recorded and the red crabs experience high mortality rates (Greenaway, 1994). The maintenance of hydration may be very important in determining the ability of the red crabs to undertake the breeding migration.

During the two migrations studied, the red crabs remained well hydrated. Although hemolymph osmolarity varied by up to 150 mOsm, the hydration state of leg muscle tissue generally varied by only 1%. The only sign of dehydration (11% decrease in muscle tissue water) was in the males during their return migration of 1993. These crabs had engaged in the strenuous activities of digging and defending their burrows during a 4-day period with no rain. The return migration of male crabs coincided with heavy rainfall, and although the male crabs were clearly drinking rain water, as seen by the hemodilution, they did not appear to rehydrate their muscle tissue before leaving the coastal terraces. A preferential defense of the extracellular over the intracellular fluid volume, previously reported for two gecarcinid species (Harris and Kormanik, 1981), may be a strategy limited to true land crabs (Taylor and Greenaway, 1994). Field studies using markers of extracellular fluid volume will be necessary to determine exact changes in hemolymph volume in migrating crabs (Harris and Andrews, 1982; Taylor and Greenaway, 1994).

Respiratory gas exchange in migrating crabs

The different durations of the migration in the two study years (5 days in 1993 *vs.* 21 days in 1995; Adamczewska and Morris, 2001) imposed very different physiological demands on the red crabs, but the respiratory changes the crabs experienced were remarkably similar. Previous inferences of the relative contribution of the gills and lungs in O_2 uptake by *G. natalis* have suggested both that the lungs are more important (Adamczewska and Morris, 1994a) and conversely that the gills and lungs play a comparable role (Adamczewska and Morris, 1998a; Farrelly and Greenaway, 1994). In both years studied here, the P_PO_2 of red crabs was consistently higher than the P_aO_2 but the pulmonary-arterial PO_2 difference decreased at the end of each day of walking. This decrease was brought about by the lower P_PO_2 (by 1- 2.5 kPa) after each day walking and indicates some diffusion limitation across the lungs. Crabs forced to exercise in the field outside of the migration season showed no such loss of pulmonary oxygenation (Adamczewska and Morris, 2000). Thus, although the lungs routinely play an important role in O_2 uptake, the gills are nonetheless recruited to a greater extent during the migration. It is clear

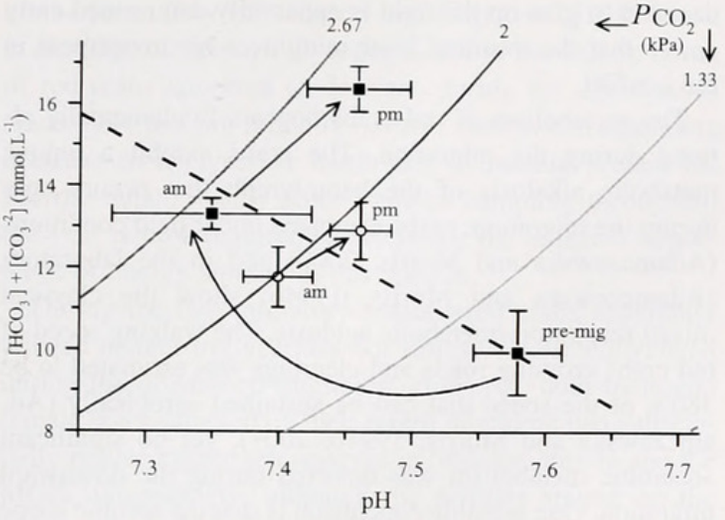

Figure 7. The pH/Bicarbonate relationship for *Gecarcoidea natalis* during two migration seasons, (■) 1993 and (○) 1995. The pre-migratory values in 1993 were assumed to be applicable to the 1995 season. The P_{CO_2} isopleths were constructed using the Henderson-Hasselbalch equation using $\alpha CO_2 = 0.03962$ and pK = 6.1. The non-bicarbonate buffer line was adopted from Adamczewska and Morris (1994a).

that hemolymph shunting occurs between the gills and lungs during locomotion in red crabs. Investigation of hemolymph flow is now essential to determine the role of these two gas exchange structures in O_2 uptake.

During the migration, the P_{O_2} of hemolymph leaving the gas exchange surfaces varied between the groups of crabs sampled (mean 3-7.7 kPa) but was within the range of values recorded previously for this species (3.7-10.7 kPa: Farrelly and Greenaway, 1994; Adamczewska and Morris, 1994a). Despite the differences in P_{O_2}, the high affinity of the Hc for O_2 in red crabs (P_{50} = 1.77 kPa: Adamczewska and Morris, 1998b) ensured Hc saturation with O_2 at the gas exchange surfaces.

Excretion of CO_2 was also maintained by walking crabs during the migration, but without any internal hypercapnia. Red crabs exercised in the field either before or after the migration season exhibited significantly decreased hemolymph CO_2 content due to a metabolic acidosis imposed on a respiratory acidosis (Adamczewska and Morris, 2000). Conversely, each day of migration resulted in an increase in hemolymph CO_2 of 2–3 mmol $\cdot$ l^{-1}, which was of metabolic rather than respiratory origin. Since the P_{CO_2} remained constant and the diffusion gradient across the respiratory surfaces was unchanged, the increase in CO_2 did not indicate impairment of CO_2 excretion.

Acid-base perturbations

More detailed analysis using the Henderson-Hasselbalch plot showed that the AM crabs experienced a relative respiratory acidosis compared with the pre-mig crabs (Fig. 7). This acidosis (~0.2 pH units) persists during the wet season even after the migration is completed and appears to be a simple consequence of the higher activity levels made possible by high relative humidity. (Adamczewska and Morris, 2000). Active red crabs must increase O_2 uptake, and consequently CO_2 excretion is facilitated by an internal hypercapnic acidosis that improves the outward P_{CO_2} gradient (*e.g.*, O'Mahoney and Full, 1984). Thus, the daily alkalosis observed in migrating red crabs in both 1993 and 1995 was superimposed on a seasonal acidosis brought about by increased basal activity. Importantly, there was no evidence of any additional respiratory acidosis as a result of walking each day during the migration (Fig. 7).

The obvious metabolic alkalosis experienced by the red crabs at the end of each day of migration contradicts all previous studies of acid-base perturbations during locomotion in crustaceans (*e.g.*, McMahon *et al.*, 1979; Smatresk *et al.*, 1979; Wood and Randall, 1981b; review: McMahon, 1981; Booth *et al.*, 1984; Greenaway *et al.*, 1988), including exercising red crabs (Adamczewska and Morris, 1998a, 2000). The alkalosis, which must originate either from the removal of H^+ from the hemolymph or the addition of metabolic base, was removed overnight, only to reappear at the end of the next day of migration. A diel de-calcification of the exoskeleton to supply metabolic base in the form of HCO_3^- seems unlikely because since there was no evidence of any change in hemolymph [Ca] between AM and PM crabs; such a change would normally accompany this process (*e.g.*, Henry *et al.*, 1981). A small increase in lactate concentration in the muscle of PM crabs in 1993 was not reflected in any increase the hemolymph. Similarly, a large increase in muscle lactate (values routinely near 6 mmol $\cdot$ kg^{-1}) seen in relatively inactive free-ranging crabs in the dry season of June 1994 was not reflected in the hemolymph (values only 0.72 mmol $\cdot$ l^{-1}). Furthermore, the muscle L-lactate concentrations of the relatively inactive dry-season crabs were nearly 4 mmol $\cdot$ kg^{-1} greater than those of more active wet-season crabs (Adamczewska and Morris, 2000). Clearly there are some aspects of the dynamics of lactate and possibly H^+ distribution that are different in *G. natalis* and require further investigation. The daily alkalosis experienced by red crabs during their downward migration would partially offset the wet-season acidosis and assist O_2 loading by increasing the oxygen affinity of the hemocyanin (Adamczewska and Morris, 1998b).

Metabolism during breeding activities

Crabs generally do not possess high aerobic capacity and tend to become anaerobic during enforced exercise (Smatresk and Cameron, 1981; Booth *et al.*, 1982; Herreid and Full, 1988; Forster *et al.*, 1989; Henry *et al.*, 1994). Hemolymph and tissue L-lactate levels may increase in excess of 30 mmol $\cdot$ l^{-1} in exercising crabs (*e.g.*, Greenaway *et al.*, 1988; Milligan *et al.*, 1989; Adamczewska and

Morris, 1994b). Since anaerobiosis accumulates acid and an O_2 debt that must be repaid later (Wood and Randall, 1981a; Ellington, 1983; Herreid, 1980), prolonged locomotion cannot depend on the anaerobic provision of energy. In migrating red crabs the L-lactate concentrations in the hemolymph and muscle tissue were generally low and comparable to those measured in free-ranging crabs during the non-migratory seasons (Adamczewska and Morris, 2000). Furthermore, L-lactate levels were similar during both of the migratory seasons, despite the fact that red crabs migrating in 1993 walked twice as far each day as those migrating in 1995 (Adamczewska and Morris, 2001). If the small diel change in muscle concentration of lactate during the 1993 migration represented anaerobiosis, this would be equivalent to only approximately 0.4 mmol ATP, or to 3–4 min of resting O_2 uptake (Adamczewska and Morris, 1994b).

Laboratory studies indicated that to cover a set distance in a fixed amount of time, red crabs are better suited to continuous travel than to periods of rapid walking interrupted by periodic rests (Adamczewska and Morris, 1998a). The mean daily walking speeds of red crabs ($1.1\ m \cdot min^{-1}$, estimated from radio-tracking during the 1993 migration) are within the range that this species is capable of supporting aerobically (Adamczewska and Morris, 1994b; 2001). However, walking speeds measured for red crabs crossing roads (*e.g.*, $6.2\ m \cdot min^{-1}$ for returning females: Adamczewska and Morris, 2001) show that sometimes during the migration the crabs walk more than 4 times faster than can be sustained aerobically under laboratory conditions. There was an approximate doubling of muscle lactate in the returning female crabs in 1993, to $6.34\ mmol \cdot kg^{-1}$, but this was again confined to the muscle, and lactate concentrations remained low in the hemolymph (Table 1). This change in muscle lactate represents only about 5 min of resting oxygen uptake.

The possession of a burrow has direct consequences for the breeding success of a male red crab. For male crabs, defending a burrow was more strenuous than prolonged locomotion during the downward migration. The basal hemolymph [L-lactate] of crabs guarding their burrows was elevated by more than $6\ mmol \cdot l^{-1}$ compared with migrating crabs. Winning fights is energetically demanding, and the levels of L-lactate (up to $35\ mmol \cdot l^{-1}$) measured after combat were comparable to the concentrations recorded after 45 min of intense exercise (Adamczewska and Morris, 1994b) or after many hours of hypoxia (Gäde, 1984; Albert and Ellington, 1985; van Aardt, 1988). *G. natalis* expends much more energy in defending burrows than the aquatic crab *Necora puber* expends in a fight under laboratory conditions (Thorpe *et al.*, 1995). The high levels of L-lactate in red crabs either indicate an extreme effort put into winning a fight or represent an accumulated sum of attempting to win many fights. Since the losers of conflicts did not show the same accumulation of L-lactate as the winners, the decision to give up the fight is apparently determined early on, so that the eventual loser minimizes his investment in the conflict.

The metabolism of red crabs appears fundamentally altered during the migration. The crabs exhibit a unique metabolic alkalosis of the hemolymph that occurs only during the migration: crabs exercised under field conditions (Adamczewska and Morris, 2000) and in the laboratory (Adamczewska and Morris, 1994b) show the classical mixed respiratory/metabolic acidosis. The walking speed of red crabs crossing roads and clearings was estimated to be 380% of the speed that can be sustained aerobically (Adamczewska and Morris, 1994b, 2001), yet no significant anaerobic metabolism was detected during the downward migration. One possible conclusion is that the aerobic scope of red crabs, which is otherwise unusually low (Adamczewska and Morris, 1994b), is specifically increased for the purpose of the migration. If so, the signals and processes by which this is achieved are yet to be elucidated.

Storage and usage of metabolic fuels

The midgut gland and muscle tissue compose a large portion of the body mass in crustaceans and are considered to be major organs for the storage of metabolic fuel (Vonk, 1960; Chang and O'Connor, 1983; England and Baldwin, 1983; Tsai *et al.*, 1984; Schirf *et al.*, 1987). In *G. natalis*, the muscle tissue stores glycogen, whereas the MGG is a major site of lipid storage (Adamczewska and Morris, 1994b; Morris, unpub. data). In animals, fatty acids are primarily stored in the form of triglycerides, and in *G. natalis* triglycerides were estimated to constitute about 70% of total lipids in the MGG ($380\ mg\ lipid \cdot g^{-1}$ dry tissue mass: S. Morris, unpub. data). The lipid stores in brachyuran crustaceans vary considerably between species, but total lipid stores in the MGG can reach $900\ mg \cdot g^{-1}$ dry mass (Giese, 1966; Heath and Barnes, 1970; Parvathy, 1971; Kucharski and DaSilva, 1991).

The glycogen stores in the muscle tissue of *G. natalis* sampled in the field (11-64 mg glucose $\cdot g^{-1}$ dry mass) were only a fraction of what this species is capable of storing ($250\ mg\ glucose \cdot g^{-1}$ dry mass, Adamczewska and Morris, 1994b) but were within the range found in other brachyuran species (8-71 $mg \cdot g^{-1}$ dry tissue mass: Giese, 1966; Pillay and Nair, 1973; Hilmy *et al.*, 1986; Kucharski and Da Silva, 1991). Since the stores of metabolic fuels in red crabs in the field were not exceptional, red crabs either lack special requirements for large energy stores under natural conditions or have no opportunity to accumulate such stores (Greenaway and Linton, 1995; Greenaway and Raghaven, 1998).

Leaf litter is the major component of the diet of red crabs. During the dry season, when the red crabs are largely confined to burrows, a thick layer of leaf litter accumulates

on the forest floor (Greenaway and Raghaven, 1998; Green *et al.*, 1999). Although glycogen stores in the muscle tissue of red crabs appeared to decrease during the dry seasons (June 1994 and pre-mig 1993), there was no corresponding decrease in triglyceride levels nor in muscle protein (S. Morris, unpub. data). More frequent sampling throughout the year is now required to determine the seasonal importance of energy storage in this species.

During the two migratory seasons red crabs apparently utilized neither triglycerides nor stores of muscle glycogen during the downward migration. However, both male and female crabs sampled on their return migration had substantially reduced glycogen reserves in both the muscle and the MGG. Interestingly, although the females stayed on the terraces 2 weeks longer than the male crabs (Hicks *et al.*, 1990; Adamczewska and Morris, 2001), the glycogen reserves were depleted to a similar extent in both sexes.

Assuming that 20% of the body mass is muscle tissue (Giese, 1966; Cameron and Wood, 1985) and using published values of $\dot{M}O_2$ (212 μmol $O_2 \cdot kg^{-1} \cdot min^{-1}$: Adamczewska and Morris, 1994b), the rate of glucose utilization in red crabs during walking would be 0.636 mmol of glucose per hour. The glycogen used by the red crabs during either the breeding activities on the terraces or the return migration was 26 and 53 mmol glucose $\cdot kg^{-1}$ in the two seasons and would be sufficient to provide fuel for about 2.5 h and 5 h of activity in 1993 and 1995 respectively. The glucose and glycogen stores utilized from the MGG would provide metabolic fuel for a further 0.5-2 h of walking. Therefore, considering that the red crabs spend a week or more engaged in breeding activities on the terraces, the glycogen stores used would only be sufficient to fuel a small portion of the active metabolism; the remainder must be supported by food intake (Greenaway and Linton, 1995; Green, 1997).

Sampling of *G. natalis* during the migration has provided unique information about the energy metabolism of these crabs and their capacity to undertake the annual migration. Perhaps surprisingly, it has become clear that the red crabs undertake the annual migration with minimal reliance on energy stores and support prolonged locomotion aerobically. In contrast, the recruitment of anaerobiosis by male red crabs while defending and maintaining their burrows indicates that these breeding activities are more energetically demanding than the actual migration. Above all, it seems clear that migrating red crabs possess a physiological capacity and exhibit physiological responses different from those of the crabs held in the laboratory and from those of non-migrating crabs in the field. Laboratory and further field-based investigations of the regulation of their metabolism are now required, especially of possible seasonal hormonal signals that might alter metabolic state.

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Literature Cited

Adamczewska, A. M., and S. Morris. 1994a. Exercise in the terrestrial Christmas Island red crab *Gecarcoidea natalis*. I. Blood gas transport. *J. Exp. Biol.* **188:** 235–256.

Adamczewska, A. M., and S. Morris. 1994b. Exercise in the terrestrial Christmas Island red crab *Gecarcoidea natalis*. II. Energetics of locomotion. *J. Exp. Biol.* **188:** 257–274.

Adamczewska, A. M., and S. Morris. 1996. The respiratory gas transport, acid-base state, ion and metabolite status of the Christmas Island blue crab, *Cardisoma hirtipes* (Dana) assessed *in situ* with respect to immersion. *Physiol. Zool.* **69:** 67–92.

Adamczewska, A. M., and S. Morris. 1998a. Strategies for migration in the terrestrial Christmas Island red crab *Gecarcoidea natalis*: intermittent *versus* continuous locomotion. *J. Exp. Biol.* **201:** 3221–3231.

Adamczewska, A. M., and S. Morris. 1998b. The functioning of the haemocyanin of the terrestrial Christmas Island red crab *Gecarcoidea natalis* and roles for organic modulators. *J. Exp. Biol.* **201:** 3233–3244.

Adamczewska, A. M., and S. Morris. 2000. Respiratory gas transport, metabolic status and locomotor capacity of the Christmas Island red crab *Gecarcoidea natalis* assessed in the field with respect to dichotomous seasonal activity levels. *J. Exp. Zool.* **286:** 552–562.

Adamczewska, A. M., and S. Morris. 2001. Ecology and behavior of *Gecarcoidea natalis*, the Christmas Island red crab, during the annual breeding migration *Biol. Bull.* **200:** 305–320.

Albert, J. L., and W. R. Ellington. 1985. Patterns of energy metabolism in the shore crab, *Menippe mercenaria*, during severe hypoxia and subsequent recovery. *J. Exp. Zool.* **234:** 175–183.

Bergmeyer, H. U. 1985. *Methods of Enzymatic Analysis.* 3rd ed. Vol. VI. Metabolites 1: Carbohydrates. VCH, Weinheim, Germany.

Bliss, D. E., J. Van Montfrans, M. Van Montfrans, and J. R. Boyer. 1978. Behavior and growth of the land crab *Gecarcinus lateralis* (Fréminville) in southern Florida. *Bull. Am. Mus. Nat. Hist.* **160:** 111–152.

Booth, C. E., and B. R. McMahon. 1985. Short communications. Lactate dynamics during locomotor activity in the blue crab, *Callinectes sapidus*. *J. Exp. Biol.* **118:** 461–465.

Booth, C. E., B. R. McMahon, and A. W. Pinder. 1982. Oxygen uptake and the potentiating effects of increased hemolymph lactate on oxygen transport during exercise in the blue crab, *Callinectes sapidus*. *J. Comp. Physiol.* **148:** 111–121.

Booth, C. E., B. R. McMahon, P. L. DeFur, and P. R. H. Wilkes. 1984. Acid-base regulation during exercise and recovery in the blue crab, *Callinectes sapidus*. *Respir. Physiol.* **58:** 395–376.

Burggren, W. W., and B. R. McMahon. 1981. Hemolymph oxygen transport, acid-base status, and hydromineral regulation during dehydration in three terrestrial crabs, *Cardisoma, Birgus* and *Coenobita*. *J. Exp. Zool.* **218:** 53–64.

Cameron, J. N., and C. M. Wood. 1985. Apparent H^+ excretion and CO_2 dynamics accompanying carapace mineralization in the blue crab (*Callinectes sapidus*) following moulting. *J. Exp. Biol.* **114:** 181–196.

Chang, E. S., and J. D. O'Connor. 1983. Metabolism and transport of carbohydrates and lipids. Pp. 263–287 in *The Biology of the Crustacea*, Vol. 5. L. H. Mantel, ed.. Academic Press, New York.

Ellington, W. R. 1983. The recovery from anaerobic metabolism in invertebrates. *J. Exp. Zool.* **228:** 431–444.

England, W. R., and J. Baldwin. 1983. Anaerobic energy metabolism in the tail musculature of the Australian yabby *Cherax destructor* (Crustacea, Decapoda, Parastacidae): role of phosphagens and anaerobic glycolysis during escape behavior. *Physiol. Zool.* **56:** 614–622.

Farrelly C. A., and P. Greenaway. 1994. Gas exchange through the lungs and gills in air breathing crabs. *J. Exp. Biol.* **187:** 113–130.

Forster, M. E., F. M. Waldron, and H. H. Taylor. 1989. Recovery from exhausting exercise in a bimodally breathing crab, *Leptograpsus variegatus* (Decapoda: Grapsidae). *J. Exp. Mar. Biol. Ecol.* **127:** 165–173.

Gäde, G. 1984. Effects of oxygen deprivation during anoxia and muscular work on the energy metabolism of the crayfish, *Orconectes limosus*. *Comp. Biochem. Physiol.* **77A:** 495–502.

Gibson, R., and P. L. Barker. 1979. The decapod hepatopancreas. *Oceanogr. Mar. Biol. Annu. Rev.* **17:** 285–346.

Gibson-Hill, C. A. 1947. Field notes on the terrestrial crabs. *Bull. Raffles Mus.* **18:** 43–52.

Giese, A. C. 1966. Lipids in the economy of marine invertebrates. *Physiol. Rev.* **46:** 244–298.

Gifford, C. A. 1962. Some observations on the general biology of the land crab, *Cardisoma guanhumi* (Latreille) in south Florida. *Biol. Bull.* **123:** 207–223.

Green, P. T. 1993. The role of Red Land Crabs in structuring rain forest on Christmas Island, Indian Ocean. Ph.D. thesis, Monash University, Clayton, Victoria, Australia.

Green, P. T. 1997. Red crabs in rain forest on Christmas Island, Indian Ocean: activity patterns, density and biomass. *J. Trop. Ecol.* **13:** 17–38.

Green P. T., P. S. Lake, and D. J. O'Dowd. 1999 Monopolization of litter processing by a dominant land crab on a tropical oceanic island. *Oecologia.* **119:** 435–444.

Greenaway, P. 1994. Salt and water balance in field populations of the terrestrial crab *Gecarcoidea natalis*. *J. Crustac. Biol.* **14:** 435–453.

Greenaway, P., and S. M. Linton. 1995. Dietary assimilation and food retention time in the herbivorous terrestrial crab *Gecarcoidea natalis*. *Physiol. Zool.* **68(6):** 1006–1028.

Greenaway, P., and S. Raghaven, 1998. Digestive strategies in two species of leaf-eating land crabs (Brachyura: Gecarcinidae) in a rain forest. *Physiol. Zool.* **71:** 36–44.

Greenaway P., S. Morris, and B. R. McMahon. 1998. Adaptations to a terrestrial existence by the Robber Crab *Birgus latro*. II. *In vivo* respiratory gas exchange and transport. *J. Exp. Biol.* **140:** 493–509.

Harris, R. R., and M. B. Andrews. 1982. Extracellular fluid volume changes in *Carcinus maenas* during acclimation to high and low environmental salinities. *J. Exp. Biol.* **99:** 161–173.

Harris, R. R., and G. A. Kormanik. 1981. Salt and water balance and antennal gland function in three Pacific species of terrestrial crab (*Gecarcoidea lalandii, Cardisoma carnifex, Birgus latro*). II. The effects of desiccation. *J. Exp. Zool.* **218:** 107–116.

Head, G., and J. Baldwin. 1986. Energy metabolism and the fate of lactate during recovery from exercise in the Australian freshwater crayfish *Cherax destructor*. *Aust. J. Mar. Freshw. Res.* **37:** 641–646.

Heath, J. R., and H. Barnes. 1970. Some changes in biochemical composition with season and during the moulting cycle of the common crab *Carcinus maenas* (L.). *J. Exp. Mar. Biol. Ecol.* **5:** 199–233.

Henry, R. P., G. A. Kormanik, N. J. Smatresk, and J. N. Cameron. 1981. The role of shell dissolution as a source of HCO_3^- for buffering hypercapnic acidosis in aquatic and terrestrial decapod crustaceans. *J. Exp. Biol.* **94:** 269–274.

Henry, R. P., C. E. Booth, F. H. Lallier, and P. J. Walsh. 1994. Post-exercise lactate production and metabolism in three species of aquatic and terrestrial decapod crustaceans. *J. Exp. Biol.* **186:** 215–234.

Herreid, C. F. 1980. Review: Hypoxia in invertebrates. *Comp. Biochem. Physiol.* **67A:** 311–320.

Herreid, C. F., and R. J. Full. 1988. Energetics and locomotion. Pp. 333–377 in *Biology of the Land Crabs*, W. W. Burggren and B. R. McMahon, eds. Cambridge University Press, New York.

Hicks, J. W. 1985. The breeding behaviour and migrations of the terrestrial crab *Gecarcoidea natalis*. (Decapoda: Brachyura). *Aust. J. Zool.* **33:** 127–142.

Hicks, J., H. Rumpff, and H. Yorkston. 1990. *Christmas Crabs*. Golden Earth Design and Printing, Singapore.

Hilmy, A. M., N. F. Abd El-Hamid, and K. H. G. Adham. 1986. Glycogen content of some organs of two marine crustaceans. *Bull. Inst. Oceanogr. Fish.* **12:** 215–229.

Klaassen, F. 1975. Ecological and ethological studies on the reproductive biology of *Gecarcinus lateralis* (Decapoda, Brachyura). *Forma Function* **8:** 101–174.

Kucharski, L. C. R., and R. S. M. Da Silva. 1991. Effect of diet composition on the carbohydrate and lipid metabolism in an estuarine crab, *Chasmagnathus granulata* (Dana, 1851). *Comp. Biochem. Physiol.* **99A:** 215–218.

McMahon, B. R. 1981. Oxygen uptake and acid-base balance during activity in decapod crustaceans. Pp. 299–335 in *Locomotion and Energetics in Arthropods*, C. F. Herreid and C. R. Fourtner, eds.. Plenum Press, New York.

McMahon, B. R., D. G. McDonald, and C. M. Wood. 1979. Ventilation, oxygen uptake and haemolymph oxygen transport, following enforced exhausting activity in the Dungeness crab *Cancer magister*. *J. Exp. Biol.* **80:** 271–285.

Milligan, C. L., P. J. Walsh, C. E. Booth, and D. G. McDonald. 1989. Intracellular acid-base regulation during recovery from locomotor activity in the blue crab *Callinectes sapidus*. *Physiol. Zool.* **62:** 621–638.

Nishida, S., Y. Takahashi, and J. Kittaka. 1995. Structural changes in the hepatopancreas of the rock lobster, *Jasus edwardsii* (Crustacea, Palinudidae) during development from the puerulus to post-puerulus. *Mar. Biol.* **123:** 837–844.

O'Mahoney, P. M., and R. J. Full. 1984. Respiration of crabs in air and water. *Comp. Biochem. Physiol.* **79A:** 275–282.

Parvathy, K. 1971. Glycogen storage in relation to the moult cycle in two crustaceans *Emerita asiatica* and *Ligia exotica*. *Mar. Biol.* **10:** 82–86.

Pillay, K. K., and N. B. Nair. 1973. Observations on the biochemical changes in gonads and other organs of *Uca annulipes, Portunus pelagicus* and *Metapenaeus affinis* (Decapoda: Crustacea) during the reproductive cycle. *Mar. Biol.* **18:** 167–198.

Schirf, V. R., T. Turner, L. Selby, C. Hannapel, P. Dela Cruz, and P. F. Dehn. 1987. Nutritional status and energy metabolism of crayfish (*Procambarus clarkii*, Girard) muscle and hepatopancreas. *Comp. Biochem. Physiol.* **88A:** 383–386.

Smatresk, N. J., and J. N. Cameron. 1981. Post-exercise acid-base balance and ventilatory control in *Birgus latro*, the coconut crab. *J. Exp. Zool.* **218:** 75–82.

Smatresk, N. J., A. J. Preslar, and J. N. Cameron. 1979. Post-exercise acid-base disturbance in *Gecarcinus lateralis*, a terrestrial crab. *J. Exp. Zool.* **210:** 205–210.

Taylor, H. H., and P. Greenaway. 1994. The partitioning of water and solutes during evaporative water loss in three terrestrial crabs. *Physiol. Zool.* **67:** 539–565.

Thorpe, E., A. C. Taylor, and F. A. Huntingford. 1995. How costly is fighting? Physiological effects of sustained exercise and fighting in swimming crabs, *Necora puber* (L.) (Brachyura, Portunidae). *Anim. Behav.* **50:** 1657–1666.

Tsai, D. E., H. Chen, and C. Tsai. 1984. Total lipid and cholesterol content in the blue crab, *Callinectes sapidus* Rathbun. *Comp. Biochem. Physiol.* **78B:** 27–31.

van Aardt, W. J. 1988. Lactate metabolism and glucose patterns in the river crab, *Potamonautes warreni* Calman, during anoxia and subsequent recovery. *Comp. Biochem. Physiol.* **91A:** 299–304.

van Aardt, W. J. 1990. Oxygen uptake and haemocyanin oxygen affinity of *Potamonautes warreni* Caman after exercise. *S. Afr. J. Zool.* **25:** 11–17.

Vonk, H. J. 1960. Digestion and metabolism. Pp. 291–316 in *Physiology of Crustacea,* Vol. 1. T. H. Waterman, ed. Academic Press, New York.

Weinstein, R. B., and R. J. Full. 1992. Intermittent exercise alters endurance in an eight-legged ectotherm. *Am. J. Physiol.* **262:** R852–R859.

Weinstein R. B., R. J. Full, and A. N. Ahn. 1994. Moderate dehydration decreases locomotor performance of the ghost crab *Ocypode quadrata. Physiol. Zool.* **67:** 873–891.

Wolcott, T. G. 1998. Ecology. Pp. 55–96 in *Biology of the Land Crabs.* W. W. Burggren and B. R. McMahon, eds. Cambridge University Press, Cambridge.

Wolcott, T. G. 1992. Water and solute balance in the transition to land. *Am. Zool.* **32:** 428–437.

Wolcott, T. G., and D. L. Wolcott. 1985. Factors influencing the limits of migratory movements in terrestrial crustaceans. *Contrib. Mar. Sci. Suppl.* **27:** 257–273.

Wood, C. M., and D. J. Randall. 1981a. Oxygen and carbon dioxide exchange during exercise in the land crab *Cardisoma carnifex. J. Exp. Zool.* **218:** 7–22.

Wood, C. M., and D. J. Randall. 1981b. Haemolymph gas transport, acid-base regulation, and anaerobic metabolism during exercise in the land crab *Cardisoma carnifex. J. Exp. Zool.* **218:** 23–35.