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Respiratory and acid–base responses during migration and to exercise by the terrestrial crab *Discoplax (Cardisoma) hirtipes*, with regard to season, humidity and behaviour

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Summary

The terrestrial crab *Discoplax hirtipes* (formerly *Cardisoma hirtipes*) exhibits a seasonally dichotomous activity pattern governed by the seasonal rainfall on Christmas Island, with a breeding migration in the wet season. Greater activity in the wet season reflects a release of constraints on ion and water balance independent of changes in exercise physiology. The respiratory responses to walking exercise by *D. hirtipes* were assessed with regard to humidity and season. In the laboratory, crabs walked 38% faster when humidity was high, while the O_2 uptake across the lung became diffusion limited despite a Ca^{2+}-induced increase in O_2 affinity of the haemocyanin. Crabs walking in 90% relative humidity exhibited a larger metabolic acidosis while re-oxidation of L-lactate was only 0.49 mmol l^{-1} h^{-1}. The wet-season crabs were more active and exhibited a respiratory acidosis compared with the quiescent dry-season crabs. The migration was close to the limit of the aerobic scope of the crabs, and the pulmonary oxygen partial pressure (P_{O_2}) was depressed, the O_2 uptake diffusion limited and the arterial–venous P_{O_2} difference diminished. Additional, enforced exercise induced a metabolic acidosis. The optimum strategy for migration would be walking continuously but at a speed within the aerobic scope. *D. hirtipes* is influenced by seasonal rain and responds to lower ambient humidity by limiting exercise. The behavioural response is paramount since the changes in respiratory status were determined primarily by the differences in commitment to, and investment in, walking and not by direct effects of humidity on respiratory physiology.

Key words: land crab, *Cardisoma*, *Discoplax*, exercise, migration, respiration, acid–base, behaviour.

Introduction

All terrestrial gecarcinid crabs, including the Christmas Island blue crab, *Discoplax hirtipes* (formerly *Cardisoma hirtipes*; Davie, 2002), migrate annually to the coast to release their eggs into the ocean (Gibson-Hill, 1947; Gifford, 1962; Klaassen, 1975; Wolcott, 1988; Hicks et al., 1990; Dela-Cruz and Morris, 1997b; Adamczewska and Morris, 1996, 2001a). On Christmas Island, during the dry season (April–October; Gray, 1995) *D. hirtipes* are restricted in their distribution to areas of freshwater seepages, sometimes more than 1 km from and over 150 m above the ocean, around which they forage for fallen leaves and fruit (Hicks et al., 1990; Adamczewska and Morris, 1996; Dela-Cruz and Morris, 1997a,b; Greenaway and Raghaven, 1998). Unlike the Christmas Island red crab, *Gecarcoidea natalis*, which migrates at the onset of the wet season (November–March), blue crabs initially disperse to forage over a wider area (Dela-Cruz and Morris, 1997b) and only once the wet season is well established (January–February) do they undertake their breeding migration (Hicks et al., 1990; S.M., personal observation).

D. hirtipes have well-developed lungs (Farrelly and Greenaway, 1992, 1993) and are competent air breathers (Adamczewska and Morris, 1996; Morris and Dela-Cruz, 1998) but require periodic immersion to facilitate nitrogen excretion (surface water for drinking is inadequate; Dela-Cruz and Morris, 1997b). Thus, the necessity for immersion apparently limits the dry-season range of *D. hirtipes.* A reduced locomotor capacity in land crabs during the dry season would further constrain their distribution, foraging ranges and ability to escape from predators.

There are several studies of exercise capacity in a variety of decapod crustaceans (for reviews, see McMahon, 1981; Herreid and Full, 1988; Full and Weinstein, 1992; Adamczewska and Morris, 2000b; Morris, 2002) but very few of migration physiology, or of seasonality, or under field conditions (e.g. Adamczewska and Morris, 2001a,b). Increasing the walking speed of terrestrial crabs induces fatigue and reduces their distance capacity (e.g. Wood and Randall, 1981a; Full and Herreid, 1984; van Aardt, 1990; Weinstein and Full, 1992; Adamczewska and Morris, 1998a).

In most crustaceans, a functional shortage of O_2 during locomotion is associated with some degree of anaerobiosis, leading to a lactacidosis that is often exacerbated by a respiratory component (e.g. Wood and Randall, 1981b; Booth et al., 1984; Greenaway et al., 1988; Forster et al., 1989; Henry et al., 1994; Adamczewska and Morris, 1998).

Seasonally variable rainfall and/or ambient humidity has received relatively little consideration as a limiting factor in exercise physiology of crabs, although dehydration is known to interfere with O_2 transport in land crabs (Burggren and McMahon, 1981). In ghost crabs, dehydration markedly decreased the maximum aerobic scope (Weinstein et al., 1994). However, the terrestrial *G. natalis* exhibited a seasonal respiratory acidosis that correlated with increased activity and respiration rate during the wet season rather than with hydration state, although hydration state may facilitate the altered behaviour (Adamczewska and Morris, 2000a). The role of ambient humidity in limiting locomotor capacity and activity is important in understanding the migration physiology of *D. hirtipes*.

The majority of studies of locomotion in crustaceans have used exhaustive exercise regimens under laboratory conditions, resulting, unsurprisingly, in a lactacidosis (e.g. McMahon et al., 1979; Greenaway et al., 1988; Forster et al., 1989; Adamczewska and Morris, 1994a,b; Morris and Adamczewska, 2002). However, field studies of *G. natalis* on Christmas Island revealed that these crabs migrated over more than 5 km without becoming anaerobic. Generally, crustaceans, including land crabs, are not adept at re-oxidising lactate and recover relatively slowly from an O_2 debt (e.g. Henry et al., 1994; Adamczewska and Morris, 1998; Morris and Adamczewska, 2002). Slow lactate oxidation is not necessarily problematic if the lactacidosis is a consequence of a 'one-off' sprinting event (e.g. predator avoidance) with a protracted recovery period. However, the blue crab migration is an endurance activity and, if the crabs become anaerobic, low rates of oxidation would lead to both more rapid and more persistent lactacidosis, which will compromise the migration. Thus, it is important to determine the extent of any such acidosis and the recovery capacity of *D. hirtipes* or whether they limit, behaviourally, migration exercise to within their maximum aerobic scope (MAS). Furthermore, it should be determined whether low humidity and dry-season conditions increase the likelihood of anaerobiosis. Is there a respiratory or energetic penalty for exercising under dry conditions or is the risk limited to problems of ion and water homeostasis (e.g. Harris and Kormanik, 1981; Dela-Cruz and Morris, 1997b), which constrain the commitment to exercise?

The migration of blue crabs on Christmas Island requires the establishment of the wet season and this is clearly a general determinant of seasonal behaviour (Hicks et al., 1990) and, thereby, of the energetic demands of locomotion. Preparation for the migration requires a complex of physiological changes (e.g. maturation of reproductive structures and gametes; Linton and Greenaway, 2000) but these are concomitant with a highly dichotomous activity level. However, it is unclear to what extent increased humidity may release constraints on behaviour and thus allow an increased locomotion and therewith the migration. Alternatively, seasonal changes in physiology may equip the crabs for migration in anticipation of the rigours of the wet season. The energetic investment in walking is seasonal and indicates a proclivity, and a greatly increased commitment, for walking during the wet season. Thus, behaviour may be modulated by ambient humidity but, at the same time, changes in underlying physiological state may also be required in order to facilitate increased levels of exercise.

To establish direct (physiological) and possible indirect (behaviourally mediated) influences of a seasonal rainfall pattern and humidity on exercise and migration in *D. hirtipes*, both field and laboratory studies were conducted in which respiratory, acid–base and metabolite status was determined. On Christmas Island, the respiratory and energetic status of *D. hirtipes* during the wet season (migration) was compared with that of crabs in the dry season (quiescent), both in their natural state *in situ* and with an additional load of 5 min enforced walking. In the laboratory, the influence of humidity was probed by comparing crabs exercised for 5 min at either 40% or 90% relative humidity. Recovery rates and duration following an exercise-induced lactacidosis were determined. The data are evaluated in regards to the seasonal ecology and breeding migration of *D. hirtipes* and land crabs generally.

Materials and methods

Investigations of walking exercise in *Discoplax hirtipes* Dana 1851 were carried out both in the laboratory and *in situ* on Christmas Island during the dry and wet seasons. *Discoplax hirtipes* were collected from Christmas Island under permit from Parks Australia. In the laboratory, the crabs were housed in communal terrariums (60 cm×1300 cm×25 cm high) at 25±1°C and at more than 80% relative humidity. The terrariums were cleaned and the crabs were fed fruit, dry dog food and dry leaves once a week. The animals were supplied with artificial Christmas Island water (Dela-Cruz and Morris, 1997) to drink and in which to immerse, and were maintained on a 12 h:12 h light:dark regime.

Exercise in the laboratory – simulations

Male and female blue crabs were used in equal numbers for experiments with body mass ranging from 331 to 557 g (mean 388±43 g). The crabs were deprived of food and water 1 day prior to experimentation. The effects of exercise at either low (40%) or high (≥90%) relative humidity (RH) on the respiratory gas exchange and metabolic status were examined. Blue crabs were sampled either while resting in their terrarium (N=6) or after 5 min of exercise in air at 40% RH (N=6) or at least 90% RH (N=6). The crabs that were exercised walked individually in an arena 1.5 m×3 m at a pace of their choosing, but gentle tactile stimulation was used to encourage crabs to continue walking (speed 2.48 and 1.80 m min^{-1} at 90% and 40% RH, respectively). Humidity was maintained using an

electric steam humidifier activated by a relay attached to a micro switch (RS Components Pty Ltd, Smithfield, NSW, Australia) mounted on a Lambrecht 194 hygrometer (Lambrecht, Göttingen, Germany) accurate to ±2.5% RH.

Sampling and analysis in the laboratory

Each blue crab was sampled for pulmonary, arterial and venous haemolymph, which was immediately analysed at 25°C for partial pressure and content of O_2 and CO_2, as well as haemolymph pH. A sample of haemolymph was frozen for measurement of osmolality, calcium and metabolites. The crabs were kept in ventilated individual terrariums with fresh drinking water for 24 h prior to experimentation. The carapace of the crabs was drilled at least 24 h prior to experiments to facilitate sampling of haemolymph from the pericardial cavity (700 μl; arterial haemolymph), from the efferent pulmonary vessel (300 μl; for vascular anatomy; see Farrelly and Greenaway, 1993) and directly from the venous sinus (800 μl; venous haemolymph). The entire sampling process required less than 30 s. Samples were taken in chilled 1 ml syringes with 21-gauge hypodermic needles and held on ice for the duration of haemolymph gas and acid–base analysis.

The partial pressure of O_2 (P_{O_2}) and CO_2 (P_{CO_2}), as well as the pH of the haemolymph, was measured using a BMS 3 MK II Blood Micro System (Copenhagen, Denmark) thermostatically controlled at 25±0.2°C and connected to a PHM73 pH/blood gas monitor (Radiometer, Copenhagen, Denmark). The electrodes were calibrated with humidified gases each day before use. The O_2 electrode was calibrated using O_2-free gas and with humidified air and the CO_2 electrode using 0.5% and 2.5% CO_2. The pH electrode was calibrated with Radiometer precision buffers of pH 7.410 (S1510) and 6.865 (S1500), accurate to ±0.005 at 25°C. Haemolymph oxygen contents ($[O_2]$) were measured using the modified Tucker chamber method (Tucker, 1967) as outlined by Bridges et al. (1979). The O_2 electrode was maintained at 32°C and connected to an oxygen meter (Strathkelvin model 781, Glasgow, Scotland). The changes in Po_2 were recorded on a pen recorder (model BD111; Kipp and Zonen, Delft, The Netherlands). The haemolymph CO_2 content was measured using a Corning 965 CO_2 analyser (Medfield, MA, USA; calibrated with HCO_3^- standard, 15 mmol l^{-1}).

The haemocyanin content of the haemolymph was measured by spectrophotometric scanning of a 10 μl haemolymph sample in 1 ml of 1% EDTA in Milli-Q water (Sydney, NSW, Australia). The peak absorbance near 338 nm was used to calculate the haemocyanin concentrations using the extinction coefficient 2.69 $E^{1\%}_{1\,cm}$ (Nickerson and Van Holde, 1971). The haemocyanin concentration was used to derive the maximum capacity for haemocyanin-bound O_2 of each sample and thereby the relative haemocyanin O_2 saturation.

An aliquot of the remaining haemolymph samples was mixed (ratio 1:1) with ice-cold 0.6 mol l^{-1} $HClO_4$ to denature proteins and was neutralised with 2.5 mol l^{-1} K_2CO_3. The denatured sample was centrifuged at 10 000 ***g*** at 4°C for 10 min and the supernatant frozen for subsequent L-lactate analysis (test kit No. 138 084; Boehringer Mannheim, Mannheim, Germany). Whole haemolymph samples were maintained at 4°C for a maximum of 15 min before freezing for later analysis for glucose (test-kit No. 510; Sigma Diagnostics, Sydney, NSW, Australia) and urate concentrations (Sigma Diagnostics test kit No. 685).

Haemolymph osmolality was measured using a vapour pressure osmometer (Wescor 5100C, Logan, UT, USA) calibrated with two precision standards, 290 and 1000 mOsm. The concentration of Ca^{2+} in the haemolymph was measured using an atomic absorption spectrophotometer (GBC 906, Melbourne, Australia) with a sample of haemolymph deproteinised with HNO_3 (0.1 mol l^{-1}; ratio 1:1). To suppress interference during measurements, samples and standards were diluted with 7.2 mmol l^{-1} $LaCl_3$.

The concentration of L-lactate from the haemolymph and the changes in the concentration of circulating glucose after 5 min of exercise were monitored at intervals for 24 h in crabs exercised at ⩾90% RH by repeated sampling (50 μl) using an ice-cold 100 μl Hamilton syringe with a 26-gauge needle inserted through the arthrodial membrane at the base of the walking legs. During recovery, blue crabs were maintained individually and supplied with a continuous flow of humidified air.

Exercise in the field – in situ

D. hirtipes were sampled on Christmas Island during two seasons: June (dry season), when the crabs were quiescent, and during the following February (wet season), when the blue crabs were engaged in the seaward breeding migration. In June, sampling was carried out at Ross Hill Gardens (10°29′11′′S, 105°40′41′′E), where the blue crabs were congregated around freshwater seepages. During the February wet season, the crabs were migrating from Ross Hill Gardens to the coast, and sampling was carried out at a lower forest terrace, approximately 500 m from Ross Hill Gardens (10°29′29′′S, 105°40′43′′E). During the dry season study period (June), RH was as low as 63% whereas in the wet season period (February) it never fell below 100% and quite often the air was supersaturated with water vapour (mist).

Two groups of crabs were sampled during each of the study seasons (N>6 for each group). The first group was the free-ranging crabs (FR); this group comprised crabs that were above ground outside of their burrows. Each crab was captured for haemolymph sampling, but any crabs that attempted to escape prior to capture were marked and excluded from the experiment. A second group of crabs was exercised for 5 min. A blue crab was selected at random and an observer approached the crab until the crab began to move away; when the crab stopped walking, the observer approached the crab again. After 5 min of this exercise the crab was captured for haemolymph sampling.

Samples were taken and analysed as described for the samples from crabs exercised in the laboratory (above) except that the samples were transported in sealed syringes on ice for the 14 min drive to the Research Station on Christmas Island

and different electrodes were used. P_{O_2} was determined with a flow-through micro oxygen probe (Microelectrodes, MI16-730, Bedford, NH, USA) connected to a PHM73 pH/blood gas monitor (Radiometer) calibrated at ambient temperature. Haemolymph pH was measured with a flow-through micro pH-probe (Microelectrodes, MI16-705), connected to the PHM73 also at ambient temperature, which remained effectively constant throughout (25±2°C). Changes in P_{O_2} of the Tucker chamber were timed with a stopwatch until a linear rate of change was recorded and the P_{O_2} then interpolated to injection time (i.e. time 0). Haemolymph CO_2 content ($[CO_2]$) was measured with a P_{CO_2} electrode (model E5037/SI) connected to a PHM73 pH/blood gas monitor using a Cameron chamber also at 32°C (Cameron, 1971) and calibrated with fresh 15 mmol l^{-1} $NaHCO_3$ standards. The changes in P_{CO_2} were recorded until a linear rate change was achieved and interpolated to injection time. A sample of the haemolymph was frozen and saved for analysis of metabolites and selected ions. Samples for Mg^{2+} analysis were prepared as for Ca^{2+} (above), as were samples for Na^+ and K^+ analysis, except they were diluted in CsCl. The Cl^- concentration was measured using a CMT10 titrator (Radiometer) calibrated with 100 mmol l^{-1} NaCl.

Muscle samples were also obtained for measurement of metabolites. A different group of blue crabs was used for tissue sampling. The crabs were encouraged to autotomise the penultimate walking leg, and the muscle tissue (0.3 g from the merus) was extracted from either FR crabs (N=8) or crabs exercised for 5 min (N=8). The muscle tissue was immediately deposited into a pre-weighed tube with 2 ml of ice-cold $HClO_4$ (0.6 mol l^{-1}) to deproteinise the sample. The vials with the muscle tissue were weighed and then homogenised with an OMNI 1000 homogeniser (Marietta, GA, USA) and frozen until further processing as described previously (Adamczewska and Morris, 1996, 1998a, 2001a). The muscle tissue was analysed for L-lactate (using Boehringer test kit no. 138 084) as well as glucose by methods described by Bergmeyer (1985). The concentrations of metabolites in tissues were expressed in mmol kg^{-1} wet tissue mass. The muscle and haemolymph samples were air-freighted to the laboratory at –40°C in dry ice.

Data analysis

All data, except the determinations of L-lactate and glucose in the haemolymph during post-exercise recovery, were independent with regard to treatment (e.g. exercise *vs* resting) and were analysed for treatment effects by analysis of variance (ANOVA). Data sets containing means with heterogenous variance (Bartletts' and Levene's tests) were log or square-root transformed before analysis. *Post-hoc* testing was by Tukey's HSD test. The changes in L-lactate and glucose concentration of the haemolymph following exercise were analysed using a one-way repeated measures design. Pulmonary, arterial and venous haemolymph samples were not completely independent within treatments since they were taken from each crab, and thus comparisons between these values were made using serial Friedman's two-factor ANOVA for ranked related samples (non-independent). This is comparable to a one-factor ANOVA and was verified using Minitab 14 in addition to Systat, which was employed for the other analyses. In all cases, P<0.05 was taken as significant. Values are presented as means ± S.E.M.

Results

Laboratory exercise studies – haemolymph gas transport and acid–base status

In the laboratory trials crabs exercised in 90% RH walked 38% faster (2.48±0.43 m min^{-1}) than the 1.80±0.29 m min^{-1} achieved by those at 40% RH (t-test, P=0.018, N=12 at each humidity). The P_{O_2} of pulmonary haemolymph (Pp_{O_2}) decreased from 12.8±0.6 kPa in crabs at rest to 5.2±1.1 and 2.5±0.2 kPa after 5 min of exercise at 40% or 90% RH, respectively (Table 1). The decline in Pp_{O_2} was greater for crabs exercised at 90% RH than for those at 40% RH. The P_{O_2} of pulmonary and arterial haemolymph was similar in the exercised crabs, but in crabs at rest the Pp_{O_2} was higher than the Pa_{O_2} by 3.8 kPa. Despite the fluctuations in the P_{O_2} of the arterial and pulmonary haemolymph, the venous P_{O_2} was low and similar in all three groups (1.05±0.12 to 1.17±0.09 kPa; Table 1). $[O_2]$ in arterial and pulmonary haemolymph was generally similar in resting crabs and in crabs exercised for 5 min (Table 1). However, while the $[O_2]_p$ of crabs at rest (0.99 mmol l^{-1}) and after 5 min of exercise at 40% RH (0.94 mmol l^{-1}) were similar, that in crabs exercised for 5 min at 90% RH was significantly greater at 1.28±0.13 mmol l^{-1} (Table 1). This difference was not reflected in haemocyanin (Hc) O_2 saturation and was due to an unusually high [Hc]. The mean $[O_2]_v$ at 0.41±0.06 mmol l^{-1} was significantly lower than the $[O_2]_a$ and $[O_2]_p$ and was similar for all three treatment groups. Despite the fluctuations in $[O_2]_p$, the Hc saturation was high (at least 87%; Table 1). The saturation of the Hc in venous haemolymph ranged from 45% in crabs at rest to 28% in the 90% RH group, which was significantly lower than that of both rested crabs and those exercised at 40% RH (Table 1).

The effects of exercise at 90% RH on haemolymph CO_2 and pH were more extreme than those at 40% RH (ANOVA; Table 1). $[CO_2]$ in the haemolymph was similar in the pulmonary, arterial and venous haemolymph within each exercise regimen (Table 1). The haemolymph $[CO_2]$ of crabs after 5 min of exercise (e.g. $[CO_2]_a$ 12.51 and 12.79 mmol l^{-1} at 40% and 90% RH, respectively) was significantly lower than in the crabs at rest (e.g. $[CO_2]_a$ 17.49 mmol l^{-1}). By contrast, the haemolymph P_{CO_2} of exercised crabs (e.g. Pv_{CO_2} 2.24 and 2.69 kPa at 40% and 90% RH, respectively) was higher than that of crabs at rest (e.g. Pv_{CO_2}=1.56 kPa; Table 1). The pH of the haemolymph was similar in pulmonary, arterial and venous haemolymph of crabs within each exercise regimen (Table 1). However, the pH of the haemolymph in crabs exercised for 5 min was significantly lower in exercised crabs (pH_v=7.35) than in those at rest (pH_v=7.59). This relative acidosis was

more pronounced in crabs exercised in 90% RH (pH_a=7.40 *vs* pH_a=7.49; Table 1).

Osmolality and metabolites in the haemolymph

Osmolality (OP) was approximately 70 mOsm greater in the laboratory crabs compared with the free-ranging *D. hirtipes* on Christmas Island (below), although haemolymph Ca^{2+} levels were lower. Exercise promoted an increase in OP from 574 to 677 mOsm after 5 min exercise at 90% RH, while the increase in OP after exercise at 40% RH was considerably smaller (Table 2). The increase in OP was reflected in a 5.1-fold increase in Ca^{2+} concentration in the haemolymph of blue crabs after 5 min exercise at 90% RH but there was no significant increase during exercise at 40% RH (Table 2). There was no detectable change in haemolymph urate concentration (0.04–0.10 mmol l^{-1}), which was within the range for field data (below), consequent to exercise in blue crabs, nor in the concentration of glucose (0.35–0.60 mmol l^{-1}). Exercise had a pronounced effect on haemolymph L-lactate concentration, which was 0.24 mmol l^{-1} in resting laboratory animals but increased to 3.68 and 5.41 mmol l^{-1} after 5 min exercise at 90% and 40% RH, respectively.

Recovery from exercise at 90% RH

Subsequent to 5 min exercise at 90% RH, L-lactate in the haemolymph continued to accumulate for at least the next 30 min and exceeded 4 mmol l^{-1} (Fig. 1). Haemolymph L-lactate was then progressively cleared and although after 5 h recovery the mean L-lactate concentration was still 1.9 mmol l^{-1}, this was no longer statistically elevated compared with values from resting crabs. L-lactate declined to 0.19 mmol l^{-1} after 24 h recovery. Haemolymph glucose varied in the haemolymph of post-exercised *D. hirtipes* differently to L-lactate (Fig. 1). The haemolymph glucose increased from 0.35 mmol l^{-1} in resting crabs for at least 2 h after exercise to

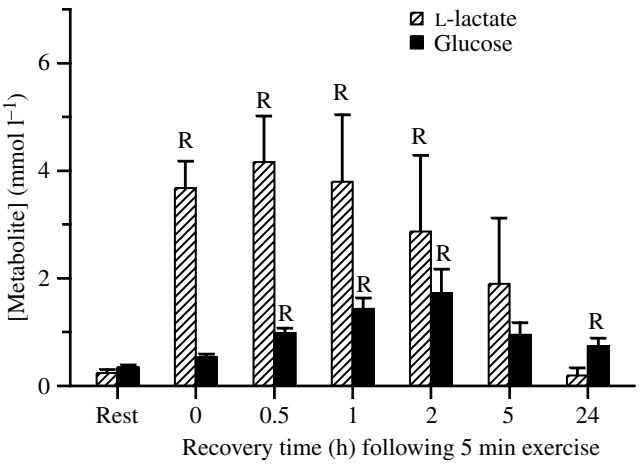

Fig. 1. The concentration of L-lactate and glucose in the haemolymph during 24 h recovery from 5 min of exercise at 90% relative humidity in the laboratory. R, significant difference between that sample group and crabs at rest (P<0.05, N=8). Time 0 is time immediately after exercise.

Table 1. *Haemolymph respiratory and acid–base status of laboratory acclimated* Discoplax hirtipes *at rest or after 5 min of exercise at either 40% or 90% relative humidity (RH)*

	P_{O_2} (kPa)			$[O_2]$ (mmol l^{-1})			% Hc O_2 saturation		
	Pulmonary	Arterial	Venous	Pulmonary	Arterial	Venous	Pulmonary	Arterial	Venous
Resting	12.75±0.64	8.88±1.67^{p}	1.05±0.12^{a,p}	0.99±0.12	0.87±0.12	0.46±0.07^{a}	96.65±6.54	83.58±4.61	44.80±4.21^{a,p}
Exercised at 40% RH	5.24±1.13†	4.73±1.26†	1.17±0.09^{a,p}	0.94±0.18	0.78±0.10	0.40±0.06^{a}	89.03±5.01	79.28±6.92	39.64±2.36^{a,p}
Exercised at 90% RH	2.49±0.22*,†	3.62±0.59†	1.12±0.13^{a,p}	1.28±0.13	1.20±0.08*,†	0.39±0.05^{a,p}	87.21±5.03	83.87±4.10	28.48±4.95^{a,p,*,†}

	P_{CO_2} (kPa)			$[CO_2]$ (mmol l^{-1})			pH		
	Pulmonary	Arterial	Venous	Pulmonary	Arterial	Venous	Pulmonary	Arterial	Venous
Resting	1.70±0.09	1.37±0.17	1.56±0.10	16.47±1.89	17.49±1.90	17.34±1.81	7.63±0.04	7.60±0.04	7.59±0.04
Exercised at 40% RH	1.76±0.18	2.10±0.15†	2.24±0.16†	13.11±1.43	12.51±1.53†	13.86±1.15	7.49±0.02†	7.45±0.03†	7.35±0.04†
Exercised at 90% RH	2.14±0.22†	2.34±0.22†	2.69±0.19*,†	12.20±0.81†	12.79±0.73†	11.89±0.62†	7.40±0.03*,†	7.40±0.03†	7.35±0.03†

There were no significant differences between pulmonary and arterial samples. Significant differences compared with arterial samples are indicated by an 'a', while differences compared with pulmonary are indicated by a 'p' above the venous sample for each treatment group. * indicates a difference in values of crabs exercised at 90% compared with those at 40% RH. † indicates a difference between crabs exercised at the respective humidity and those at rest. Differences between treatments were assessed by parametric analysis (ANOVA) with *post-hoc* testing (Tukey's HSD pair-wise comparison), while differences within treatments (i.e. haemolymph type) were assessed using Friedman's two-factor ANOVA for related samples, a non-parametric equivalent to one-factor ANOVA. N=8 for each treatment; total N=64.

Table 2. *Osmolality (OP), calcium, urate, L-lactate and glucose concentration in the haemolymph of* D. hirtipes *during various exercise regimes carried out in the laboratory*

		OP (mOsm)	Ca^{2+} (mmol l^{-1})	Urate (mmol l^{-1})	L-lactate (mmol l^{-1})	Glucose (mmol l^{-1})
Resting	90% RH	573.6±6.8	3.11±0.67	0.04±0.005	0.24±0.06	0.35±0.04
5 min exercise	40% RH	601.5±15*,†	4.94±0.91†	0.101±0.048	5.41±0.70*,†	0.6±0.31
	90% RH	676.9±4.9*	15.82±0.54*	0.069±0.021	3.68±0.49*	0.53±0.06

Exercise for 5 min under either 40% or 90% relative humidity (RH) and resting values at 90% RH are compared. Values are means ± S.E.M.
*, different from resting value; †, different from 90% RH exercised value.

1.73 mmol l^{-1}, and this hyperglycaemia persisted even after 24 h recovery, at which time the concentration was still 0.74 mmol l^{-1} (Fig. 1).

In situ assessment of exercise in D. hirtipes *on Christmas Island*

In each of the sample groups, the P_{O_2} in the arterial and pulmonary haemolymph was similar but decreased significantly in the venous samples (Table 3). In June (dry), the Pp_{O_2} of exercised crabs (6.5±1.8 kPa) was significantly lower than in the FR crabs (12.0±2.8 kPa). By contrast, during February (wet), the mean Pp_{O_2} of FR and the exercised crabs was similar (combined mean 5.5±1.2 kPa; Table 3).

Like the P_{O_2}, $[O_2]$ in the haemolymph was similar in pulmonary and arterial haemolymph (Table 3). However, while the $[O_2]_p$ of FR and exercised crabs was similar in June, in February (wet) the $[O_2]_p$ of exercised crabs (0.66±0.04 mmol l^{-1}) was lower than in the FR (migrating) crabs (1.00±0.15 mmol l^{-1}). The venous $[O_2]$ ranged from 0.26 to 0.36 mmol l^{-1} and was not significantly different among any of the groups (Table 3).

Despite the differences in P_{O_2} and $[O_2]$ between some treatments, the Hc was always well saturated at the gas exchange surfaces in all sample groups (pulmonary Hc O_2>95%) but decreased to below 30% in the venous haemolymph of crabs exercised in the dry season (Table 3).

There was no difference in $[CO_2]$ between the pulmonary, arterial and venous haemolymph within any of the sample groups (Table 3). While the mean haemolymph $[CO_2]$ in crabs sampled in June was similar in FR and exercised crabs (e.g. $[CO_2]_v$=16.70±0.90 and 16.60±1.02 mmol l^{-1} respectively), during the February wet season the $[CO_2]$ of exercised crabs (e.g. $[CO_2]_v$=11.38±0.57 mmol l^{-1}) was significantly lower than in FR crabs ($[CO_2]_v$=17.28±1.86 mmol l^{-1}).

The pH of pulmonary, arterial and venous haemolymph was similar within each of the sample groups, but exercise induced a haemolymph acidosis (Table 3). The haemolymph of crabs sampled in June (dry) had a pH_v of 7.60±0.03, but after 5 min of exercise this decreased to pH_v 7.26±0.08. Similarly, in crabs sampled in February (wet), the haemolymph pH_v of 7.45±0.03, for example, decreased to an even lower value of 6.99±0.04. While the relative acidosis induced by 5 min enforced walking was similar in both seasons, the initial haemolymph pH of FR crabs sampled in February was significantly lower than that of the FR crabs sampled in June (Table 3).

Haemolymph and tissue metabolites

The concentration of glucose in the haemolymph of FR crabs sampled in February (wet season; 0.22±0.03 mmol l^{-1}) was almost twice that of FR crabs sampled in June (dry; 0.13±0.02 mmol l^{-1}; Table 4). Likewise, the increase in haemolymph glucose after 5 min of exercise in February (0.14 mmol l^{-1}) was twice that measured in June (Table 4). While the glucose concentration in the muscle of FR crabs was comparable with the concentrations in the haemolymph, after 5 min of exercise the increase in muscle [glucose] was much greater at 1.1 mmol kg^{-1} in June and 0.7 mmol kg^{-1} in February (Table 4).

The concentration of urate in the haemolymph was similar in both seasons (0.075–0.121 mmol l^{-1}) and did not change after 5 min of exercise (Table 5). Haemolymph L-lactate was similar in the two sampling seasons and increased from a mean of 0.76±0.17 mmol l^{-1} in FR crabs to a mean of 9.2±1.2 mmol l^{-1} after 5 min of exercise (Table 4). The concentration of L-lactate in the muscle tissue was consistently higher than in the haemolymph and increased from a mean (combining both dry and wet season data) of 2.3±0.81 mmol kg^{-1} in resting crabs to a mean of 13.9±2.5 mmol kg^{-1} after 5 min of exercise (Table 4).

Osmotic and salt balance

There was no difference between the haemolymph OP of *D. hirtipes* sampled in the dry season and those sampled in the wet season (Table 5). Exercising blue crabs for 5 min promoted an increase in haemolymph osmolality of 73.1 mOsm in June and 58.8 mOsm in February (Table 5). Similarly, there was no seasonal variation in haemolymph Ca^{2+} concentration, but the 5 min exercise promoted a 40–45% increase in circulating Ca^{2+} levels (Table 5). During the June sampling season, exercised *D. hirtipes*, compared with FR crabs, showed no change in Na^+ and Cl^-, but Mg^{2+} and K^+ were increased by 31% and 22%, respectively (Table 5).

Discussion

The behaviour of *Discoplax hirtipes* is an important determining component in the extent of walking exercise, both in the laboratory at differing relative humidity and during different seasons. The varying commitment that the crabs make to walking and thus on metabolic demand is manifest in the respiratory, acid–base and metabolite status of exercised and naturally migrating crabs.

Respiratory competence of the lungs in supporting exercise

Afferent systemic haemolymph O_2 saturation was not a simple limitation to exercise in *D. hirtipes.* In the laboratory, the Pp_{O_2} and Pa_{O_2} values in the haemolymph in resting *D. hirtipes* were similar to previous *in situ* values (Adamczewska and Morris, 1996) and to those in quiescent crabs in the field during the dry season. The haemolymph of *D. hirtipes* has a high O_2-carrying capacity (~1 mmol l^{-1}) and is normally well saturated leaving the lungs (Farrelly and Greenaway, 1994; Adamczewska and Morris, 1996). The Hc in *D. hirtipes* has a very high affinity for O_2 (P_{50}=0.62 kPa at pH 7.8 and 25°C; Dela-Cruz and Morris, 1997a) and saturates at P_{O_2} values well below those in resting crabs.

The index L_{diff} (Piiper, 1982; Taylor and Taylor, 1992) assesses diffusion limitation to gas exchange and, for terrestrial crabs, is generally between 0.4 and 0.5 (Innes and Taylor, 1986, Taylor and Taylor, 1992; Adamczewska and Morris, 1998). *D. hirtipes* resting in the laboratory or quiescent in the field showed typical L_{diff} values of 0.41±0.03 and 0.44±0.14, respectively, characteristic of an air-breather (Morris and Dela-Cruz, 1998).

The relative haemolymph flow through the lungs of resting *D. hirtipes* was 82.1%, with only 17.9% through the gills (S.M., unpublished observation) as determined using an injected radiolabelled micro-sphere method (e.g. Taylor and Greenaway, 1984). The gills may be important in the excretion of CO_2 (Farrelly and Greenaway, 1994) but this could not be substantiated (see also Dela-Cruz and Morris, 1997a). The only significant (Friedmans test) decrease in haemolymph [CO_2] was during lung transit (–0.87±0.36 mmol l^{-1}), which supports the contrary suggestion that CO_2 excretion is over the lungs. The [CO_2] in the haemolymph of resting *D. hirtipes* was between 16.5 and 17.3 mmol l^{-1} and was very similar to both previous laboratory (Farrelly and Greenaway, 1994) and field values (Adamczewska and Morris, 1996). Similarly, the P_{CO_2} and pH values of the two resting groups of *D. hirtipes* (laboratory and dry season field crabs) were similar to those in previous *in situ* studies (Adamczewska and Morris, 1996).

The influence of humidity on exercise in the laboratory

Rather than low humidity exacerbating the demands of exercise on respiration, increasing the humidity encouraged the crabs to a greater commitment to walking. In the laboratory, exercised *D. hirtipes* exhibited pronounced reductions in the haemolymph P_{O_2}, especially in the Pp_{O_2}, which was reduced by 59% and 80% in crabs exercised at 40% RH and 90% RH, respectively. These decreases are consistent with a partial failure of the lungs to oxygenate the haemolymph, especially in crabs exercising in air at 90% RH. The L_{diff} for animals exercised at 40% RH increased to 0.77±0.07, and at 90% RH to 0.93±0.02, indicating severe diffusion limitation in

Table 3. *Haemolymph respiratory and acid–base status of* Discoplax hirtipes *sampled on Christmas Island during June (dry season) or February (wet season)*

	P_{O_2} (kPa)			[O_2] (mmol l^{-1})			% Hc O_2 saturation		
	Pulmonary	Arterial	Venous	Pulmonary	Arterial	Venous	Pulmonary	Arterial	Venous
June – FR	12.00±2.79	10.26±2.69	0.89±0.05^{a,p}	0.92±0.09	0.86±0.13	0.36±0.08^{a,p}	100.9±8.6	92.2±11.3	44.9±9.5^{a,p}
June – exercised	6.51±1.84†	5.44±1.83†	1.76±0.24^{†,p}	1.10±0.13	1.01±0.15	0.33±0.04^{a,p}	99.3±11.5	89.4±7.3	29.7±2.3^{†,a,p}
February – FR	5.75±1.65*	3.93±1.55*	0.73±0.08^{a,p}	1.00±0.15	0.91±0.08	0.36±0.07^{a,p}	105.3±3.0	101.0±7.1	39.4±4.0^{a,p}
Feb – exercised	5.32±0.80	4.10±0.61	0.96±0.23*^{,a,p}	0.66±0.04*,†	0.68±0.03*,†	0.26±0.05^{a,p}	95.3±11.5	99.2±10.7	41.2±11.4^{a,p}

	[CO_2] (mmol l^{-1})			pH		
	Pulmonary	Arterial	Venous	Pulmonary	Arterial	Venous
June – FR	15.17±1.88	16.26±1.57	16.70±0.90	7.60±0.03	7.62±0.02	7.60±0.03
June – exercised	14.64±1.21	15.25±1.33	16.60±1.02	7.30±0.07†	7.31±0.08†	7.26±0.08†
February – FR	17.34±1.82	16.14±1.81	17.28±1.86	7.46±0.02*	7.48±0.03*	7.45±0.03*
Feb – exercised	11.02±0.66*,†	11.18±0.82*,†	11.38±0.57*,†	7.09±0.07*,†	7.11±0.07*,†	6.99±0.04*,†

Pulmonary, arterial and venous haemolymph samples were taken from free-ranging crabs (FR) or crabs exercised for 5 min. There were no differences between pulmonary and arterial haemolymph in any of the parameters measured. Significant differences between venous and arterial samples are indicated by an 'a', while differences between venous and pulmonary are indicated by a 'p' above the venous sample for each treatment group. † indicates a significant difference between FR and exercised crabs within each season (June or February). * indicates a significant difference between seasons (June *vs* February) for either exercised crabs or for rested crabs. Differences between treatments were assessed by parametric analysis (ANOVA) with *post-hoc* testing (Tukey's HSD pair-wise comparison), while differences within treatments (i.e. haemolymph type) were assessed using Friedman's two-factor ANOVA for related samples, a non-parametric equivalent to one-factor ANOVA. N=8 for each treatment; total N=64.

Table 4. *Haemolymph and leg muscle tissue concentration of glucose and L-lactate in* D. hirtipes *sampled on Christmas Island during June (dry season) or February (wet season)*

		[Glucose]		[L-lactate]	
		Haemolymph (mmol l^{-1})	Leg muscle (mmol kg^{-1})	Haemolymph (mmol l^{-1})	Leg muscle (mmol kg^{-1})
June (dry)	FR	0.13±0.02	0.12±0.03	0.84±0.14	2.09±0.65
	Exercised	0.20±0.02†	1.20±0.29†	9.47±1.64†	14.57±3.50†
February (wet)	FR	0.22±0.03*	0.16±0.03	0.68±0.20	2.42±0.96
	Exercised	0.35±0.07*,†	0.82±0.08†	8.97±0.74†	13.3±2.5†

Haemolymph and leg muscle were taken from free-ranging crabs (FR) or crabs exercised for 5 min. † indicates a significant difference between FR and exercised crabs within each season (June or February). * indicates a significant overall difference between seasons (June *vs* February) for either FR or exercised treatment groups. Two-factor ANOVA; N=8 for each treatment; total N=64.

the crabs exercising under humid conditions. In exercising *G. natalis*, L_{diff} increased from 0.53 to 0.77 (Adamczewska and Morris, 1998) and in exercised *Potamonautes warreni* from 0.57 to 0.77 (Adamczewska et al., 1997). If L_{diff} increases in *D. hirtipes* during severe exercise then this correlates with a 38% faster walking speed in crabs under 90% compared with 40% RH. This large effect of laboratory exercise in lowering haemolymph P_{O_2} was not reflected in the oxygen content of *D. hirtipes* haemolymph, which remained generally high (the increase in $[O_2]$ in crabs exercised at 90% RH was due to unusually high [Hc]). The venous Hc O_2 saturation was significantly lower in the crabs exercised at 90% RH compared with both controls and those exercised at 40% RH. This relative lowering of venous Hc oxygenation is consistent with comparatively greater respiratory demand in that group, consequent on their faster locomotion.

The increased haemolymph calcium in both laboratory and field crabs may explain how Hc-O_2 saturation remained high despite the internal hypoxia. The O_2 affinity of *D. hirtipes* Hc is significantly improved by increased haemolymph Ca ($\Delta\log P_{50}/\Delta\log[Ca^{2+}]$=–0.45 at pH 7.4) but is not sensitive to L-lactate (Dela-Cruz and Morris, 1997a). By employing the Bohr coefficient for the pH sensitivity of O_2 binding by *D. hirtipes* Hc (θ=–0.57; Dela-Cruz and Morris, 1997a), the P_{50} of *D. hirtipes* Hc in laboratory exercised crabs at 90% RH would be 1.06 kPa. Incorporating the effect of increased Ca^{2+} reduced the P_{50} to 0.51 kPa in *D. hirtipes* exercised at 90% RH, which is an improvement of the Hc affinity for O_2 by 52%. In crabs exercised at 40% RH the Ca^{2+} increase was less, thus the potentiation of Hc O_2 affinity was proportionately less and correlated with the less severe internal hypoxia.

In *Cardisoma guanhumi*, fatigue set in at a walking speed of 3 m min^{-1} (Herreid et al., 1979), which was only slightly faster than 2.48 m min^{-1} by *D. hirtipes* at 90% RH and was thus likely close to the maximum speed for *D. hirtipes*. While haemolymph L-lactate increased after 5 min exercise in the laboratory, 3–5 mmol l^{-1} is not high for land crabs (e.g. Greenaway et al., 1988; Adamczewska and Morris, 1994, 1998). Short-term, vigorous exercise affected urate oxidase and increased haemolymph urate in *G. natalis* (Adamczewska and Morris, 1998) but in *D. hirtipes* 5 min laboratory exercise was insufficient to alter [$urate^-$]. Furthermore, there was no significant hypergylcaemia until after the exercise demand had ceased.

In crustaceans, low rates of lactate reoxidation (0.8–2.6 mmol l^{-1} h^{-1}) are the norm (Wood and Randall, 1981b; Forster et al., 1989; Henry et al., 1994; Adamczewska and Morris, 1998) but the rate of 0.49 mmol l^{-1} h^{-1} in *D. hirtipes* exercised in the laboratory was unusually slow, although Henry et al. (1994) reached a similar conclusion for

Table 5. *The osmolality (OP) and the concentration of Ca^{2+}, Na^+, Cl^-, Mg^{2+}, K^+ and urate in the haemolymph of* D. hirtipes *sampled on Christmas Island*

		OP (mOsm)	Concentration (mmol l^{-1})					
			Ca^{2+}	Na^+	Cl^-	Mg^{2+}	K^+	Urate
June (dry)	Free ranging	501.1±22.0	5.0±0.4	264.4±6.0	264.8±6.9	3.8±0.5	5.2±0.1	0.093±0.011
	5 min exercise	574.2±46.2*	9.1±1.2*	284.9±13.9	276.0±8.0	4.9±0.4*	7.5±0.3*	0.075±0.012
February (wet)	Free ranging	518.8±13.3	6.2±0.7	N/A	N/A	N/A	N/A	0.121±0.027
	5 min exercise	577.6*	10.6*	N/A	N/A	N/A	N/A	0.115±0.018

The OP, Ca^{2+} and urate were measured for crabs sampled during the dry season of 1994 or during the wet season, while Na^+, Cl^-, Mg^{2+} and K^+ were determined for crabs sampled in the dry season. The crabs were sampled either during their normal behaviour or after 5 min exercise (N>8). Values are means ± S.E.M.

*Significant effect of exercise.

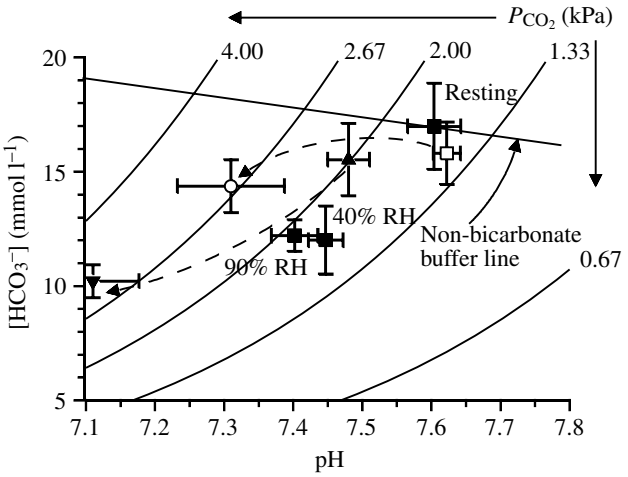

Fig. 2. The pH/[HCO_3^-] diagram for acid–base variables measured in the arterial haemolymph of *Discoplax hirtipes* sampled in the field during the dry season (open square, free-ranging; open circle, 5 min exercise) and the wet, migration, season (triangles, free-ranging; inverted triangles, 5 min exercise), compared with laboratory values for resting blue crabs and those exercised for 5 min at either 40% or 90% relative humidity (filled squares). The acid–base perturbations after 5 min of exercise are indicated by the broken lines with arrowheads (without implying acid–base state at times within the 5 min exercise period). The non-bicarbonate buffer line is provided (Dela-Cruz and Morris, 1997a). The P_{CO_2} isopleths were calculated according to the Henderson–Hasselbalch equation using αCO_2= 0.3068 μmol l^{-1} Pa^{-1} and pK_a=6.08.

C. guanhumi. Thus, in the long term, migrating *D. hirtipes* would be severely constrained to within the MAS. In *D. hirtipes*, mobilised glucose accumulates in the haemolymph while lactate is being re-oxidised. The primary site for glucose mobilisation in land crabs appears to be the leg muscles (Morris and Adamczewska, 2002). The simplest explanation is that, subsequent to short-term exercise, glucose continues to flux from the muscle of *D. hirtipes* under conditions where O_2 supply is restored and L-lactate can slowly re-enter into the respiratory pathway, obviating the need for elevated glycolysis.

The consequences of exercise were obvious in the haemolymph CO_2 status and associated acid-base state. The general consequence of 5 min exercise by *D. hirtipes* in the laboratory was decreased [CO_2] and pH together with increased P_{CO_2}, which, with the aid of the Henderson–Hasselbalch diagram (Fig. 2), was seen to include a large metabolic (lactate) acidosis.

These data are also consistent with different work loads (walking speeds) between the groups and/or differential lactate and H^+ efflux rates. Assuming that the L-lactate efflux into the haemolymph was accompanied by a stoichiometric efflux of H^+, the haemolymph pH due to metabolic acid [$pH_{a(m)}$]could be predicted for each crab (for method, see Greenaway et al., 1988; as derived from Wood et al., 1977). The haemolymph of crabs exercised at 90% RH was pH_a=7.40, which was not different to the pH_a=7.45 in those crabs exercised at 40% RH (Table 1; Fig. 2). The corresponding increases in haemolymph lactate were 3.44 and 5.17 mmol l^{-1}, respectively, which provided a statistically unchanged pH_a=7.48 for the crabs in 40% RH but a higher pH_a=7.51±0.02 in those crabs exercised in 90% RH (paired *t*-test, *T*=–4.63, *P*=0.002). Thus, in *D. hirtipes* exercised at 90% RH, there appears to be a preferential retention of L-lactate over H^+ by the tissues, perhaps as a consequence of the severity and brevity of the exercise period (see table 2 in Adamczewska et al., 1997). When land crabs were exercised to exhaustion, the L-lactate efflux exceeded that of H^+ (e.g. Greenaway et al., 1988; Adamczewska and Morris, 1994b) whereas *G. natalis* under non-exhausting conditions (Adamczewska and Morris, 1998) exhibit values similar to those from *D. hirtipes*.

Behavioural responses to humidity are paramount since the differences between the *D. hirtipes* exercised at 40% and 90% RH were not directly debilitating effects of low humidity but rather are due to an increased proclivity to exercise at higher humidity. The increased commitment to walking in 90% RH accounts for the relative internal hypoxia as well for the deeper hypercapnic lactacidosis.

Exercise in the field and relevance to the seasonal migration

The free-ranging *D. hirtipes* during the dry season were constrained in their distribution around freshwater seepages, were relatively inactive and moved only short distances (Hicks et al., 1990; Greenaway and Raghaven, 1998; S.M., unpublished radio-tracking data). Free-ranging *D. hirtipes* in the wet season were migrating and walked persistently but not continuously during the daylight hours. The establishment of the wet season is a requirement for the migration (Hicks et al., 1990; S.M., personal observations), and the resultant seasonal differences in the behaviour of free-ranging crabs correlate with changes in the haemolymph respiratory gas status. Thus, the relative humidity does not affect O_2 transport directly but instead determines the behaviour of the animal and thereby the demands that the crabs then make on their oxygen uptake and transport systems.

The haemolymph P_{O_2} of free-ranging crabs in June was not different from that measured in crabs at rest in the laboratory, implying that the *D. hirtipes* were indeed quiescent in the field during the dry season. However, in the wet season, the P_{O_2} values of migrating crabs declined markedly and were similar to those of exercised crabs in the laboratory. Thus, while L_{diff} was 0.44 in quiescent dry season crabs, the uptake of O_2 was more severely diffusion limited in the migrating *D. hirtipes* (L_{diff}=0.75). Superimposing 5 min exercise on the quiescent dry season animals further reduced their haemolymph P_{O_2} and increased L_{diff} to 0.95, as in the laboratory exercised crabs. In the wet season animals that were migrating, the extra 5 min exercise load failed to elicit any further reduction in P_{O_2}, and L_{diff} remained at 0.78.

The low P_{O_2} of the haemolymph in naturally migrating crabs was again not manifested in low O_2 content because of the high affinity of the Hc for O_2. The apparent decreased O_2 content in crabs exercised during February was due to relatively low

[Hc] since the % saturation was unaffected (Table 3). In June (dry season), subsequent to the 5 min exercise, the increase in Ca^{2+} content would have decreased the P_{50} (increased O_2 affinity) from 1.21 to 0.91 kPa, and in the migrating wet season crabs from 1.60 to 1.18 kPa. Actively foraging *G. natalis* on Christmas Island in the wet season exhibited a relative haemolymph hypoxia (2.9 kPa) compared with the relatively inactive crabs in the dry season, which correlated with activity (Adamczewska and Morris, 2000a).

Migrating crabs in the wet season maintained low L-lactate levels, indistinguishable from those in June during the dry season. Therefore, the breeding migration of blue crabs is completely aerobic, and within the MAS. Migrating *G. natalis* also exhibited low and constant haemolymph lactate levels (Adamczewska and Morris, 2001b) and this may be a feature of gecarcinid land crabs. The elevated O_2 demand of migrating crabs (Feb) compared with those confined to around the freshwater springs in June is reflected in a relative hyperglycaemia typical of exercised crustaceans (England and Baldwin, 1983; Morris and Adamczewska, 1994b, 2002). In both June and February, the 5 min imposed exercise elevated haemolymph lactate to a similar extent, at least 9 mmol l^{-1}, and tissue [lactate] also increased similarly in both groups despite the obvious differences in the haemolymph P_{O_2} changes. The data are consistent with migrating *D. hirtipes* walking close to their aerobic limit during which time pulmonary P_{O_2} is maximally depressed, O_2 uptake diffusion limited, and the arterial–venous (a–v) P_{O_2} difference minimised (albeit with little effect on the a–v Hc O_2 difference). Extra exercise imposed a requirement that exceeds the aerobic scope and requires anaerobic supplementation.

As with the haemolymph oxygenation, the CO_2 and acid–base status of relatively inactive crabs during the dry season was similar to that of resting crabs in the laboratory (Fig. 2). Red crabs, *G. natalis*, on Christmas Island showed a seasonal respiratory acidosis such that actively foraging crabs in the wet season accumulated an extra 3 mmol l^{-1} of CO_2 and a decrease in pH of 0.13 compared with the relatively inactive crabs in the dry season (Adamczewska and Morris, 2002a). The migrating *D. hirtipes* were similarly acidotic compared with the quiescent dry-season animals due to a respiratory acidosis with no evidence of any metabolic component (Fig. 2). Consequently, requiring the dry season (June) animals to exercise elicited both a respiratory component to the ensuing acidosis (as exhibited by migrating crabs) and a metabolic component, as evidenced by the accumulated lactate. The fundamentally different basic respiratory state of the wet-season animals, close to their aerobic limit, compared with dry-season crabs meant that when the additional exercise was imposed on migrating blue crabs the acidosis was primarily metabolic in origin.

Adamczewska and Morris (1998) concluded that burst locomotion has significant disadvantages if it exceeds the MAS and if the crabs, like *D. hirtipes*, have slow rates of lactate re-oxidation (e.g. Henry et al., 1994; Morris and Adamczewska, 2002). Migrating *D. hirtipes* remain aerobic but exhibit a hypoxic respiratory acidosis compared with those in the dry season. Any extra energetic demand requires the recruitment of anaerobiosis and a debt that, in *D. hirtipes*, would require many hours to repay and impair subsequent exercise capacity. The best strategy for migrating blue crabs would appear similar to that of *G. natalis*, to walk continuously but not to exceed the MAS. During the dry season, extensive walking is resisted by either species. In *D. hirtipes*, the seasonal activity is inextricably linked to ion and water homeostasis, which modify the extent of activity which the animals can engage in and, thereby, the seasonal metabolic and respiratory demands. It is apparent from the laboratory trials and field observations that *D. hirtipes* responds to ambient humidity and limits, even under imposed locomotion, the commitment to exercise when at lower RH. This humidity sensitivity is integral to the seasonal behaviour and thus to the resulting routine metabolic demands in different seasons and to migration of the crabs.

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