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STRATEGIES FOR MIGRATION IN THE TERRESTRIAL CHRISTMAS ISLAND RED CRAB *GECARCOIDEA NATALIS*: INTERMITTENT *VERSUS* CONTINUOUS LOCOMOTION

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Summary

The terrestrial red crab *Gecarcoidea natalis* undertakes an annual breeding migration and must sustain locomotion for prolonged periods. The migrating crabs must travel a specific distance in a fixed time and can either walk at a constant speed or walk faster for short periods and then pause to feed or rest. To simulate the potential differences between continuous and intermittent locomotion during the migration, red crabs were sampled after walking at a voluntary speed for 5 or 20 min without pausing or after 20 min of enforced walking intermittently at approximately twice that speed. The respiratory and metabolic status of the crabs was investigated during the different exercise regimes to assess which strategy might be more advantageous during the migration.

The gills and lungs appeared to function similarly in gas exchange, and the P_{O_2} in the haemolymph was 8.2 kPa which fully saturated the haemocyanin with O_2. The uptake of O_2 by red crabs was diffusion-limited and the diffusion coefficient (L_{diff}) varied from 0.53 in resting crabs to 0.8 post-exercise. Post-exercise, red crabs experienced a mixed respiratory/metabolic acidosis which was greatest (0.2 pH units) in crabs walking intermittently, i.e. at a higher speed. Haemolymph L-lactate concentrations peaked at 5 mmol l^{-1} immediately post-exercise in the intermittent exercise group, whereas after 20 min of continuous exercise haemolymph L-lactate continued to increase, reaching a maximum of 2.5 mmol l^{-1} at 1 h post-exercise. L-Lactate recovered slowly to basal levels within 5 h. The maximum rate of L-lactate clearance from the haemolymph was only 1.75 mmol l^{-1} h^{-1}, and short pauses in exercise were insufficient for substantial L-lactate reoxidation. Exercise regimes in the laboratory were within the locomotor speeds determined for migrating red crabs, which overall have a mean walking speed close to their aerobic limit but periodically pause and also exceed this limit by three- to fourfold.

Key words: *Gecarcoidea natalis*, Christmas Island red crab, haemolymph, intermittent exercise, migration strategy.

Introduction

The terrestrial Christmas Island red crab *Gecarcoidea natalis* is remarkable for the mass breeding migration it must make each year. During this migration, the crabs may travel up to 5 km in as many days to reach the coast and must maintain locomotor activities for extended periods, up to 12 h each day (Hicks, 1985; Green, 1997; Adamczewska, 1997). The migration of the red crabs occurs at the end of the dry season and is triggered by the arrival of the monsoon rains, but the breeding activities are also synchronised with the lunar cycle. Thus, the red crabs must walk to the coast in time to synchronise their breeding with the lunar cycle and, consequently, must walk a pre-determined distance in a fixed period.

During the migration, a broad spectrum of locomotor strategies is possible, ranging from continuous locomotion during the daylight hours to intermittent locomotion during which these crabs walk as fast as possible, perhaps becoming fatigued, and then rest at intervals. Previous studies of exhausting exercise in *G. natalis* have shown this species to have a relatively low scope for increasing the level of aerobic exercise (Adamczewska and Morris, 1994*b*). Radio-tracking studies revealed that during the migration the mean walking speed of crabs is near the maximum aerobic speed (MAS), while field observations showed crabs to pause for minutes or even hours before engaging in periods of higher-speed walking (Adamczewska, 1997; A. M. Adamczewska and S. Morris, unpublished observations). It would thus appear that the locomotion of migrating *G. natalis* is a mixed strategy whereby some pauses are interspersed with periods of locomotion exceeding the MAS. While intermittent locomotion during the migration may be necessary, it might also be advantageous, for example by increasing distance

capacity (Weinstein and Full, 1992) and also by providing periods during which migrating crabs may feed to replenish or supplement their energy stores. The latter may be of significance since food can become a limiting resource while the island red crab population congregates near the shore for 2 weeks of breeding activity. However, if the speed required to compensate for pausing significantly exceeds the MAS, then there would presumably be metabolic disadvantages and costs as a consequence.

Several aspects of locomotion in crustaceans, such as metabolic costs, are relatively well studied (for reviews, see McMahon, 1981; Herreid and Full, 1988). Respiratory gas transport and acid–base perturbations during exercise have been examined in a range of terrestrial crustaceans (e.g. Cameron and Mecklenburg, 1973; Wheatly *et al.* 1986; Greenaway *et al.* 1992; Adamczewska and Morris, 1994*a*; Adamczewska *et al.* 1997). Land crabs possess both gills and lungs, which are variously used for respiratory gas exchange. Analysis of respiratory gases in the haemolymph provides important information regarding the gas exchange across the gills and/or lungs and on the diffusion limitations of oxygen moving into the tissues (e.g. Innes and Taylor, 1986*b*; Greenaway *et al.* 1988; McMahon and Burggren, 1988; Farrelly and Greenaway, 1994; Henry, 1994). During periods of increased O_2 demand, inadequacies of the gas exchange surfaces become apparent if oxygenation of the arterial/pulmonary haemolymph decreases while the external partial pressure of O_2 remains high (e.g. Innes and Taylor, 1986*b*; Taylor and Taylor, 1992). In addition to a normal Bohr shift (pH sensitivity), the respiratory pigment, haemocyanin, in crustaceans is variously sensitive to Ca^{2+}, L-lactate and urate, which may assist O_2 transport (for reviews, see Morris, 1991; Morris and Bridges, 1994). L-Lactate, urate and Ca^{2+} all influence the O_2 affinity of the haemocyanin in *G. natalis* (Adamczewska and Morris, 1998). In most crustaceans, a functional shortage of O_2 during locomotion is associated with some degree of anaerobiosis (for reviews, see Herreid and Full, 1988; Forster *et al.* 1989; Henry *et al.* 1994). In addition to creating an O_2 debt, anaerobiosis results in haemolymph acidosis, which is often exacerbated by a respiratory component (e.g. Wood and Randall, 1981*b*; Booth *et al.* 1984; Greenaway *et al.* 1988; Forster *et al.* 1989). Thus, crustaceans experience fatigue during exercise, and their distance capacity decreases with increased speed of locomotion (Wood and Randall, 1981*a*; Full and Herreid, 1984; van Aardt, 1990).

Most previous studies of locomotion in crustaceans have been aimed at determining the limitations on exercise capacity and have therefore used exhaustive exercise regimes (e.g. McMahon *et al.* 1979; Greenaway *et al.* 1988; Forster *et al.* 1989; Adamczewska and Morris, 1994*a*,*b*). Under natural conditions, exercise to near exhaustion may occur during predator avoidance but is unlikely to occur during routine foraging activities or during a migration. In fact, the few studies of crustaceans under natural conditions, in the field, have demonstrated that crabs usually move intermittently and walk at speeds that can be sustained aerobically (Full and Weinstein, 1992; Weinstein, 1995). Since intermittent locomotion increases distance capacity in at least some crustaceans (Weinstein and Full, 1992), the use of exercise regimes in which crabs are required to walk continuously at high speed may not be appropriate for assessing the distance capacities and limitations of animals under ecologically relevant conditions.

Not only is *G. natalis* an excellent crustacean model for addressing the physiology of locomotor strategies, but the ecology of red crabs, including their annual migration, is potentially dependent on their exercise capacity and the strategies employed to maximise this. The effects of continuous compared with intermittent walking, to achieve the same net distance in the same time, on respiratory gas transport and on the energetics of locomotion were investigated under controlled laboratory conditions to determine which of these locomotor strategies may be more advantageous during the migration of the red crabs.

Materials and methods

Protocol and exercise regimes

Male and female red crabs *Gecarcoidea natalis* (Pocock) with a body mass ranging from 114 to 232 g (190±6 g; mean ± S.E.M., N=50) were collected from Christmas Island and maintained in the laboratory as described previously (Adamczewska and Morris, 1994*a*). The carapace of red crabs was drilled a minimum of 24 h prior to experiments to allow haemolymph sampling from the pericardial cavity (700 µl, arterial haemolymph), from the efferent pulmonary vessel (300 µl, pulmonary haemolymph) (Farrelly and Greenaway, 1993) and directly from the venous sinus (800 µl, venous haemolymph). The puncture holes in the carapace were immediately resealed with vacuum grease to prevent blood loss, the entire process requiring less than 30 s. The samples were taken with 1 ml plastic syringes fitted with 21 gauge hypodermic needles which were chilled prior to collection and held on ice for the duration of haemolymph gas and acid–base analysis. Each crab was housed in an individual box with a continuous supply of humidified air and fresh drinking water for 24 h prior to experimentation. The effects of continuous or intermittent exercise on the respiratory gas and metabolic status of red crabs were examined. Red crabs were sampled either while resting (N=6) or after a period of exercise. Three different exercise regimes were used with a sample size of at least six in each case: (1) 5 min of exercise without pausing; (2) 20 min of exercise without pausing; and (3) 20 min of exercise with pauses for rest.

The crabs were exercised in a runway 2.5 m long, 20 cm wide and 20 cm high. A crab was placed at one end of the runway, and the far end of the runway was covered to provide a darkened retreat towards which the crab would walk. By alternately exposing and covering the opposite ends of the runway it was possible to encourage the crabs to walk almost

continuously. Therefore, the crabs were exercised for 5 or 20 min without pausing (continuous exercise) and they generally walked at their own chosen speed. The mean walking speeds were 1.9±0.12 m min^{-1} for the 5 min exercise (mean ± S.E.M., N=6) and 1.6±0.11 m min^{-1} (N=6) for the 20 min exercise. Gentle tactile stimulation was used infrequently and only if a crab stopped walking.

The 20 min of exercise with pausing (intermittent exercise) was organised on the basis of the speed used by the red crabs during the 20 min of continuous exercise, during which they travelled 32.5 m. Therefore, the crabs exercised intermittently were required to walk the same overall distance. The intermittent exercise regime was divided into six periods of 185 s and terminated in one period of 90 s. During each of the 185 s intervals, the crab was required to walk a distance of 5 m. During the last 90 s of the exercise period, each crab had to walk the remaining 2.5 m.

The difference between the continuous and intermittent exercise regimes was that the crabs exercised intermittently had to walk at a faster speed but were allowed to rest after walking a distance of 5 m. Each bout of exercise for the intermittent group consisted of stimulating a crab to walk up and down the runway by gentle tactile stimulation and allowing the crab to rest in a darkened retreat at the end of the runway for the remainder of the 185 s interval. Consequently, the faster a crab walked, the longer were the rest intervals. The mean time taken to walk the 5 m was approximately 85 s, but ranged from 70 to 110 s. During the last 90 s of exercise, every crab walked the remaining 2.5 m in less than 60 s and therefore had a minimum of 30 s rest before sampling. A crab sampled in one exercise regime was not used in any of the other treatment groups.

Haemolymph and tissue sampling and analysis

The haemolymph samples were analysed immediately for partial pressure and content of O_2 and CO_2, as well as blood pH, using a BMS 3 MK II Blood Micro System thermostatted 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. The CO_2 electrode was calibrated using two humidified gases, one with 0.5 % CO_2 and the other 2.5 % CO_2 (precision CIG certified analysis). The pH electrode was calibrated with Radiometer precision buffers of pH 7.410 (S1510) and 6.865 (S1500), accurate to ±0.005 pH units at 25 °C.

Blood oxygen contents (C_{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). The changes in the partial pressure of oxygen (P_{O_2}) were recorded on a pen recorder (Kipp and Zonen, model BD111). The haemolymph CO_2 content was measured using a Corning 965 CO_2 analyser (calibrated with HCO_3^- standard, 15 mmol l^{-1}).

The haemocyanin content of the haemolymph was measured by spectrophotometric scanning of a 10 µl blood sample in 1 ml of Milli-Q water. The peak absorbance near 338 nm was used to calculate the haemocyanin concentrations using the extinction coefficient $2.69E^{1\%}_{1cm}$ (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 0.6 mol l^{-1} $HClO_4$ to denature proteins and neutralised with 2.5 mol l^{-1} K_2CO_3. The denatured sample was centrifuged at 10 000 ***g*** for 10 min, and the supernatant was used for L-lactate analysis (Boehringer Mannheim test kit no. 138 084). Whole haemolymph samples were also analysed for glucose (Sigma Diagnostics test kit no. 510) and urate (Sigma Diagnostics test kit no. 685) concentrations.

Haemolymph osmotic pressure was measured using the Wescor 5100C vapour pressure osmometer. The osmometer was calibrated daily with two precision standards, 290 and 1000 mosmol kg^{-1} from Wescor, containing NaCl. The concentration of calcium in the haemolymph was measured using an atomic absorption spectrophotometer (GBC 906, Melbourne, Australia) using 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} $LaCl_3$ at a ratio of approximately 1:42.

The disappearance of L-lactate from the haemolymph was monitored for 24 h after exercise in the crabs exercised for 20 min by repetitive sampling (50 µl), using a 26 gauge needle inserted through the arthrodial membrane at the base of the walking legs.

To assess the metabolic status of muscle tissue, leg muscle samples were obtained by encouraging a crab to autotomise the second walking leg. The muscle tissue from the leg was immediately removed (approximately 0.3 g) and 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 (6 mm generator) and then with a glass homogeniser (Wheaton type) to obtain a finer homogenate. The homogenates were centrifuged in a Hereaus 17R centrifuge at 4 °C for 15 min at 5300 ***g*** and the supernatant was removed. The pellet was resuspended in 0.7 ml of 0.4 mol l^{-1} $HClO_4$ and centrifuged again. The second supernatant was removed, pooled with the first and the combined supernatant was neutralised with 0.8 ml of K_2CO_3 (3.75 mol l^{-1}). The solution was kept in an ice bath for 1 h, again centrifuged, and the final supernatant removed and used for analysis of L-lactate (Boehringer Mannheim test kit no. 138 084) and glucose as described by Bergmeyer (1985). Concentrations of metabolites in muscle tissue were expressed in mmol kg^{-1} wet tissue mass. Leg muscle samples were obtained from different crabs from those used for haemolymph samples.

Statistical analysis of haemolymph respiratory gas status

and of changes in haemolymph and tissue metabolite and ion concentrations in crabs at rest or exercised under the various exercise regimes was by one- or two-factor analysis of variance (ANOVA). Prior to ANOVA, the homogeneity of variances was confirmed using Bartlett's χ^2-test. *Post-hoc* testing was by Tukey's test or specific contrast analysis. The disappearance of L-lactate from the haemolymph after exercise was analysed by univariate repeated-measures ANOVA, and *post-hoc* testing was by 'C-MATRIX' contrast analysis (SYSTAT for Windows). Difference in the diffusion limitation coefficient L_{diff} were assessed by repeated-measures ANOVA and paired *t*-tests. The significance level was taken as P=0.05, and all data are presented as means ± S.E.M.

Results

Gas transport and acid–base status in the haemolymph

The oxygen contents (C_{O_2}) of arterial and pulmonary haemolymph were similar in resting crabs and in crabs exercised either continuously or intermittently. The mean C_{O_2} in arterial haemolymph (Ca_{O_2}) was 0.81±0.09 mmol l^{-1} but decreased significantly in venous haemolymph (Fig. 1A). The O_2 content in the venous haemolymph (Cv_{O_2}) of quiescent red crabs appeared to be higher (0.54±0.05 mmol l^{-1}) than in the exercised groups (0.38±0.01 mmol l^{-1}), although this difference was not statistically significant at the normal fiducial value ($F_{3,83}$=2.465, P=0.068). The haemocyanin was fully saturated with O_2 in pulmonary and arterial haemolymph (mean 100±3 %), but saturation decreased significantly in venous haemolymph to 81 % in quiescent crabs and only 44 % in crabs exercised intermittently for 20 min (Fig. 1B). The oxygenation status of the haemolymph did not differ statistically between the exercise regimes. Consistent with the C_{O_2} data, the mean partial pressure of O_2 (P_{O_2}) was similar in pulmonary (8.28±1.3 kPa) and arterial (8.24±0.94 kPa) haemolymph but decreased significantly in venous haemolymph to only 2.23±0.17 kPa (Fig. 1C).

The CO_2 contents (C_{CO_2}) were similar in the pulmonary, arterial and venous haemolymph within each exercise regime (Fig. 2A). The mean haemolymph C_{CO_2} was also very similar between the treatment groups and ranged from 13.9±0.8 mmol l^{-1} in the quiescent crabs to 12.1±1.0 mmol l^{-1} in crabs exercised for 5 min. Likewise the partial pressures of CO_2 (P_{CO_2}) were similar in the pulmonary, arterial and venous haemolymph within each treatment group (Fig. 2B). However, the mean haemolymph P_{CO_2} in crabs exercised intermittently for 20 min (1.90±0.15 kPa) was significantly greater than that in quiescent crabs (1.38±0.10 kPa) and in crabs exercised continuously for 20 min (1.37±0.13 kPa; Fig. 2B).

The pH values were similar in pulmonary, arterial and venous haemolymph within each exercise regime (Fig. 3). The haemolymph pH in each exercise group was significantly different from that in all other groups; thus, pH was greatly dependent on exercise and the exercise regime. The mean haemolymph pH decreased with an increase in the duration of exercise from 7.62±0.03 in quiescent crabs to 7.48±0.03

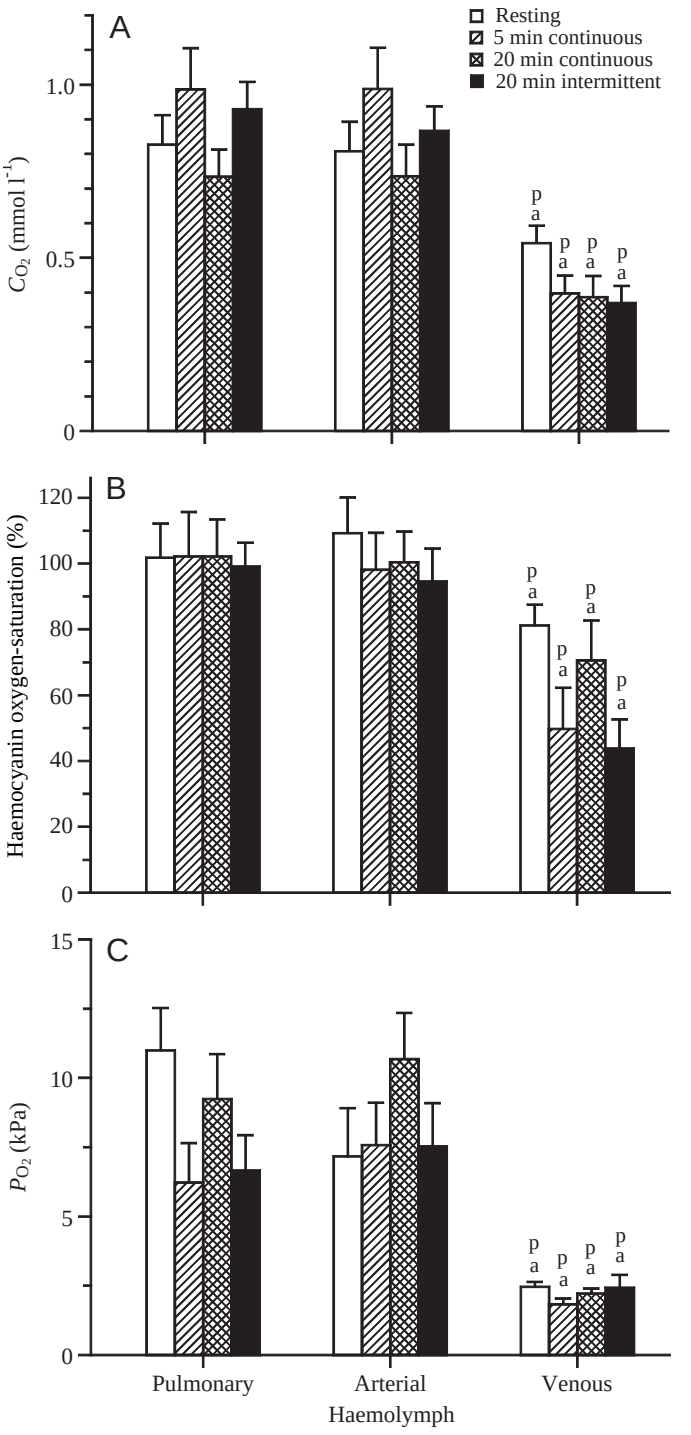

Fig. 1. (A) Oxygen content (C_{O_2}) in the haemolymph (mmol l^{-1}), (B) haemocyanin O_2-saturation and (C) oxygen partial pressure (P_{O_2}) (kPa) in the haemolymph of quiescent *Gecarcoidea natalis* or after various exercise regimes. Crabs exercised for 20 min either continuously or intermittently were required to walk the same overall distance. There were no significant differences between pulmonary and arterial samples. Significant differences between venous and arterial samples are indicated by an 'a', while significant differences between venous and pulmonary samples are indicated by a 'p' above the venous sample for each treatment group. Values are means + S.E.M. (N=6 in each treatment).

in crabs exercised for 20 min continuously (Fig. 3). Interestingly, the haemolymph of crabs exercised intermittently was significantly more acidic (7.36±0.03) than that of crabs exercised continuously for the same period.

Metabolite concentrations in the haemolymph and leg muscle

The glucose concentration in the haemolymph was significantly greater after 20 min of intermittent exercise (0.74±0.06 mmol l^{-1}) than in quiescent crabs (0.36±0.06 mmol l^{-1}; Fig. 4A). The increase in glucose concentration in the haemolymph after 20 min of intermittent locomotion was reflected in an increase of 0.20±0.03 mmol kg^{-1} in the muscle samples (Fig. 4A).

The increase in glucose concentration after 20 min of intermittent exercise was accompanied by a significant increase in L-lactate concentration (Fig. 4B). The concentration of L-lactate in the haemolymph increased from 0.23±0.1 mmol l^{-1} in quiescent crabs to 5.0±1.1 mmol l^{-1} after 20 min of intermittent exercise; in the muscle, L-lactate concentration increased by 3.3±0.6 mmol kg^{-1} during the same period (Fig. 4B). Interestingly, [L-lactate] in the muscle tissue was the same for crabs exercised continuously for either 5 min (2.2±0.8 mmol kg^{-1}) or 20 min (2.1±0.8 mmol kg^{-1}). The relationship between L-lactate concentration in the haemolymph and that in the leg muscle tissue was similar for each exercise regime.

The pattern of L-lactate clearance from the haemolymph was slightly different in crabs recovering from either continuous or intermittent exercise for 20 min (Fig. 5). Immediately after 20 min of continuous exercise, the L-lactate concentration in the haemolymph was 0.9±0.3 mmol l^{-1}, a value not statistically different from that of quiescent crabs (0.23±0.1 mmol l^{-1}). However, thereafter, L-lactate levels continued to increase during recovery from continuous exercise to reach 2.47±1.0 mmol l^{-1} at 1 h after exercise. In contrast, after 20 min of intermittent exercise, the L-lactate levels were significantly elevated (5.0±1.1 mmol l^{-1}) compared with those of quiescent crabs and there was no further increase during recovery. L-Lactate levels in both continuously and intermittently exercising crabs returned to resting concentrations within 5 h post-exercise (Fig. 5).

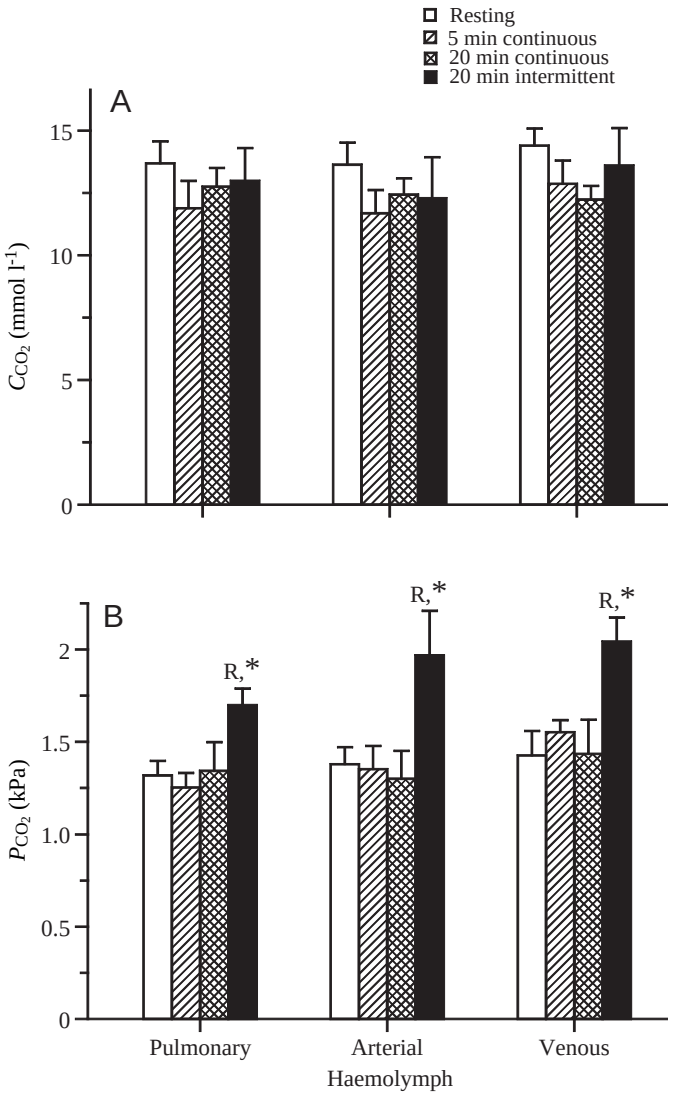

Fig. 2. (A) CO_2 content (C_{CO_2}) (mmol l^{-1}) and (B) CO_2 partial pressure (P_{CO_2}) (kPa) in the haemolymph of *Gecarcoidea natalis* at rest or after exercise. Crabs exercised for 20 min either continuously or intermittently were required to walk the same overall distance. An 'R' indicates a significant difference compared with the crabs at rest. A difference between crabs exercised for 20 min either intermittently or continuously is designated with an asterisk. Values are means + S.E.M. (N=6 in each treatment).

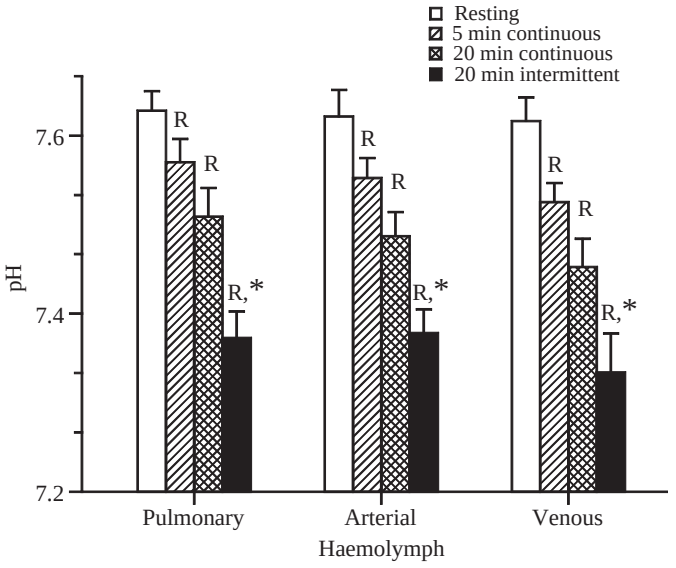

Fig. 3. Haemolymph pH of *Gecarcoidea natalis* at rest or after exercise. Crabs exercised for 20 min either continuously or intermittently were required to walk the same overall distance. An 'R' indicates a significant difference compared with crabs at rest. A difference between crabs exercised for 20 min either intermittently or continuously is designated with an asterisk. Values are means + S.E.M. (N=6 in each treatment).

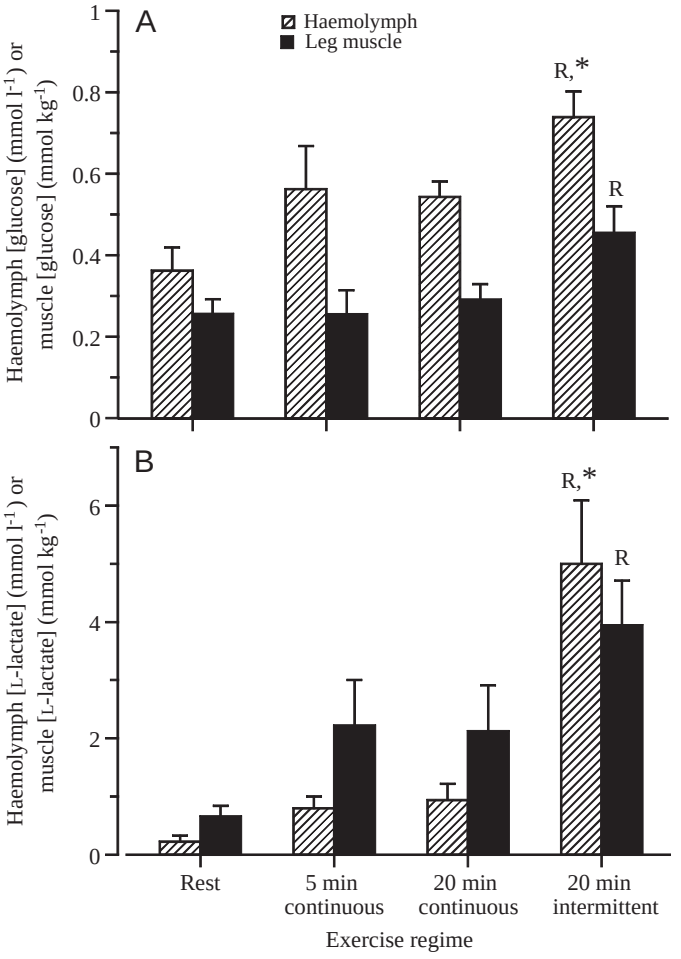

Fig. 4. (A) The concentration of glucose (mmol l^{-1}) in the haemolymph and leg muscle (mmol kg^{-1}) of *Gecarcoidea natalis* either at rest or after exercise. Note that values for haemolymph and muscle are shown on the same scale but that different units apply. (B) The concentration of L-lactate (mmol l^{-1}) in the haemolymph and leg muscle (mmol kg^{-1} muscle) of *G. natalis* either at rest or after exercise. Crabs exercised for 20 min either continuously or intermittently were required to walk the same distance. An 'R' above a column indicates a significant difference from crabs at rest. An asterisk designates a significant difference between crabs exercised either intermittently or continuously for 20 min. The comparisons were separate for the haemolymph and muscle tissue. Values are means + S.E.M. (N=6 in each treatment).

Although the urate concentration in the haemolymph of crabs exercised intermittently for 20 min was 0.081±0.01 mmol l^{-1} compared with 0.043±0.008 mmol l^{-1} in quiescent crabs, these values were not statistically different ($F_{3,28}$=2.737, P=0.062). The mean calcium concentration in the haemolymph of crabs exercised for 5 min (20.0±0.9 mmol l^{-1}) was significantly greater than that of quiescent crabs (16.5±0.8 mmol l^{-1}; Table 1). The mean osmotic pressure was 753±20 mosmol kg^{-1}, and there were no significant differences among the experimental groups (Table 1).

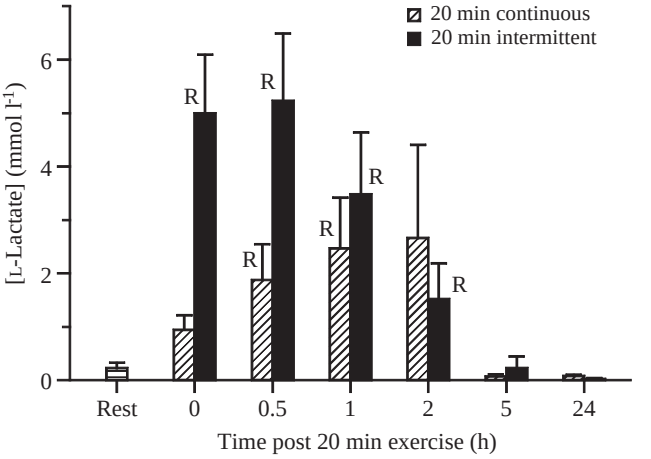

Fig. 5. Haemolymph L-lactate concentration (mmol l^{-1}) during 24 h of recovery from 20 min of either continuous or intermittent exercise. Time zero is immediately after 20 min of exercise. An 'R' above a column indicates a significant difference between the exercised group and crabs at rest. Values are means + S.E.M. (N=6 in each treatment).

Discussion

The relative importance of gills and lungs in gas exchange

The oxygenation of the haemolymph clearly increased after it has passed across the gas-exchange surfaces. The mean C_{O_2} in pulmonary haemolymph of red crabs at rest (0.83 mmol l^{-1}) was within 0.02 mmol l^{-1} of the arterial values and was comparable to values reported previously (Farrelly and Greenaway, 1994; Adamczewska and Morris, 1994*a*). In contrast, the mean P_{O_2} of the pulmonary haemolymph (P_{PO_2}=8.28 kPa) was markedly higher than previously reported for *G. natalis* (P_{PO_2}=3.7 kPa; Farrelly and Greenaway, 1994) but well within the range of arterial P_{O_2} reported for other terrestrial decapods, e.g. 3.8–12.6 kPa (Wood and Randall, 1981*b*; Farrelly and Greenaway, 1987, 1994; Morris and Adamczewska, 1996; Adamczewska *et al.* 1997).

The surface area of the lungs is only 12 % that of the gills but it presents a 10- to 30-fold thinner barrier to gas diffusion (Farrelly and Greenaway, 1992, 1993). Farrelly and Greenaway (1994) suggested similar lung and gill function in resting crabs, but Adamczewska and Morris (1994*a*) showed that the lung is more important during exercise. These findings could be a combined consequence of preferential redirection of haemolymph flow between the lungs and the gills (Taylor and Greenaway, 1984; Al-Wassia *et al.* 1989; Airriess and McMahon, 1994; Morris and Airriess, 1998) and of the resistance within the beds of the lacunae that limit the volume of haemolymph flowing through the lungs (Rajashekhar and Wilkens, 1991; Taylor and Taylor, 1992). The direct measurement of haemolymph flow in land crabs is now essential to determine conclusively the contribution of the lungs and gills in gas exchange.

The diffusion resistance of the gas-exchange surfaces has

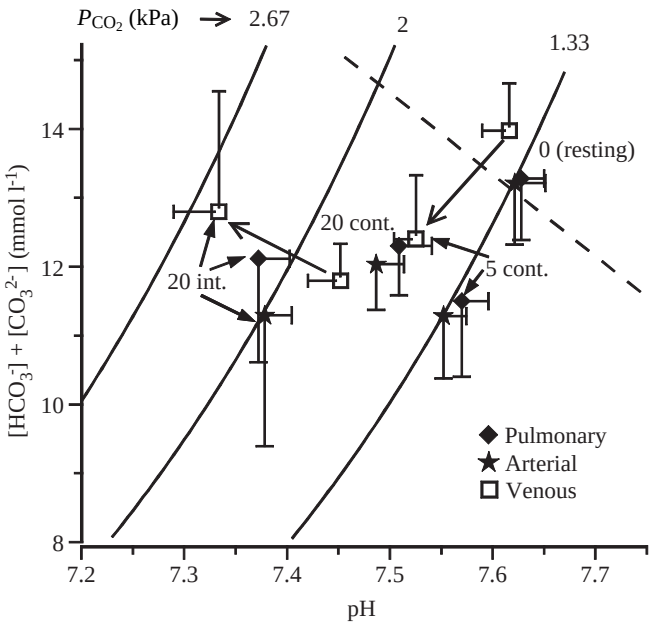

Fig. 6. The pH/HCO_3^- relationship for *Gecarcoidea natalis* haemolymph at 25 °C, showing the response to exercise *in vivo*. Values for the pulmonary, arterial and venous haemolymph of crabs at rest after 5 or 20 min of continuous (cont.) exercise or 20 min of intermittent (int.) exercise are shown. The P_{CO_2} isopleths were derived from measurements *in vitro* and the Henderson–Hasselbalch relationship using α_{CO_2}=0.299 mmol l^{-1} kPa^{-1} and pK=6.1, where α is the solubility coefficient and pK is the apparent dissociation constant. The broken line is the non-bicarbonate buffer line derived from the data of Adamczewska and Morris (1994*a*). Values are means ± S.E.M. (N=6 in each treatment/time).

been quantified using the equilibrium coefficient L_{diff} (Piiper, 1982; Taylor and Innes, 1988):

$$L_{diff} = (P_{B_{O_2}} - Pa/p_{O_2})/(P_{B_{O_2}} - Pv_{O_2}) ,$$

where $P_{B_{O_2}}$ is the partial pressure of O_2 within the branchial chamber, Pa/p_{O_2} is the partial pressure in either the arterial or pulmonary haemolymph and $P_{B_{O_2}}$–Pv_{O_2} is the difference in the partial pressure between the branchial chamber air and that of the venous haemolymph. A value of zero for L_{diff} represents a minimal diffusion barrier, while a value of one represents a severe barrier to diffusion. The gas-exchange surfaces of crustaceans are generally considered to be diffusion-limited, and in aquatic species L_{diff} normally ranges from 0.48 to 0.87. For terrestrial crabs, L_{diff} tends to be lower, between 0.44 and 0.63 (reviewed by Innes and Taylor, 1986*b*; Taylor and Taylor, 1992). In *G. natalis*, the diffusion limitations of the lungs alone and of the combined branchial and pulmonary circuits (paired *t*-test, repeated-measures ANOVA) were similar (Table 2), and for crabs at rest the L_{diff}(lungs) value of 0.53 was typical for land crabs (Taylor and Taylor, 1992). The greater L_{diff}(lungs) value of 0.77 for crabs exercised for 20 min intermittently compared with crabs at rest (L_{diff}=0.53) supports the premise that, during intermittent exercise, haemolymph flow increases and the lungs become more diffusion-limited. This limitation was not observed in animals exercised continuously for 20 min, but L_{diff}(lungs) was 0.8 after only 5 min of exercise (Table 2). Similar evidence for increased diffusion limitation in intermittently but not continuously exercised animals was apparent in the combined lung and gill data (Table 2). Clearly differences existed after 20 min between continuous and intermittent exercise; L_{diff} for the intermittently exercised group was more similar to that of the group exercised for 5 min. In terrestrial crustaceans, ventilation is driven by CO_2 (e.g. Burggren and McMahon, 1988). It is likely that CO_2-induced hyperventilation may initially lag behind a developing hypoxia, as well as varying between exercise regimes, and some high L_{diff} values may therefore be transients or artefacts. A difference in L_{diff} between 0.8 and 0.6 could, for example, be explained if the P_{O_2} within the lung lumen decreased to 13 kPa.

Oxygen supply during exercise

A rapid increase in the rate of O_2 uptake ($\dot{M}_{O_2}$) together with a high factorial aerobic scope (FAS=maximal $\dot{M}_{O_2}$/resting $\dot{M}_{O_2}$) is generally associated with active animals using primarily aerobic metabolism during exercise (e.g. Herreid, 1981; Full, 1987; van Aardt, 1991). *G. natalis* has a relatively low FAS of 2–3.5 (Adamczewska and Morris, 1994*b*; S. Morris and J. Hughes, unpublished data) compared with that of other crustaceans (FAS range 2.6–6: McMahon, 1981; Innes and Taylor, 1986*a*; Adamczewska and Morris, 1994*b*). A low FAS limits the increase in walking speed if anaerobiosis is to be avoided. Increased O_2 supply to the tissues must be met either by increasing the arteriovenous O_2 content difference (Ca_{O_2}–Cv_{O_2}) and/or by increasing cardiac output (McMahon *et al.* 1979; Booth *et al.* 1982; Hamilton and Houlihan, 1992).

Table 1. *Osmotic pressure and calcium concentration in the haemolymph of* Gecarcoidea natalis *during various exercise regimes carried out in the laboratory*

	Quiescent	5 Cont.	20 Cont.	20 Int.
[Calcium] (mmol l^{-1})	16.5±0.8^A	20.0±0.9^A	17.0±0.8	18.8±1.1
Osmotic pressure (mosmol kg^{-1})	743±9	770±18	708±17	791±35

The exercise regimes indicate continuous exercise for 5 min (5 Cont.), 20 min (20 Cont.) or intermittent exercise for 20 min (20 Int.).
Values are means ± S.E.M. (N=6 for each treatment).
Values sharing a superscripted letter are significantly different.

Table 2. *The concentration of haemocyanin-bound O_2 in the pulmonary haemolymph (Hc–O_2), the arterial–venous C_{O_2} difference (Ca_{O_2}–Cv_{O_2}) and the diffusion limitation coefficient (L_{diff}) for* Gecarcoidea natalis *for the pulmonary circuit alone (lungs) and for the combined pulmonary and branchial circuits (lungs and gills) at rest and during various exercise regimes*

Exercise regime	Hc–O_2 pulmonary (mmol l^{-1})	(Ca_{O_2}-Cv_{O_2}) (mmol l^{-1})	L_{diff}, lungs	L_{diff}, gills and lungs
Quiescent	0.70±0.09 (7)	0.27±0.07^{E,F} (6)	0.53±0.10^{C,D} (7)	0.70±0.10 (6)
5 min continuous	0.87±0.11 (6)	0.59±0.12^{E} (6)	0.80±0.11^{D} (6)	0.72±0.11 (6)
20 min continuous	0.65±0.11 (6)	0.35±0.11 (7)	0.59±0.10^{A} (7)	0.54±0.09^{B} (7)
20 min intermittent	0.85±0.09 (8)	0.56±0.10^{F} (8)	0.77±0.06^{A,C} (8)	0.72±0.07^{B} (8)

Values are means ± S.E.M.
The values in parentheses are the number of replicates.
Values sharing superscripted letters are significantly different.

Additionally, a greater proportion of the haemolymph can be redirected into the arteries supplying the walking limbs (Airriess and McMahon, 1994; De Wachter and McMahon, 1996).

Subjecting the Ca_{O_2}–Cv_{O_2} values, calculated for each crab, to ANOVA showed that crabs at rest differed from those in the exercised groups, for example, when compared with the 5 min exercised crabs (P=0.017; *post hoc* contrast test). This difference occurred primarily since, while during exercise the arterial haemolymph remained 100 % O_2-saturated (Fig. 1A,B), the venous O_2 reserves decreased by 0.16 mmol l^{-1} for all the exercise groups (Fig. 1A). The Ca_{O_2}–Cv_{O_2} difference did not increase with intensity of exercise but instead could be significantly correlated with haemocyanin concentration which determines [haemocyanin–O_2] (Table 2) and thus maximum O_2-carrying capacity (Pearson correlation value r=0.787, Bartlett χ^2=16.917, Bonferroni-adjusted P<0.001) according to: Ca_{O_2}–Cv_{O_2}=0.0348+0.4969[haemocyanin]. Additionally, a large Ca_{O_2}–Cv_{O_2} difference was associated with a high L_{diff}(lungs) value (Table 2). Since the lungs of *G. natalis* are diffusion-limited, in haemolymph with a high capacity for O_2, proportionally more O_2 would be bound to haemocyanin and consequently the haemolymph will have a lower P_{O_2} than haemolymph with a low O_2-carrying capacity. Thus, a difference in the calculated L_{diff} would occur despite exactly the same amount of O_2 diffusing across the gas-exchange surfaces.

Urate and calcium concentrations showed a tendency to increase in exercised crabs and both potentiate the O_2 affinity of *G. natalis* haemocyanin (Adamczewska and Morris, 1998) but, given the efficacy of the gas-exchange organs in maintaining arterial oxygen loading in exercised red crabs, there seems little advantage in increasing the O_2 affinity of the haemocyanin. Instead, reductions in haemocyanin O_2-affinity might improve unloading at the tissues. While there was an increased acidification of the haemolymph with an increase in exercise intensity (Fig. 3), the pH-sensitivity of O_2 binding by haemocyanin in *G. natalis* is low (Adamczewska and Morris, 1998). Importantly, the effect of L-lactate on O_2 binding by haemocyanin is reversed in *G. natalis* and so decreases O_2 binding, thereby facilitating O_2 delivery (for a detailed analysis, see Adamczewska and Morris, 1998). Some anaerobiosis and L-lactate production during exercise may actually serve to assist O_2 release at high venous P_{O_2}.

Anaerobiosis during intermittent and continuous exercise

While increased speed of locomotion promotes an increase in $\dot{M}_{O_2}$, it also tends to be associated with increased anaerobiosis and fatigue (Wood and Randall, 1981*a*,*b*; van Aardt, 1990; Adamczewska and Morris, 1994*b*). During exercise, despite the high O_2-saturation of the haemocyanin at the gas-exchange surfaces and venous O_2 reserves greater than 0.35 mmol l^{-1}, *G. natalis* exhibited a functional anaerobiosis, particularily during the higher-speed intermittent exercise. The haemolymph L-lactate concentration recorded for *G. natalis* after 20 min of intermittent exercise (mean 5 mmol l^{-1}) was generally lower, however, than that reported previously for this and other species exercised for comparable periods (range 8–19 mmol l^{-1}: McMahon *et al.* 1979; Booth *et al.* 1982; van Aardt, 1990; Adamczewska and Morris, 1994*b*; Henry *et al.* 1994), indicating that the exercise regimes in the present study were less dependent on anaerobiosis.

Intermittent locomotion provides a possible advantage in the opportunity to repay part of any anaerobic debt that has been incurred during walking (e.g. Brooks *et al.* 1973). The voluntary walking speed of red crabs exercised continuously was close to the MAS (110 %) and promoted minimal anaerobiosis, while crabs walking intermittently walked faster at approximately 230 % of the MAS. Thus, the primary disadvantage of the intermittent locomotion was that it required *G. natalis* periodically to exceed MAS, promoting elevated glycolysis, hyperglycaemia and the accumulation of an O_2 debt. In the field, the mean daily walking speed estimated for crabs engaged in their migratory activites using radio-tracking data was 1.1 m min^{-1}, which is within the estimated MAS (Adamczewska, 1997; A. M. Adamczewska and S. Morris, unpublished data). However, walking speeds recorded for crabs during their migration in open areas exceeded the MAS by fourfold (e.g. 4.7 m min^{-1}, Adamczewska and Morris, 1994*b*; Adamczewska, 1997; 5.4 m min^{-1}, Hicks, 1985) and, thus, were considerably greater

than the walking speed of crabs exercised intermittently in the laboratory.

Low rates of L-lactate reoxidation of 0.8–2.6 mmol l^{-1} h^{-1} are common in crustaceans (Wood and Randall, 1981*b*; Forster *et al.* 1989; Henry *et al.* 1994). In red crabs, the fastest rate of L-lactate clearance from the haemolymph (1.75 mmol l^{-1} h^{-1}) was within this range and thus the duration of the pauses during intermittent exercise was clearly insufficient for substantial L-lactate oxidation. The accumulation of L-lactate *per se* is probably not the cause of fatigue (e.g. Putnam, 1979). Fatigue during exercise could arise from a shortage of metabolic fuel, from acidosis and from a reduction in the availability of high-energy phosphates, but typically in exercised crustaceans glucose concentrations increase and remain high (England and Baldwin, 1983; Adamczewska and Morris, 1994*b*).

The diminishing high-energy phosphate stores certainly result in fatigue (Onnen and Zebe, 1983; Krause and Wegner, 1996), and the restoration of arginine phosphate stores and the ATP pools occurs more quickly after exercise than the clearance of L-lactate (Wood and Randall, 1981*b*; Ellington, 1983; Gäde, 1984; Head and Baldwin, 1986; Henry *et al.* 1994). Thus, regeneration of high-energy phosphate stores during the pauses in intermittent exercise may be sufficient to increase the distance capacity compared with continuously exercised animals.

CO_2 and acid–base balance during various exercise regimes

The C_{CO_2} and P_{CO_2} values recorded for *G. natalis* were typical of other land crabs (Wood and Randall, 1981*b*; McMahon and Burggren, 1988; Greenaway *et al.* 1988; Adamczewska and Morris, 1994*a*; Farrelly and Greenaway, 1994), but the measurements in red crabs did not allow conclusive determination of the relative importance of the gills and lungs in CO_2 excretion (for a review, see Henry, 1994). The increase in P_{CO_2} during intermittent exercise served, however, to increase the outward diffusion gradient for CO_2 and apparently to maintain CO_2 excretion.

G. natalis experienced a mixed metabolic/respiratory post-exercise acidosis, as is common for other crustaceans (e.g. McDonald *et al.* 1979; Smatresk and Cameron, 1981; Booth *et al.* 1984; Forster *et al.* 1989; Greenaway *et al.* 1992). Assuming stoichiometric L-lactate and H^+ flux, it was possible to determine the haemolymph pH decrease attributable to anaerobiosis (Wood *et al.* 1977; Greenaway *et al.* 1988). In crabs exercised for 5 min, the acidosis was purely metabolic (Fig. 6). The proportion of the acidosis experienced by crabs exercised continuously for 20 min that could be attributed to L-lactate production was only 6 %, but increased to 36 % after 2 h of recovery. In contrast, in crabs exercised intermittently, L-lactate accounted for 57 % of the haemolymph acidosis, reflecting the consequences of walking speed exceeding the MAS and elevated anaerobiosis. These results, nonetheless, are in marked contrast to those of previous studies on exhaustive exercise in *G. natalis* and *Birgus latro*, where the concentrations of L-lactate in the haemolymph predicted a greater acidosis than actually occurred (Greenaway *et al.* 1988; Adamczewska and Morris, 1994*b*). Furthermore, the post-exercise acidosis exhibited by *G. natalis* in the present study (0.06–0.24 pH units) was generally low compared with previous values measured in air-breathing crustaceans (e.g. 0.2–0.7 pH units; Wood and Randall, 1981*b*; Wheatly *et al.* 1986; Greenaway *et al.* 1988; Forster *et al.* 1989; Adamczewska and Morris, 1994*a*). The relatively small acid–base perturbations caused by non-exhausting exercise in the present study indicate that the chosen exercise regimes, including the intermittent strategy, were well within the capacity of red crabs and can be used in interpretation of the ethology of this species.

Under natural conditions, non-migrating *G. natalis*, like other crabs, move intermittently when foraging (Weinstein and Full, 1992; Weinstein, 1995; Adamczewska, 1997). Nonetheless, after 20 min of intermittent exercise, red crabs had higher L-lactate levels, a higher P_{CO_2} and were significantly more acidotic than crabs exercised continuously for 20 min. The main disadvantage of intermittent burst locomotion is that it increases the reliance on anaerobiosis, but red crabs possess a large anaerobic capacity (Adamczewska and Morris, 1994*b*) and, if required, could accommodate a sizeable O_2 debt each day. This debt could be repaid while resting overnight but represents an extra net overall cost. It remains possible that intermittent locomotion during which walking speeds do not exceed the MAS might offer advantages over continuous locomotion but such net speeds would be inadequate and, importantly, are exceeded on a daily basis by migrating red crabs. Whether there are real advantages to intermittent locomotion during the migration requires further analysis of feeding behaviour and food requirements during the migration. Whether these outweigh the disadvantages and attendant costs of a regular lactacidosis will need to be resolved by sampling crabs during their migratory activities.

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