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FIDDLER CRAB EXERCISE: THE ENERGETIC COST OF RUNNING SIDEWAYS

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SUMMARY

The fiddler crab, *Uca pugilator*, used sideways octapedal locomotion during 15 min of treadmill exercise. At each velocity tested (0·06, 0·11 and 0·16 km h^{-1}), oxygen consumption ($\dot{V}_{O_2}$) showed only a modest, sluggish elevation; a 'steady-state' was never attained. The highest $\dot{V}_{O_2}$ recorded, 0·22 mlO_2 g^{-1} h^{-1}, was 4·4 times the resting rate. Net whole body lactate (WBL) was found to increase at a constant rate throughout the exercise period.

During recovery, $\dot{V}_{O_2}$ and WBL removal followed a similar time course and returned to pre-exercise rates in 30–45 min. Although the fate of lactate after exercise is unknown for crustaceans, calculations suggest that not enough oxygen is consumed by the crab during recovery to oxidize lactate completely to CO_2 and H_2O. A gluconeogenic fate is compatible with the data.

As running velocity was increased, V_{O_2} increased only slightly, while the net rate of WBL production showed a substantial elevation. At low velocity aerobic metabolism accounted for 60 % of the ATP produced when aerobic metabolism and anaerobic fermentation are considered. Anaerobic fermentation dominated at medium and high velocity and produced 60 and 70 % of the ATP, respectively.

The minimum cost of transport, the least amount of energy required to transport a given mass a distance, was determined using both aerobic and anaerobic sources. This estimation of locomotion economy for *Uca pugilator* was within the range predicted for a vertebrate of a similar mass.

INTRODUCTION

Study of the energetics of terrestrial locomotion has primarily focused on vertebrates (Bennett, 1978, 1980; Bennett & Ruben, 1979; Taylor, 1977). Yet, their morphological and physiological design represent only one possible solution to the problem of locomotion. Invertebrates possess tremendous diversity in body shape, leg number, style of locomotion, respiratory and circulatory systems as well as the neuromuscular systems producing leg movement (Herreid & Fourtner, 1981). The study of invertebrates promises greatly to broaden our understanding of the energetic strategies of locomotion.

Key words: Crab locomotion, aerobic metabolism, anaerobic metabolism.

The following investigation focuses on a mobile invertebrate group, the brachyuran crustaceans. Members of this taxon which have invaded land use sideways octapedal locomotion. This study defines the metabolic design of the fiddler crab, *Uca pugilator*, under rigorously controlled conditions. Several parameters of exercise were analysed.

(1) *Exercise performance*. This was accomplished by running crabs to fatigue on a motor-driven treadmill to determine the range of sustainable velocities.

(2) *Aerobic response to exercise*. By measuring the kinetics of oxygen consumption ($\dot{V}_{O_2}$) while the crabs ran at known velocities, we were able to obtain data directly analogous to that for exercising vertebrates. Previous work on the land crabs *Cardisoma guanhumi* and *Gecarcinus lateralis* (Herreid, Lee & Shah, 1979; Herreid, O'Mahoney & Full, 1983) suggests that the aerobic response of crustaceans to locomotion is sluggish and reduced in magnitude. However, data on the ghost crab, *Ocypode guadicaudii*, indicate that some species of crabs are capable of a rapid and substantial aerobic response to exercise (Full & Herreid, 1983). Our selection of the fiddler crab expands the number of crustacean species studied as we attempt to define the types of aerobic responses in arthropods.

(3) *Anaerobic response to exercise*. The major indicator of anaerobic metabolism in crustaceans is L-lactate, as in vertebrates (McMahon, 1981; see Long, 1976 for lactate stereospecificity). Alternative anaerobic pathways, such as those found in molluscs and annelids, have not been demonstrated in crustaceans (Graham, Mangum, Terwilliger & Terwilliger, 1983; Zebe, 1982). The anaerobic capacity of crustaceans has been described primarily with respect to hypoxia (Bridges & Brand, 1980). Under this stress *Uca pugilator* has demonstrated ample anaerobic capacity; 26 h of anoxia can result in a 20-fold increase in the anaerobic end-product, lactic acid (Teal & Carey, 1967). Few studies have considered anaerobic metabolism during terrestrial locomotion in crustaceans (Burke, 1979; Wood & Randall, 1981*b*) and no extensive examination of this energy component has been reported. In this study we attempted to determine the fiddler crab's anaerobic abilities during exercise by analysis of whole body lactate (WBL). Analysis by this method avoids the problems of lactate compartmentalization encountered when only blood levels are measured (Bennett & Licht, 1972).

(4) *Recovery after exercise*. In addition to determining oxygen uptake and WBL kinetics during exercise, WBL clearance and its poorly understood relationship with recovery $\dot{V}_{O_2}$ were also examined.

(5) *Cost of locomotion*. Both aerobic and anaerobic ATP production were used to estimate the minimum cost of locomotion and compare this value to vertebrates using vastly different strategies for terrestrial locomotion.

MATERIALS AND METHODS

Animals

The crab, *Uca pugilator*, was collected at Beaufort, North Carolina and sent by bus to our laboratory in Buffalo, N.Y. The crabs were maintained in excellent condition in the laboratory at 24 °C in large aquaria with moist sand and 50 % sea water. The aquaria were kept in an environmental chamber set on a 12 h light: 12 h dark cycle. The animals were fed TetraMin fish food and insect larvae. Only intermoult mal

were used for experimentation. The average mass of a crab used for the experiments on aerobic metabolism was $2{\cdot}26 \pm 0{\cdot}48$ g (S.D.) and for anaerobic metabolism, $1{\cdot}82 \pm 0{\cdot}37$ g (S.D.).

Exercise performance

Fiddler crabs were exercised on a motor-driven treadmill to determine the range of velocities which could be sustained for a minimum of 15 min. The crabs, after a 10-min rest period on the treadmill, were run to fatigue at a given velocity. Fatigue was defined as that time when a crab (1) did not maintain pace with the treadmill, (2) dragged its abdomen and (3) did not respond to three successive prodding attempts. Running velocities ranged from 0·06–0·40 km h^{-1}. At these velocities animals adjusted their gait immediately to match the motion of the treadmill belt. Exercise velocities below 0·16–0·17 km h^{-1} were sustained for over 15 min by all animals and therefore were selected for evaluation of both aerobic and anaerobic metabolism (Fig. 1). At these lower velocities most crabs ran very well for 15 min, maintaining a position at the middle of the treadmill belt. Runs longer than 15 min or below 0·06 km h^{-1} could not be used for metabolic studies because most animals displayed erratic locomotion. Experiments in which the animals did not run consistently were discarded.

Aerobic metabolism

Fiddler crabs were exercised at three velocities on a treadmill enclosed in a respirometer. $\dot{V}_{O_2}$ was determined by open flow respirometry.

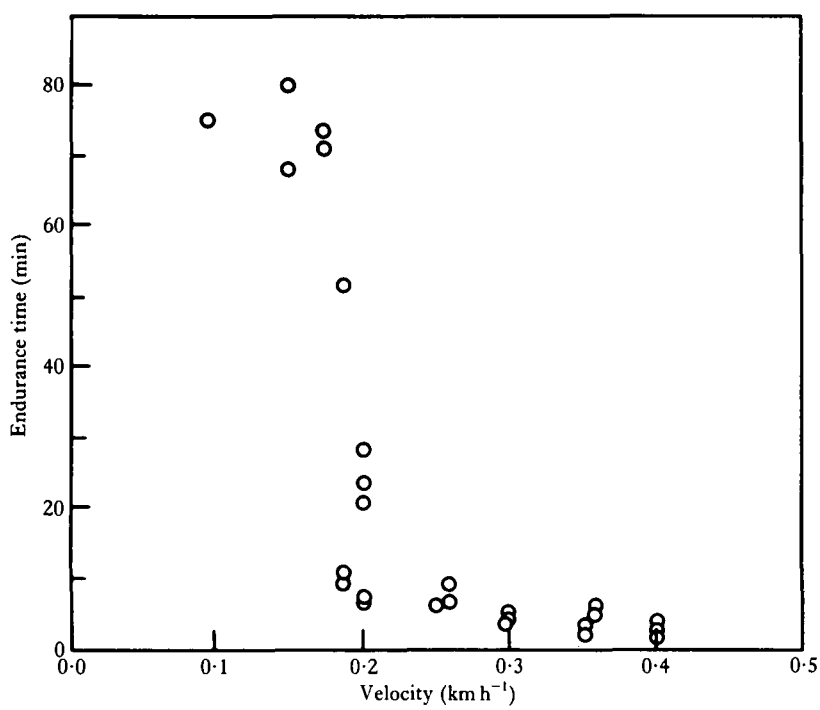

Fig. 1. Treadmill endurance time or time to fatigue as a function of velocity. Each data point represents a separate trial.

Protocol

Animals were initially placed in a treadmill respirometer for a 30-min rest period. The rest period was followed by a 15-min exercise bout at one of three velocities: 0·06, 0·11 or 0·16 km h^{-1}. A 60-min recovery period followed the run. Each crab was tested at all three velocities. $\dot{V}_{O_2}$ was monitored continuously during rest, run and recovery.

Oxygen consumption measurements

All measurements were made at 24 °C. Animals were exercised in a Lucite respirometer which contained a variable speed treadmill (Herreid, 1981). Gas was drawn through the chamber and a room air reference line at 55 ml min^{-1}. Both air streams were dried by tubes containing Drierite. The fraction of oxygen present in the excurrent flow from the chamber was compared to the fractional oxygen concentration of the reference line by a two-channelled oxygen analyser (Applied Electrochemistry) which was interfaced with an integrating chart recorder (Linear Instruments Model 282). From the continuous recordings of the difference in fractional oxygen concentration (room air – animal chamber line) and flow rate, instantaneous $\dot{V}_{O_2}$ was computed (see Bartholomew, Vleck & Vleck, 1981; Full & Herreid, 1983; Herreid, Prawel & Full, 1981). Mass-specific $\dot{V}_{O_2}$ was determined by equation (3a) of Withers (1977) assuming a mean respiratory quotient (RQ) of 1·0. The fractional error in $\dot{V}_{O_2}$ is ± 9 % if RQ is taken to be 1·0 but is actually 0·50 or 1·50. Calculation of instantaneous $\dot{V}_{O_2}$ allows estimation of rapid changes in $\dot{V}_{O_2}$ given the 'washout' characteristics of the system. The response time of the 125-ml chamber was approximately 3 min to 50 % full scale deflection. This was determined by addition of gas of a precisely known concentration of O_2 (20·861 ± 0·001 % O_2). The delay time ranged from 20–30 s.

For each trial, instantaneous $\dot{V}_{O_2}$ values were calculated at 1-min intervals. A curve fitting technique, interpolation using the cubic spline method, was executed on an Apple II+ computer (Warme, 1981). This method fits a cubic polynomial through each of four successive points. All $\dot{V}_{O_2}$ values were corrected to STPD conditions.

Anaerobic metabolism

WBL content of fiddler crabs was determined at specified time intervals during exercise at three velocities and in recovery.

Protocol

All crabs were given a 30-min rest period on the treadmill before exercise. The animals were run at one of three velocities (0·06, 0·11 or 0·16 km h^{-1}) for a given duration. The duration of exercise ranged from 2 to 15 min. After the exercise bout the crab was rapidly removed from the treadmill and prepared for WBL analysis. Also, WBL content was determined in control animals: (1) after removal from holding tank, (2) after a 30-min rest period on the treadmill and (3) after 3, 5, 15 and 75 min of rest in addition to the initial 30-min rest period. Any experiment in which a crab struggled was aborted.

In separate experiments concerning WBL removal during recovery all animals wer

n for 15 min. Then after a specified recovery period (2–60 min) the crab was quickly taken from the treadmill and prepared for WBL analysis as in the exercise trials.

Whole body lactate preparation

WBL content was determined by a modification of the procedure developed by Bennett & Licht (1972). At the conclusion of an exercise bout or control experiment the animal was carefully removed from the treadmill and frozen in liquid nitrogen. The time required for this process was less than 2 s. Immediately upon freezing the animal was pulverized in a mortar pre-cooled with liquid nitrogen. Subsequently the tissue powder was placed in a 0·6 N-perchloric acid solution whose volume equalled five times the animal's mass. The extract was then homogenized and allowed to incubate on ice for 30 min with frequent stirring. After this period the homogenate was centrifuged for 10 min in a refrigerated centrifuge at 10 000 rev./min (12 000 ***g***). The supernatant was filtered and stored in a refrigerator for less than 2 days before analysis.

Lactate analysis

All chemicals used were obtained from Sigma Chemical Co. The quantitative determination of L-lactate in the supernatant was accomplished by a specific spectrophotometric assay (Sigma diagnostic kit No. 826-UV). The glycine-hydrazine buffer (Stock No. 826-3; pH = 9·2) was selcted for use and modified as follows: (1) EDTA was added to produce a 12 mM concentration; (2) concentrated HCl was introduced dropwise until a pH of 9·0 was reached.

All reaction mixtures were incubated for 45 min at 25·0 °C. After the incubation period absorbance was determined on a Beckmann Dual-Beam Spectrophotometer.

Assay performance characteristics

The performance characteristics of the assay were evaluated with respect to reproducibility, recovery and storage.

Reproducibility. Preliminary experiments on lactate determination showed a significant drift of absorbance readings over time. Similar results have been observed by Graham *et al.* (1983) for *Cancer magister* where a stable endpoint was not reached even after 32 h. The drifting endpoint in the assay appears to be a result of side reactions catalysed by metal ions (Engel & Jones, 1978). Addition of EDTA in high concentration (over 10 mM) to the glycine-hydrazine buffer along with a decrease in pH to 9·0 minimizes side reactions (Engel & Jones, 1978). Our experiments and those of Graham *et al.* (1983) conducted with the modified glycine-hydrazine buffer support these contentions. By following the increase in absorbance at 340 nm over time a stable endpoint was attained at 40–45 min for both control and animal samples.

Reproducibility between measurements of a given sample was assessed by duplicate tests. The percentage error between duplicate measurements was 2·2 %. A paired *t*-test of the duplicates over a range of values did not reveal a significant difference in lactate content ($t = 0{\cdot}02$, $P = 0{\cdot}5$).

Recovery. Recovery of lactate was examined by addition of known amounts of lactate one-half of the crab homogenate. Lactate content was determined for both samples.

The concentration of lactate in the lactate-added sample could be predicted from the sum of the known lactate added plus that present in the original sample. The predicted lactate content of the lactate-added samples was not significantly different from the experimentally obtained values as shown by a paired t-test ($P > 0{\cdot}05$).

Storage. A series of experiments was undertaken to determine the length of time a sample could be stored in a refrigerator at 0–5 °C. Crab supernatant was analysed for lactate content immediately after preparation and at 2 and 4 days after preparation. Lactate content was found not to change after 2 days of storage, but was significantly elevated after 4 days. This finding does not agree with the time reported for human serum in the Sigma technical bulletin No. 826-UV which indicates a stability period of 7 days. Our experience with other crab species indicates that stability is a problem. Consequently, unless stability tests are reported one must view results with suspicion whenever storage is involved.

Calculation of energy equivalents

In order to examine the relative energetic contributions of aerobic and lactate pathways to exercise, all data were converted to a common scale, ATP equivalents (see McGilvery, 1979):

1 mol lactate = 1·50 mol ATP
1 mol oxygen = 6·33 mol ATP.

These relationships are based on glycogen being the fuel source (Hohnke & Scheer, 1967; Teal & Carey, 1967).

In recovery lactate can be removed by complete oxidation or gluconeogenesis. Complete oxidation of 1 mol of lactate was assumed to require 3 mol oxygen. The oxygen necessary for conversion of 1 mol of lactate to glycogen was taken to be 0·6 mol oxygen (McGilvery, 1979).

RESULTS

Oxygen consumption

The $\dot{V}_{O_2}$ of eight *Uca pugilator* was monitored 30 min prior to exercise. During this period animals showed little if any movement in the treadmill respirometer. The average resting $\dot{V}_{O_2}$ for the 15 min prior to exercise was $0{\cdot}049 \pm 0{\cdot}010$ (S.E.) $mlO_2\ g^{-1}\ h^{-1}$. This rate of uptake is comparable to that found by other investigators of *Uca* respiration (Brown, Bennett & Webb, 1951; Teal & Carey, 1967).

At the onset of exercise $\dot{V}_{O_2}$ increased over the resting level at each velocity (Fig. 2). $\dot{V}_{O_2}$ continued to rise during the entire exercise period. The peak $\dot{V}_{O_2}$ value was attained after the completion of exercise. A 'steady-state' oxygen consumption ($\dot{V}_{O_{2ss}}$) was not observed during 15 min of running. Two records of exceptional runners at low velocity did show that a $\dot{V}_{O_{2ss}}$ was attained in 20–25 min. The pattern of $\dot{V}_{O_2}$ increase can be evaluated by calculating the time required for $\dot{V}_{O_2}$ to rise to 50 % of the value measured at the end of exercise ($t_{1/2}$ on-response; this is not the same value as $t_{1/2}$ used in mammalian exercise because no 'steady-state' was reached). The $t_{1/2}$ on-response for the range of velocities examined was approximately 4–5 min.

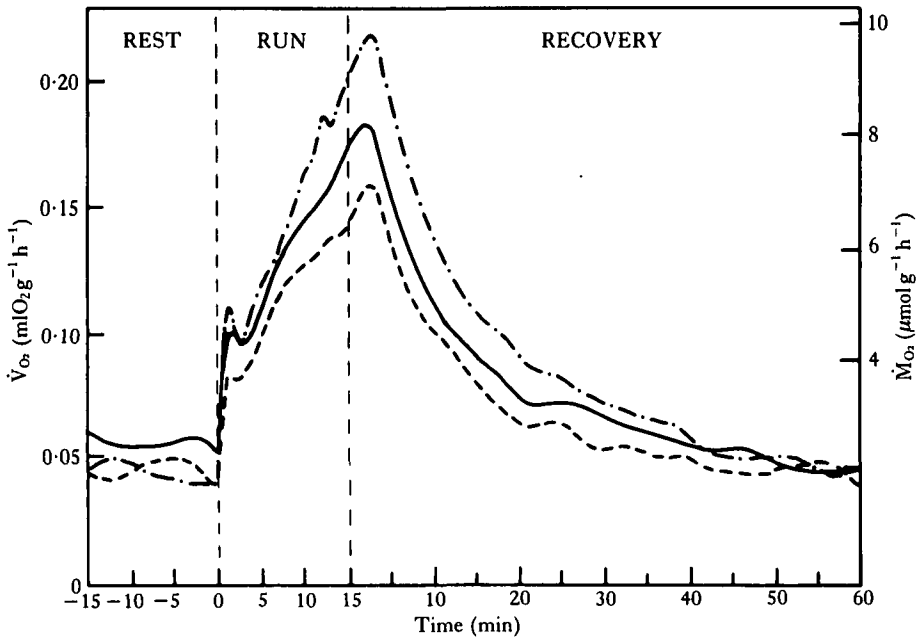

Fig. 2. Oxygen consumption ($\dot{V}_{O_2}$) of fiddler crabs on a treadmill during rest, exercise and recovery. The upper, middle and lower curves represent crabs running at velocities of 0·16, 0·11 and 0·06 km h^{-1} respectively. Each curve is the mean of 7–8 animals. Standard error bars are not included for clarity. The average standard error for all three velocities was 0·009 ± 0·004 mlO_2 g^{-1}h^{-1} (S.D.).

The $\dot{V}_{O_2}$ during recovery began to decline after 3 min and fell to near resting levels in 30–45 min. The time necessary for 50 % recovery ($t_{1/2}$ off-response) ranged from 7 to 10 min.

The highest rate of $\dot{V}_{O_2}$ observed was 0·22 mlO_2 g^{-1}h^{-1} after exercise at 0·16 km h^{-1}. This rate of $\dot{V}_{O_2}$ was 4·4 times the resting $\dot{V}_{O_2}$ level.

In Fig. 3 three parameters of the aerobic response to exercise are compared at different velocities. The parameters include: (1) the net volume of oxygen consumed during exercise, (2) oxygen deficit and (3) oxygen debt (excess post-exercise oxygen).

The net volume of oxygen used during exercise was calculated by subtracting the resting $\dot{V}_{O_2}$ from the area under the $\dot{V}_{O_2}$ exercise curve (Fig. 2). Net exercise V_{O_2} increased with an increase in velocity [$F(2,20) = 4{\cdot}7$, $P = 0{\cdot}05$]. A one-way analysis of variance was used to test if this and other parameters were different at the three velocities studied. An analysis of variance test for linearity was employed for the relationship of V_{O_2} *versus* the velocity of locomotion. The hypothesis of a linear relationship was not rejected for the net exercise V_{O_2} [$F(1,20) = 0{\cdot}45$, $P = 0{\cdot}51$].

O_2 deficit refers to the lag in O_2 uptake during the initial phase before $\dot{V}_{O_{2ss}}$ is reached (Stainsby & Barclay, 1970). Its magnitude is determined by the difference between two values: (1) the theoretical abrupt rise in $\dot{V}_{O_2}$ which should occur if a $\dot{V}_{O_{2ss}}$ were reached instantly at the start of exercise and (2) the actual increase in $\dot{V}_{O_2}$. Since *Uca pugilator* lacked a 'steady-state', we selected the best estimate available for this energy demand at each velocity. O_2 deficit was, therefore, calculated as the difference between: (1) a theoretical instantaneous $\dot{V}_{O_{2ss}}$ (energy demand),

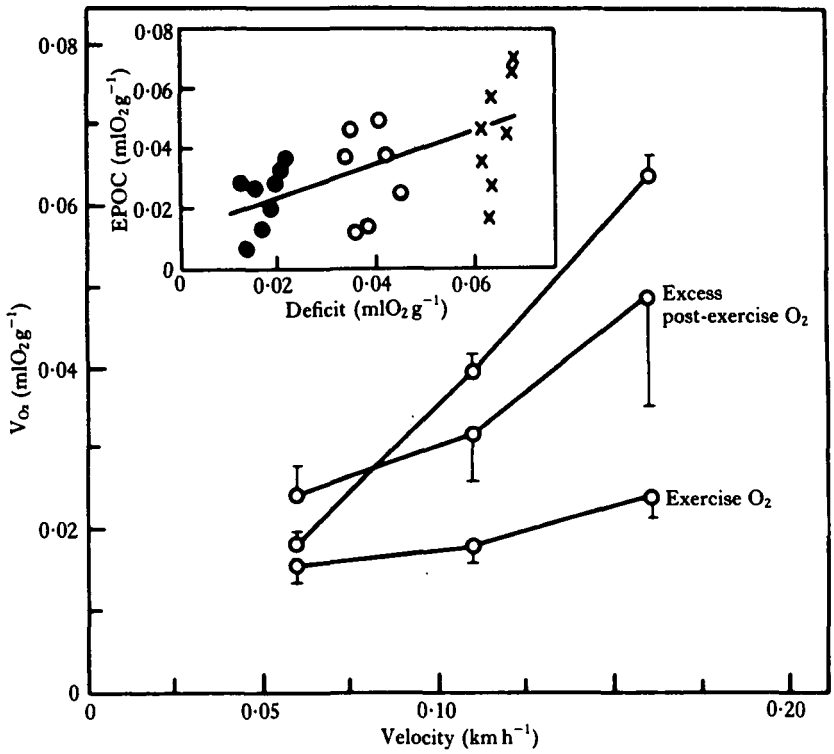

Fig. 3. Volume of oxygen (V_{O_2}) in exercise, deficit and post-exercise periods at three velocities of locomotion. Vertical bars represent ±S.E. of mean. Inset. Relationship between excess post-exercise oxygen consumption (EPOC) and the volume of oxygen represented by the O_2 deficit. Data point symbols represent three running velocities: 0·16 (×), 0·11 (○) and 0·06 (●) km h^{-1}.

estimated at the end of exercise from the sum of the actual $\dot{V}_{O_2}$ and oxygen equivalents for net WBL production, and (2) the net volume of oxygen consumed by the animal during the entire exercise period (see Fig. 5). The magnitude of the O_2 deficit showed a significant increase with increased velocity [$F(2,20) = 405{\cdot}5$, $P < 0{\cdot}05$]. The hypothesis of a linear relationship was also not rejected [$F(1,20) = 1{\cdot}71$, $P = 0{\cdot}21$].

Oxygen debt is the excess post-exercise oxygen consumption (EPOC) or the area under the $\dot{V}_{O_2}$ recovery curve above rest. EPOC increased with running velocity [$F(2,21) = 7{\cdot}5$, $P < 0{\cdot}05$]. The increase in EPOC was linear [i.e. hypothesis not rejected; $F(1,19) = 0{\cdot}60$, $P = 0{\cdot}45$] and EPOC values were similar to the O_2 deficit but exceeded that of exercise. A significant correlation was found between EPOC and O_2 deficit ($t = 4{\cdot}17$, $P < 0{\cdot}05$, $r = 0{\cdot}67$; Fig. 3 Inset).

Lactate production and removal

Net WBL content increased during the exercise period at each velocity (Fig. 4). A stepwise polynomial regression analysis (Zar, 1974) showed that a linear relationship was the best fit function in each case ($P = 0{\cdot}05$). A least squares regression analysis was performed on the data at each exercise intensity level (Table 1). Unexercised control animals showed no increase in WBL. Four animals ran consistently for 30 m

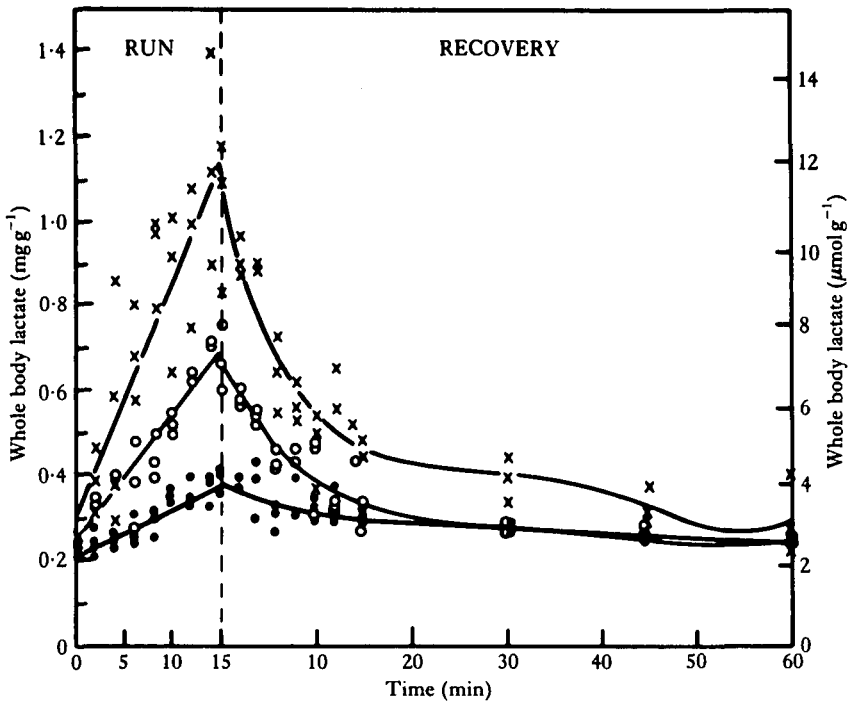

Fig. 4. Time course of net whole body lactate content during rest (t = 0), exercise and recovery at three velocities. Each data point is the value for a separate trial. Data point symbols represent the three running velocities: 0·16 (×), 0·11 (○) and 0·06 (●) km h^{-1}. The equations for each function are found in Table 1.

Table 1. *Equations expressing net whole body lactate content as a function of time during exercise and recovery at three velocities*

Velocity (km h^{-1})	Exercise (net production)	Recovery (net removal)
0·06	WBL = 0·011(±0·002)t + 0·120 (N = 28, r = 0·90)	WBL = 0·65 − 0·03t + 7·4 × 10^{-4}t^2 − 9·3 × 10^{-6}t^3 + 4·1 × 10^{-8}t^4 (N = 33, r = 0·81)
0·11	WBL = 0·030(±0·003)t + 0·242 (N = 27, r = 0·95)	WBL = 1·91 − 0·13t + 4·0 × 10^{-3}t^2 − 5·4 × 10^{-5}t^3 + 2·6 × 10^{-7}t^4 (N = 33, r = 0·91)
0·16	WBL = 0·056(±0·011)t + 0·316 (N = 27, r = 0·86)	WBL = 3·39 − 0·25t + 7·7 × 10^{-3}t^2 − 1·1 × 10^{-4}t^3 + 5·2 × 10^{-7}t^4 (N = 33, r = 0·94)

WBL represented in units of mg g^{-1} and t represented in units of min.
± Values in parentheses are 95 % confidence intervals for the slope.

at low velocity (0·06 km h^{-1}). WBL content in these crabs was not elevated over the values found at 15 min.

The rate of net WBL production during exercise was determined from the slope of the relationship between WBL content and time (Fig. 4). The slopes of the regression

line for each velocity were significantly different from one another as shown by an analysis of covariance [$F(2,76) = 31{\cdot}9$; $P = 0{\cdot}001$]. As indicated by the slope values in Table 1, the net rate of WBL production increased with velocity.

WBL content declined to resting levels in approximately 60 min of recovery (Fig. 4). A stepwise polynomial regression analysis showed that a fourth-order polynomial best described the WBL decline over time ($P < 0{\cdot}10$). The polynomial equations for each exercise velocity were generated by a curve fitting programme (Warme, 1981) and are presented in Table 1. Fig. 6 shows the derivative of the best-fit curves for the decrease in net WBL content during recovery. This represents the instantaneous rate of WBL removal. In Fig. 6 the net rate of WBL removal decreases with time at each exercise intensity level and approaches zero at approximately 30 min into recovery.

DISCUSSION

Exercise performance

Crabs display a unique style of locomotion. They run sideways. Fiddler crabs use this method when they are startled and flee back into their burrows or during slow walking as they travel many metres to feed. All eight walking legs are involved and the gait is equivalent to an alternating tetrapod (Barnes, 1975). This pattern of locomotion was observed in *Uca pugilator* at all velocities.

At velocities between 0·06 and 0·16 km h^{-1} all crabs maintained a consistent gait for 15 min. When the treadmill velocity was increased to 0·17–0·20 km h^{-1}, the crabs could not sustain the high stepping frequencies required and the time to fatigue declined greatly (Fig. 1). Fiddler crab exercise performance can be directly compared to only one other crustacean species, the ghost crab, *Ocypode gaudichaudii*. The smaller members of this highly aerobic crab species (mass = 2·8 g), tested under identical conditions, could run at velocities over 0·30 km h^{-1}, twice that of *Uca pugilator*, before a substantial decline in endurance time was osberved (Full & Herreid, 1983). In large land crabs endurance time decreases dramatically in a similar range of velocities (V); for example in *C. carnifex* (50–300 g) V = 0·20–0·30 km h^{-1} (Wood & Randall, 1981*a*), in *C. guanhumi* (150 g) V = 0·18 km h^{-1}, and in *G. lateralis* (50 g) V = 0·16 km h^{-1} (Herreid *et al.* 1979, 1983). The range of maximum sustainable velocities can serve as an indicator of an animal's capacity for activity. It is not clear what morphological or physiological constraint is operating to limit activity at these velocities. With data on only a few species, it appears that crabs fall near the low end of the range of sustained running velocities when compared to poikilotherms such as lizards (Bennett, 1980; John-Alder & Bennett, 1981).

Exercise period

Aerobic metabolism

Many animals power sustained locomotion primarily by aerobic metabolism. This is best exemplified in homoeothermic vertebrates and insects where $\dot{V}_{O_2}$ kinetics during terrestrial locomotion show only short time lags, small O_2 deficits and a $\dot{V}_{O_{2ss}}$ which represents the total energy required to run at a given velocity (Brackenbury & Avery, 1980; Cerretelli *et al.* 1979; Herreid, 1981; Marconi *et al.* 1982). In addition

variety of mammals running on treadmills can elevate $\dot{V}_{O_2}$ at least 10-fold over their already high resting levels (Taylor *et al.* 1980).

It has been suggested that crustaceans are limited in their abilities (1) to remove O_2 from the medium because of the low diffusive conductance of the chitinous gill and (2) to deliver O_2 convectively by way of an open circulatory system (McMahon, 1981; Taylor, 1982). In spite of such predictions, some crustaceans are capable of rapid increases in $\dot{V}_{O_2}$ with aerobic factorial scopes of 10-fold or greater (Full & Herreid, 1983; Rutledge & Pritchard, 1981). $\dot{V}_{O_2}$ in the ghost crab, *Ocypode guadichaudii*, has been shown to rise quickly to a 'steady-state' at all running velocities. The maximum $\dot{V}_{O_2}$ was nearly 12 times the resting level (Full & Herreid, 1983).

However, for *Uca pugilator* and the majority of crustaceans studied thus far, aerobic capacity seems limited (Booth, McMahon & Pinder, 1982; Burke, 1979; Herreid, 1981; McMahon, McDonald & Wood, 1979; Wood & Randall, 1981*a*). Large land crabs, such as *C. guanhumi* and *G. lateralis*, show a slow increase in $\dot{V}_{O_2}$ during treadmill locomotion with no 'steady-state' being attained in 10–20 min; $t_{1/2}$ on to peak $\dot{V}_{O_2}$ range from 4–6 min (Herreid *et al.* 1979, 1983). Aerobic factorial scopes at the conclusion of heavy exercise ($0{\cdot}16$–$0{\cdot}18\,km\,h^{-1}$) for both species were modest (e.g. three- to five-fold increases). The aerobic response of *U. pugilator* to exercise is similar to these large land crabs. Aerobic capacity is limited; $0{\cdot}22\,ml O_2\,g^{-1}\,h^{-1}$ was the largest rate recorded. The volume of oxygen used during exercise increased only slightly as a function of velocity (Fig. 2). The peak $\dot{V}_{O_2}$ at high velocity ($0{\cdot}16\,km\,h^{-1}$) was approximately 4·4 times the resting values (Fig. 2). Comparable factorial increases have been reported in fiddler crabs during diurnal and tidal activity rhythms. Brown *et al.* (1951) observed the greatest $\dot{V}_{O_2}$ values in the morning and at low tide, the time when crabs leave their burrows to feed at the water's edge.

The $\dot{V}_{O_2}$ kinetics of *Uca pugilator* are sluggish ($t_{1/2}$ on-response = 4–6 min) and no 'steady-state' is attained during 15 min of locomotion (Fig. 2). $\dot{V}_{O_2}$ kinetics have been most rigorously examined and are best understood for exercising humans (Stainsby & Barclay, 1970). A delayed $\dot{V}_{O_{2ss}}$, like that of *Uca pugilator*, has been described for sedentary humans forced to exercise with arms or legs (Cerretelli, Pendergast, Paganelli & Rennie, 1979; Cerretelli *et al.* 1977). For these untrained subjects to meet their energy demands during the large O_2 deficit, glycolysis is accelerated leading to lactate build-up. As seen below, an analogous situation is present in *Uca pugilator* and probably other crab species with a similar aerobic response.

Anaerobic metabolism

In exercising vertebrates anaerobic fermentation supplements aerobic ATP production, increasing the scope for activity (Bennett, 1978, 1980). Lactate accumulates in the blood primarily under three exercise conditions: (1) early in submaximal exercise during the O_2 deficit period (Cerretelli *et al.* 1979; Gleeson, 1980), (2) during $\dot{V}_{O_{2ss}}$ at submaximal work intensities greater than 50 % maximal $\dot{V}_{O_2}$ (Nagle *et al.* 1970; Seeherman, Taylor, Maloiy & Armstrong, 1981) and (3) at work levels exceeding maximal $\dot{V}_{O_2}$ (Margaria *et al.* 1963; Bennett, 1978). Lactate is probably produced in exercising crustaceans under similar conditions.

The major role that lactate fermentation plays in fiddler crab locomotion can be seen by first considering the time course of WBL accumulation. Net WBL increased

early in the exercise period and continued to increase at a constant rate until the end of the run (Fig. 4). The marked dependence on anaerobic metabolism is not unexpected in light of the slow, modest increases in $\dot{V}_{O_2}$ during exercise. Haemolymph lactate accumulation has been measured by Wood & Randall (1981*b*) for the land crab, *C. carnifex*, after 10 min of treadmill exercise at a sustained, submaximal velocity ($0{\cdot}14\,km\,h^{-1}$). These authors state that $\dot{V}_{O_{2ss}}$ was probably not attained during the exercise bout and certainly such is the case for the related species, *C. guanhumi* (Herreid *et al.* 1979). Consequently, as in exercising vertebrates, lactate accumulation is likely in crustaceans during the O_2 deficit period. Moreover, the lack of a $\dot{V}_{O_{2ss}}$ in the fiddler crab suggests that aerobic metabolism is inadequate to meet the energetic requirement throughout the 15-min exercise period (Fig. 2).

The relationship of anaerobic metabolism and exercise intensity in crustaceans is poorly understood. Numerous studies on crustaceans report significant increases in haemolymph lactate after high-intensity activity, probably approaching maximal $\dot{V}_{O_2}$ (Burke, 1979; McMahon *et al.* 1979; McDonald, McMahon & Wood, 1979; Phillips, McKinney, Hird & McMillan, 1977; Smatresk & Cameron, 1981; Wood & Randall, 1981*b*). In mammals only exercise above 50 % maximal $\dot{V}_{O_2}$ results in accumulation of blood lactate. Blood lactate shows a rapid, near-linear elevation for exercise at or exceeding maximal $\dot{V}_{O_2}$ (Seeherman *et al.* 1981). Net WBL in the fiddler crab increased in a linear fashion even at the lowest velocity ($0{\cdot}06\,km\,h^{-1}$; see Fig. 4). When velocity was increased the net rate of WBL production increased further. This indicates that anaerobic metabolism in *Uca pugilator* may contribute to the activity energetics of behaviour patterns of even relatively low intensity. The range of activity powered solely by aerobic metabolism appears to be narrow.

ATP production

The relative contribution of anaerobic and aerobic processes to exercise over time can be directly compared if an equivalent measure, the rate of ATP produced, is adopted. For *Uca pugilator* the pathway of lactate production yields ATP at a constant rate from the onset of exercise (Fig. 5). The major contribution of this pathway to the summed rate of ATP production (fermentation rate plus aerobic rate) is found early in exercise. It should be stressed that the anaerobic contribution from fermentation is estimated from the *net* WBL production, as lactate has been shown to be both produced and utilized during exercise (Gleeson & Bennett, 1982; Issekutz, Shaw & Issekutz, 1976). Lactate which is produced and subsequently oxidized would be represented in the aerobic contribution.

Aerobic metabolism yields progressively greater amounts of ATP in the later stages of exercise. Thus, the summed rate of ATP production increases throughout exercise, reaching a maximum at the end of the period. Since there is no evidence to suggest that energy demand increases with time, this maximum rate is taken to be the best estimate of the energy demand for the complete duration of the run (Fig. 5). Under these circumstances, a portion of the O_2 deficit is still unaccounted for. This volume of oxygen probably represents the contribution of O_2 stores and high-energy phosphate compounds (e.g. ATP and arginine phosphate).

The accuracy of the energy demand estimate will depend on the state of O_2 and high-energy phosphate stores. The predicted energy demand will be an underestimat

the contribution of these stores greatly exceeds that shown in Fig. 5. This is unlikely even if maximally estimated stores are totally depleted (Herreid, 1981). The energy demand may be an overestimate if the depleted stores are significantly replenished during exercise. These caveats aside, data from a few exceptional runners at low velocity suggest that the energy demand prediction based upon our maximum

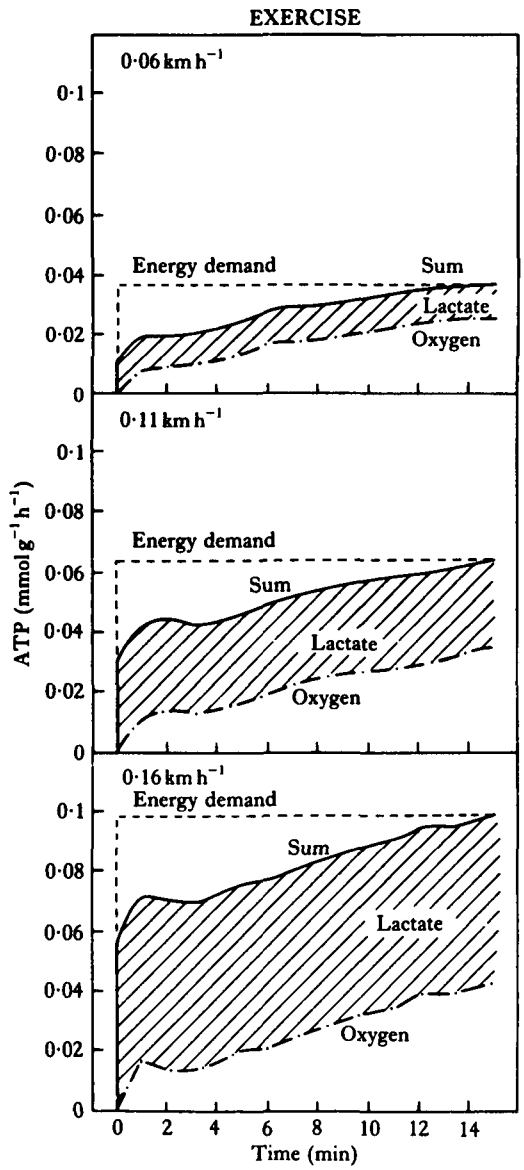

Fig. 5. Net rate of ATP production for aerobic metabolism (oxygen), lactate fermentation (lactate) and the sum of the two over a 15-min exercise period. The dashed line represents the estimated energy demand when all components of O_2 deficit (probably O_2 and high-energy phosphate stores plus lactate production) along with $\dot{V}_{O_2}$ are included. Top, middle and lower graphs represent three exercise velocities.

summed ATP rate is reasonable. In these crabs a $\dot{V}_{O_2ss}$ comparable to the energy demand estimate is attained in 20–25 min while net WBL production approaches zero.

As *Uca pugilator* was run faster on the treadmill the net rate of WBL production increased significantly, while $\dot{V}_{O_2}$ showed only a small increase with velocity. Fig. 8 shows the relative contribution of anaerobic and aerobic ATP production for all three velocities. The more intense the exercise, the greater was the proportion of ATP produced from lactate fermentation. This finding is similar to data on exercising mammals at velocities where O_2 delivery approaches maximal; near this point only small increases in O_2 uptake are observed and the rate of lactate production in the blood increases linearly with velocity (Margaria *et al.* 1963; Seeherman *et al.* 1981).

Recovery

Enhanced energy production and utilization does not come to a halt immediately after exercise. An elevated $\dot{V}_{O_2}$ during recovery is common in vertebrates and may last from 30 min to well over 6 h (Bennett, 1978). In *Uca pugilator* the decline in $\dot{V}_{O_2}$ to pre-exercise values was not observed until 30–45 min (Fig. 2).

The volume of oxygen consumed above resting values during recovery has been traditionally referred to as the oxygen debt (Knuttgen, 1971). The return of $\dot{V}_{O_2}$ to resting levels after exercise was once thought to possess two components which simply repayed the oxygen debt incurred (O_2 deficit) at the onset of exercise (Margaria, Edwards & Dill, 1933). The initial, fast repayment component (alactacid O_2 debt) was believed to be due to the resynthesis of high-energy phosphates and the second, slow component (lactacid O_2 debt) due to the extra oxygen required for the conversion of lactate to glycogen. Recent studies do not support the classical hypothesis; in fact it has been suggested that the term EPOC be used in place of oxygen debt (Brooks & Gaesser, 1980; Gleeson, 1980; Segal & Brooks, 1979). Substantial evidence exists that numerous factors contribute to one or both of the 'components' of EPOC (Knuttgen, 1971; Stainsby & Barclay, 1970). The relationship between O_2 deficit and EPOC for the fiddler crab is shown in Fig. 3. The magnitude of O_2 deficit and EPOC are similar at each velocity. This is consistent with the original hypothesis of O_2 debt. Whether this observation is simply fortuitous or an indicator of an actual coupling awaits clarification of EPOC components in crustaceans.

A major controversy in the O_2 debt hypothesis involves the lactacid component. In the classical interpretation, based on studies of *in vitro* amphibian muscle, the lactacid component was thought to consist of the additional oxygen required for the oxidative removal of 20% of the lactate produced during exercise (Knuttgen, 1971). The energy derived from lactate oxidation could then provide for the conversion of the remainder of lactate (80%) to glycogen. While some studies support this conclusion, others do not. Hermansen & Vaage (1977) have found that 75% of the lactate was converted to glycogen after heavy exercise in man. Yet, just the reverse was demonstrated for treadmill exercising rats; over 80% of the radioactively labelled lactate was oxidized to CO_2 and H_2O (Brooks, Brauner & Cassens, 1973; Brooks & Gaesser, 1980). A gluconeogenic path of lactate removal has been suggested in some lizards (Gleeson, 1982), salamanders (Hutchison, Trurney & Gratz, 1977) and snakes (Gratz & Hutchison, 1977). This requires a close correlation of $\dot{V}_{O_2}$ and lactat

removal. Yet, in other lower vertebrates EPOC returns to resting levels well before lactate significantly declines (Bennett, 1978; Gleeson, 1980).

The fate of lactate after exercise in crustaceans is unknown (Phillips *et al.* 1977). Lactate removal after activity is usually slow (8–24 h) as is the return of $\dot{V}_{O_2}$ to pre-exercise rates (McMahon, 1981). In *U. pugilator* net WBL content declined to resting levels in approximately 1 h (Fig. 4). The net rate of the WBL removal during recovery can be computed by taking the derivative of the WBL *versus* time function (Fig. 4). Fig. 6 shows that the maximal net rate of WBL removal occurs immediately after exercise and approaches zero in 30 min. WBL clearance in the fiddler crab is clearly content dependent. The greater the amount of lactate that is present, such as after exercise at the high velocity, the faster is its rate of removal.

The net rate of WBL removal and $\dot{V}_{O_2}$ show a similar time course during the recovery period (Figs 2 and 6). The $t_{1/2}$ off-responses for the $\dot{V}_{O_2}$ pattern range from 7–10 min. The same range of times is found if one examines the time to 50 % WBL clearance rate. Again, the correspondence in time course suggests a possible causal relationship. Assuming that complete oxidation and gluconeogenesis are the two most probable paths, the question becomes which fate of lactate is compatible with EPOC in *U. pugilator*? An estimation of the oxygen required to remove an amount of lactate over a given time interval can be made using the net WBL clearance equations (Table 1) and assuming a standard stoichiometric relationship for the O_2 used in either lactate oxidation or gluconeogenesis. Fig. 7 shows a cumulative record of the actual oxygen

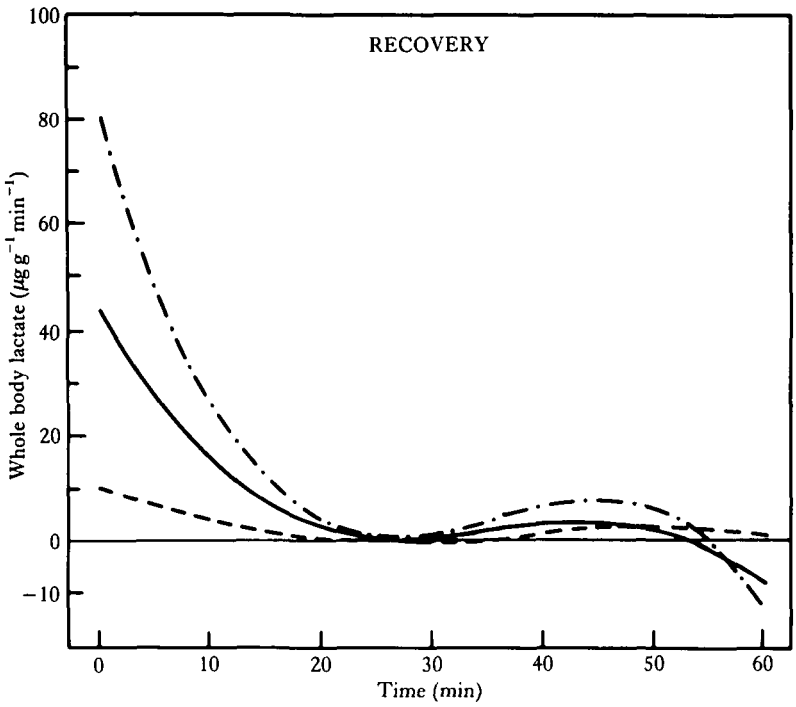

Fig. 6. Net rate of whole body lactate removal during a 60-min recovery period after exercise at 0·16 (—·—), 0·11 (—) and 0·06 (– –) km h^{-1}. Horizontal line represents the rate at which no lactate removal or production occurs.

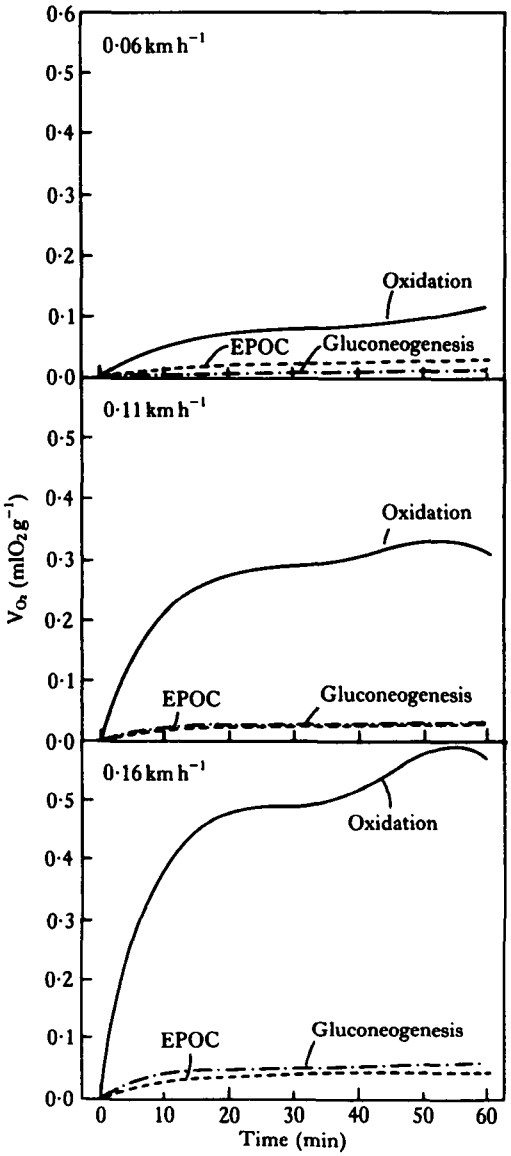

Fig. 7. Cumulative volume of oxygen required during a 60-min recovery for net lactate removal by oxidation (—) and gluconeogenesis (—·—) compared to the actual amount used (EPOC; --). Top, middle and lower graphs represent three exercise velocities.

consumed (EPOC) and the amount predicted for WBL removed by either oxidation or conversion to glycogen. The oxygen required for direct oxidation of WBL to CO_2 and H_2O greatly exceeds that actually consumed. In contrast, the O_2 requirement predicted for gluconeogenesis is quite similar to that actually found in recovey. Therefore, analysis of EPOC and WBL clearance in fiddler crabs indicates that the classical interpretation of lactate removal *via* gluconeogenesis may be appropriate.

Cost of transport

The minimum cost of transport (M_{run}), the amount of oxygen required to move a gram of animal over a kilometre distance, has commonly been used to evaluate an animal's economy of locomotion (Taylor, Schmidt-Nielsen & Raab, 1970; Taylor, Heglund & Maloiy, 1982). The M_{run} value has the advantage of being independent of an animal's resting $\dot{V}_{O_2}$ and range of running velocities, thus allowing comparison among different species. In a number of mammals, birds and reptiles $\dot{V}_{O_{2ss}}$ increases linearly with velocity (Taylor, 1977). M_{run} is frequently determined by calculating the slope of the $\dot{V}_{O_{2ss}}$ *versus* velocity relationship or by dividing the $\dot{V}_{O_{2ss}}$ (less the resting rate) by velocity at the velocity where the minimum value is reached. In either case, the $\dot{V}_{O_{2ss}}$ must be assumed to represent the total amount of energy required to locomote at a given velocity. This assumption is generally warranted for the more aerobic vertebrates exercising on treadmills (Rome, 1982; Seeherman *et al.* 1981).

M_{run} for terrestrial locomotion has been determined in only one crustacean, the ghost crab, *Ocypode gaudichaudii* (Full & Herreid, 1983). This species rapidly attains a $\dot{V}_{O_{2ss}}$ during exercise and exhibits a linear increase in $\dot{V}_{O_{2ss}}$ with running

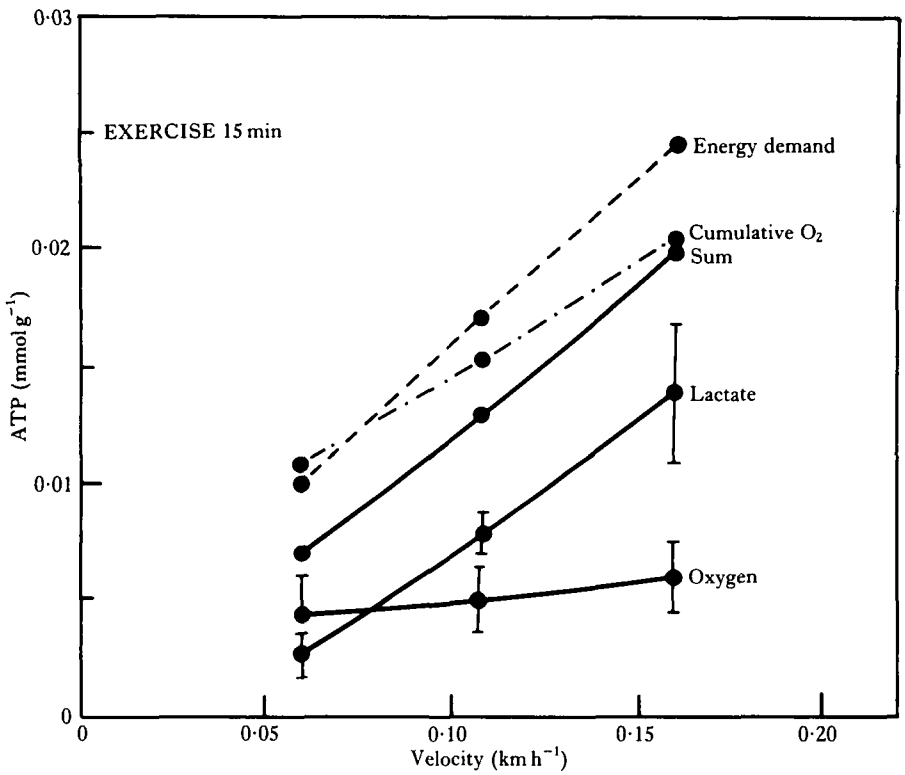

Fig. 8. Estimated amount of ATP produced from aerobic metabolism (oxygen), lactate fermentation (lactate) and the sum of both aerobic metabolism and anaerobic fermentation (sum) for 15 min of exercise. The cumulative net O_2 line (—·—) includes the sum of exercise and recovery V_{O_2} minus the resting rate. The top line (––) represents the best estimate of energy demand at a given velocity (see Fig. 5; included are estimates of other O_2 deficit components). Error bars at each velocity represent 95 % confidence intervals.

velocity. Other crab species, such as *C. gaunhumi* and *G. lateralis*, do not attain a $\dot{V}_{O_2ss}$ and therefore no comparable M_{run} value can be calculated. Estimated M_{run} values for these two species have been determined by using the cumulative net V_{O_2} (sum of exercise and recovery V_{O_2} minus the resting V_{O_2}; see Herreid, 1981). This approach assumes the oxygen used during recovery completely repays the oxygen deficit incurred during exercise and is therefore subject to the same criticisms as the classical O_2 debt hypothesis.

The fiddler crab does not meet the $\dot{V}_{O_2ss}$ criteria for the standard calculation of M_{run}. However, our energetic analysis does allow estimation of both aerobic and anaerobic components. Both $\dot{V}_{O_2}$ and net WBL production show significant increases with velocity (Figs 3 and 4). The total amount of ATP derived from lactate fermentation plus that from V_{O_2} during 15 min of exercise can be calculated for each exercise velocity by integrating under the ATP production curves in Fig. 5. At low velocity, V_{O_2} accounted for 60 % of the ATP produced by the sum of aerobic and fermentation processes (Fig. 8). At medium and high velocity, lactate fermentation dominated and contributed 60 and 70 % to the summed ATP values, respectively. Since both sets of ATP values increased in a linear fashion with velocity, an M_{run} value can be estimated for aerobic and anaerobic metabolism. The aerobic M_{run} value is only one-third that of the anaerobic fermentation value. The M_{run} value for the summed ATP relationship is $1{\cdot}8\,ml O_2\,g^{-1}\,km^{-1}$ (mmol ATP converted to $ml O_2$).

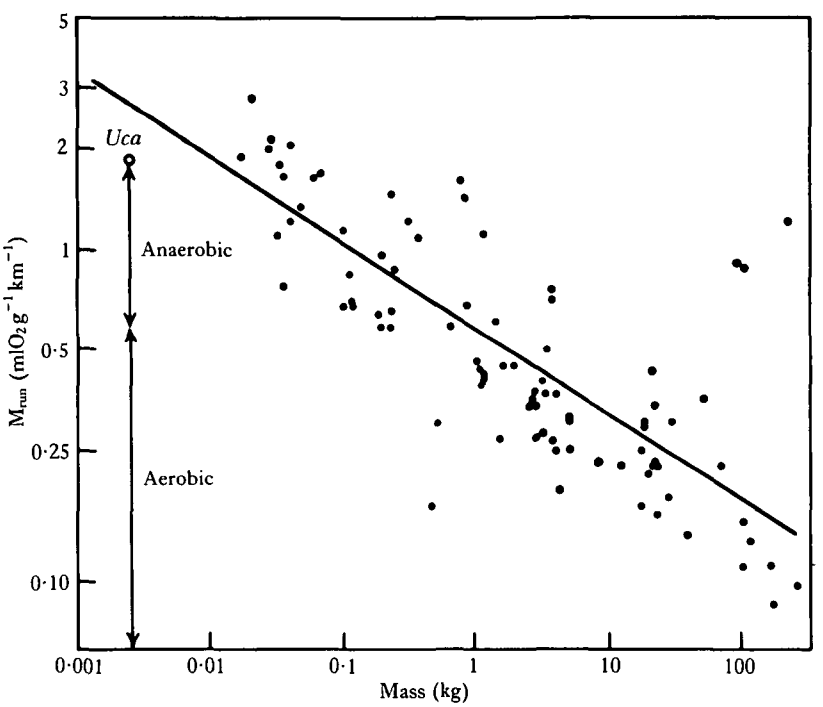

Fig. 9. Log minimum cost of locomotion *versus* log body mass. Points and regression line are taken from Taylor, Heglund & Maloiy (1982) and represent 62 species of birds and mammals. The relative contributions of aerobic and anaerobic metabolism for *Uca pugilator* are shown. Note the actual anaerobic contribution is three times that of the aerobic but appears smaller due to the log scale.

The economy of locomotion can also be determined for our best estimate of the actual energy demand (see Figs 5 and 8). This M_{run} value includes other possible components of the O_2 deficit – O_2 and high-energy phosphate stores in addition to lactate fermentation – and further emphasizes the major role of energy sources not reflected in $\dot{V}_{O_2}$. However, when an M_{run} value is calculated from our estimate of energy demand, then a cost ($2{\cdot}2\,ml O_2\,g^{-1}\,km^{-1}$) only somewhat greater than the sum M_{run} is found. It is interesting to note that in *Uca pugilator* the M_{run} value determined by the cumulative net V_{O_2} method ($1{\cdot}0\,ml O_2\,g^{-1}\,km^{-1}$) is comparable in magnitude to the other two estimations (Fig. 8). It appears that even when estimated by different approaches, locomotion economy for *Uca pugilator* falls within the range predicted for a running vertebrate with a similar mass (Fig. 9).

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