

Intermittent exercise alters endurance in an eight-legged ectotherm

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Weinstein, Randi B., and Robert J. Full. Intermittent exercise alters endurance in an eight-legged ectotherm. *Am. J. Physiol.* 262 (Regulatory Integrative Comp. Physiol. 31): R852–R859, 1992.—Most animals move intermittently, yet many proposed performance limitations of terrestrial locomotion are based on steady-state measurements and assumptions. We examined the effect of work-rest transitions by exercising the ghost crab, *Ocypode quadrata* (28.1 ± 8.1 g), intermittently on a treadmill at 0.30 m/s, a supramaximal speed [i.e., greater than the speed that elicits the maximal rate of oxygen consumption ($\dot{V}O_2$)]. Duration of the exercise and pause periods, ratio of exercise to pause, and speed during the exercise period were varied to determine the effect on performance. Crabs fatigued after 7.5 min of continuous running, a distance capacity (i.e., total distance traveled before fatigue) of 135 m. When the task was done intermittently with 2-min exercise and 2-min pause periods, the crabs fatigued after 87 min (a total distance of 787 m), representing an 5.8-fold increase in distance capacity compared with continuous exercise at the same absolute speed (0.30 m/s) and a 2.2-fold increase in distance capacity compared with continuous exercise at the same average speed (0.15 m/s). Pause periods <30 s did not result in greater distance capacity compared with continuous exercise at the same average speed. Longer (3–5 min) and shorter exercise periods (≤ 30 s) decreased distance capacity. Leg muscle lactate increased 10-fold to 15 $\mu\text{mol/g}$ leg during intermittent exercise. However, significant amounts of lactate were cleared from the leg during the brief pause periods. The average $\dot{V}O_2$ during intermittent exercise was not statistically different from maximal $\dot{V}O_2$. The aerobic cost of intermittent exercise, both per time and per distance, was equal to or greater than the aerobic cost of continuous exercise at the same average speed. Further examination of dynamic physiological adjustments, such as in intermittent exercise, is likely to alter the performance limits established under a steady-state paradigm.

oxygen consumption; muscle; lactate; arthropods

STEADY-STATE OXYGEN CONSUMPTION ($\dot{V}O_2$) increases with speed during constant-speed terrestrial locomotion until a maximal rate of consumption ($\dot{V}O_{2\text{max}}$) is attained. Endurance of continuous locomotion has been shown to be correlated with the maximal capacities of an animal for aerobic metabolism (4). Body size (36, 55), body temperature (39, 40), and the metabolic cost of locomotion (23) all affect $\dot{V}O_{2\text{max}}$ or the speed at which it is attained [i.e., maximum aerobic speed (MAS) (39)]. Exercise at speeds below the MAS (i.e., submaximal speeds) is considered sustainable and can be maintained by aerobic metabolism. Locomotion at speeds greater than MAS (i.e., supramaximal speeds) requires supplemental energy provided by anaerobic metabolism and is considered unsustainable, because it rapidly leads to exhaustion. In mammals (53), lizards (39, 40), salamanders (20), and crabs (21), endurance declines considerably at speeds approaching the MAS.

Results from studies on constant-speed steady-state exercise have been used 1) to develop design principles for oxygen and energy transport (57), 2) to advance hypotheses concerning the evolution of endothermy (6),

and 3) to predict natural locomotory behavior (5, 37). However, steady-state continuous exercise could represent “an artificial situation for many animals” (2) and the systems that support activity. Few animals move continuously. Most animals start and stop; that is, they move intermittently. Studies of intermittent exercise indicate that alternating periods of high-intensity exercise with pause periods, during which low-intensity or no work is done, can alter metabolic responses and endurance (52). Early studies of intermittent exercise on humans showed that when a heavy (i.e., supramaximal) work load was divided into short exercise and pause periods, the heavy work load was “transformed to a submaximal load on circulation and respiration and could be well tolerated” (1). The duration of the exercise and pause periods had profound effects on endurance and lactate accumulation (13, 14). Significant lactate accumulation and phosphagen depletion in humans can be prevented by performing intermittent exercise with short exercise periods (i.e., <30 s) and longer pause periods (16, 19, 44, 51).

These previous studies lead us to propose that examination of intermittent locomotion and dynamic physiological adjustments should be the next paradigm used to evaluate the performance limits of oxygen and energy transport systems in exercising animals. Moreover, use of intermittent protocols could be used to improve predictions of natural locomotory behavior previously derived from steady-state tests.

In the present study we examine the effect of intermittent exercise on the performance of an eight-legged ectotherm. We selected the ghost crab, *Ocypode quadrata*, for several reasons. First, the ghost crab has a low $\dot{V}O_{2\text{max}}$ and MAS relative to endotherms. The MAS for the ghost crab is a walking speed and reflects the inability of the ghost crab to sustain even a fast walking pace (8, 21). We hypothesize that intermittent locomotion will be of greater consequence in ectotherms and may represent a behavior that either reduces the limitations associated with a low $\dot{V}O_{2\text{max}}$ and MAS or imposes additional constraints. Second, much is known about the ghost crab's locomotor performance with respect to biomechanics, energetics, endurance, and muscle biochemistry (8, 21, 26, 27). Third, observations of ghost crabs in their natural habitat suggest that these active animals move intermittently, making frequent starts and stops rather than moving continuously for long distances along the beach (T. G. Wolcott, personal communication). Finally, several studies have been published on the continuous steady-state exercise performance of the ghost crab (8, 21, 24), and we have access to the same population of crabs used in these studies.

MATERIALS AND METHODS

Animals

The ghost crab, *O. quadrata* [28.1 ± 8.1 (SD) g], was collected from the beaches surrounding the Duke University Marine Laboratory in Beaufort, NC. Individual crabs were housed in plastic

containers filled with 30–50% seawater to a depth of 1 cm. The animals were kept in an environmental chamber on an 14:10 h light-dark photoperiod at 25°C. The crabs were fed fresh fish two or three times a week, and their containers were cleaned and filled with fresh seawater 24 h after feeding. Only intermolt crabs were used. Individuals were kept in captivity <5 wk. To maximize the number of experimental trials, crabs were randomly assigned to three or four exercise groups (see below). As a result, no individual exercised at all protocols. Each individual rested for at least 5 days between trials.

$\dot{V}O_2$ and Distance Capacity Protocol

Resting $\dot{V}O_2$. $\dot{V}O_2$ was measured as the crabs rested quietly in a treadmill respirometer for 30 min. Metabolic rate was determined for 10 min before each exercise trial. The $\dot{V}O_2$ value during the final 6 min of the preexercise period was averaged to obtain the resting $\dot{V}O_2$ ($\dot{V}O_{2\text{ rest}}$). All trials were conducted at 25°C.

Continuous exercise. The treadmill speeds selected for the present study were based on previous investigations of *O. quadrata* (21). Crabs were exercised continuously to fatigue on a treadmill at 0.3 or 0.4 m/s. A speed of 0.18 m/s elicits $\dot{V}O_{2\text{ max}}$ and is therefore the MAS for these crabs (21). The speeds selected for continuous exercise, 0.30 (170% MAS) and 0.40 m/s (220% MAS), exceed MAS and were supramaximal. Measurements of $\dot{V}O_{2\text{ max}}$, MAS, and endurance during continuous exercise on crabs from the present study were not significantly different from those reported previously (21). The population of ghost crabs used in the present study was from the same crab population used by Full (21).

Intermittent exercise. Crabs exercised intermittently to fatigue by alternating exercise periods with pause periods. Four variables were manipulated in the intermittent exercise trials: 1) exercise duration (E), 2) pause duration (P), 3) E-to-P ratio, and 4) speed during the exercise period. Intermittent exercise protocols are outlined in Table 1 and include the corresponding absolute (AbS) and average speeds (AvS) for comparison with continuous exercise.

$\dot{V}O_2$ Measurements

Crabs were exercised in a miniature treadmill-respirometer. Air flow rate was 300 ml/min. Air leaving the chamber was dried with calcium sulfate (Drierite), and CO_2 was removed with asbestos (Ascarite). The oxygen concentration was monitored with an O_2 analyzer (Ametek S3A), which was interfaced with a personal computer (Macintosh II) by data acquisition hard-

ware and software (LabView, National Instruments). Mass-specific $\dot{V}O_2$ was calculated from the O_2 concentration (35). Calculations of instantaneous $\dot{V}O_2$ (21, 36) were not significantly different from rates determined without the correction (58). Measurements of $\dot{V}O_2$ were only made for the exercise protocols where the exercise period was at least 120 s.

Distance Capacity Measurements

Crabs were considered to be fatigued when they could no longer keep pace with the treadmill belt, even when prodded (21). Data from trials during which crabs walked erratically were discarded. Distance capacity (i.e., the total distance traveled continuously or intermittently before fatigue) was used as the index of endurance, instead of time to fatigue, because the term endurance usually connotes only continuous activity. The distance capacity for each trial was calculated from the treadmill speed and the number and duration of exercise intervals completed. The distance capacity for intermittent exercise was compared with continuous exercise at the same AbS used during the exercise intervals and with continuous exercise at the same AvS.

Muscle Lactate Protocol and Measurements

After the $\dot{V}O_2$ and distance capacity measurements were made, crabs were divided into four groups: rest (R), continuous exercise (CE), intermittent exercise (IE), and intermittent pause (IP; Fig. 1). In the R group, animals rested in the treadmill chamber for 30 min before being frozen in liquid nitrogen. The CE group of crabs was exercised continuously to fatigue on the treadmill at 0.30 m/s and then was immediately frozen in liquid nitrogen. The IE protocol consisted of 2-min exercise periods (speed 0.30 m/s) alternated with 2-min pause periods. The crabs in the IE group were frozen in liquid nitrogen upon completion of the fourth exercise period. Crabs in the IP group were frozen at the end of the fourth pause period. Running direction was recorded to document whether legs were leading or trailing because the crabs run sideways.

Whole animals were frozen rapidly (i.e., <2 s) in liquid nitrogen and stored at $-80^\circ C$ for <1 wk before tissue preparation and analysis. Leading and trailing third walking legs, along with the associated extrinsic musculature (i.e., within the body), were dissected free in a cold room ($-5^\circ C$) and immediately homogenized in 4 vol of perchloric acid. After centrifugation the resulting supernatant was neutralized with K_2CO_3 . Leg lactate concentration was determined according to Full and Herreid (25) with modifications from Prestwich (49).

Statistical Analysis

Unless noted, values are reported as means \pm SE.

Distance capacity. Statistical comparisons at submaximal AvS values were made by comparing 95% confidence intervals

Table 1. Intermittent exercise protocols

Protocol No.	Exercise Period, s	Pause Period, s	Exercise-to-Pause Period Ratio	AbS, m/s	AvS, m/s
Constant ratio					
1	30	30	1	0.30	0.15
2	120	120	1	0.30	0.15
3	180	180	1	0.30	0.15
4	240	240	1	0.30	0.15
5	300	300	1	0.30	0.15
Varied pause duration					
6	120	30	4	0.30	0.23
7	120	60	2	0.30	0.19
2	120	120	1	0.30	0.15
8	30	5	6	0.30	0.25
9	30	15	2	0.30	0.19
1	30	30	1	0.30	0.15
10	30	60	0.5	0.30	0.10
Varied speed					
11	5	10	0.5	0.30	0.10
12	5	10	0.5	0.40	0.13

AbS, absolute speed (i.e., speed during exercise period); AvS, average speed for a complete exercise-pause cycle.

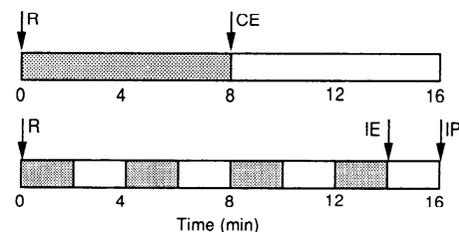


Fig. 1. Muscle metabolite protocol. Stippled bars, exercise periods; open bars, pause periods. Speed during exercise intervals was 0.30 m/s. Experimental groups are indicated by arrows; $n = 6$ crabs/group. R, rest group; CE, continuous exercise group; IE, intermittent exercise group; IP, intermittent pause group. Samples were taken after 30 min rest within the chamber (R), after continuous exercise to fatigue (CE), after the 4th intermittent exercise period (IE), and after the 4th intermittent pause period (IP).

for the total distance covered before fatigue during intermittent exercise with the 95% confidence intervals for the total distance covered before fatigue during continuous exercise at the same submaximal AvS values. For the comparison at 0.30 m/s, a two-tailed Mann-Whitney U test was performed.

Aerobic cost. We performed analysis of variance to determine the effect of experimental condition. For comparisons between treatments we used Scheffé's F test.

Muscle lactate. We performed an analysis of variance to determine the effect of the experimental condition on muscle lactate concentration. For comparisons between treatments we used Scheffé's F test. A paired t test was conducted to determine differences between leading and trailing legs within each condition.

RESULTS

$\dot{V}O_2$

$\dot{V}O_{2\text{ rest}}$. $\dot{V}O_{2\text{ rest}}$ at 25°C was 0.18 ± 0.05 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ ($n = 5$). This resting rate was not significantly different from data collected by Full (21).

Continuous exercise. After the onset of exercise, $\dot{V}O_2$ increased rapidly to a steady state ($\dot{V}O_{2\text{ ss}}$; Fig. 2A). The time to attain 50% of the $\dot{V}O_{2\text{ ss}}$ ($t_{1/2\text{ on}}$) was 38.5 ± 7.8 s ($n = 4$). This value for $t_{1/2\text{ on}}$ was not significantly different from data collected by Full (21), where $t_{1/2\text{ on}}$ was 28.2 s.

Intermittent exercise. For crabs exercising intermit-

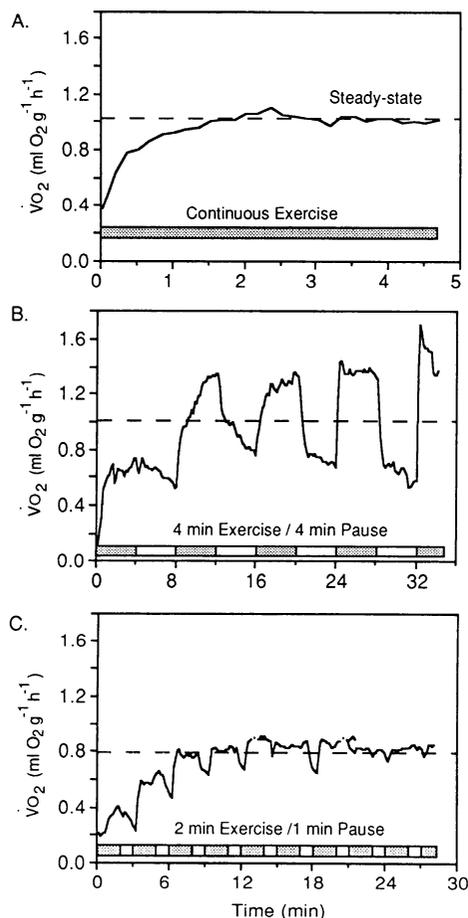


Fig. 2. Oxygen consumption ($\dot{V}O_2$) kinetics for continuous (A) and intermittent (B and C) exercise for 3 different individuals. Exercise protocols are indicated on each graph as exercise periods (stippled bars) and pause periods (open bars). Dashed lines represent values for average $\dot{V}O_2$.

tently, $\dot{V}O_2$ increased as the crab exercised and decreased as the crab paused (Fig. 2, B and C). Longer exercise and pause periods resulted in greater oscillations in $\dot{V}O_2$. Exercise intervals lasting ≥ 2 min tended to result in a steady state. Even the longest pause periods, 5 min, did not result in complete aerobic recovery (i.e., $\dot{V}O_2$ did not return to $\dot{V}O_{2\text{ rest}}$).

Aerobic Cost of Exercise

The average aerobic cost of intermittent exercise was determined by integrating segments of the intermittent exercise record that contained at least one complete exercise-pause cycle in which the sum of the increase and decrease in $\dot{V}O_2$ were within a constant percentage of the average $\dot{V}O_2$ of the animal. This average $\dot{V}O_2$ (aerobic cost) was expressed both per unit time (ml $O_2 \cdot g^{-1} \cdot h^{-1}$) and per distance (ml $O_2 \cdot g^{-1} \cdot km^{-1}$).

Aerobic cost per time. For continuous exercise, $\dot{V}O_{2\text{ ss}}$ increases linearly as exercise speed increases [Fig. 3A (21)]. At 0.18 m/s, the crab attained its $\dot{V}O_{2\text{ max}}$, and further increases in speed did not result in subsequent

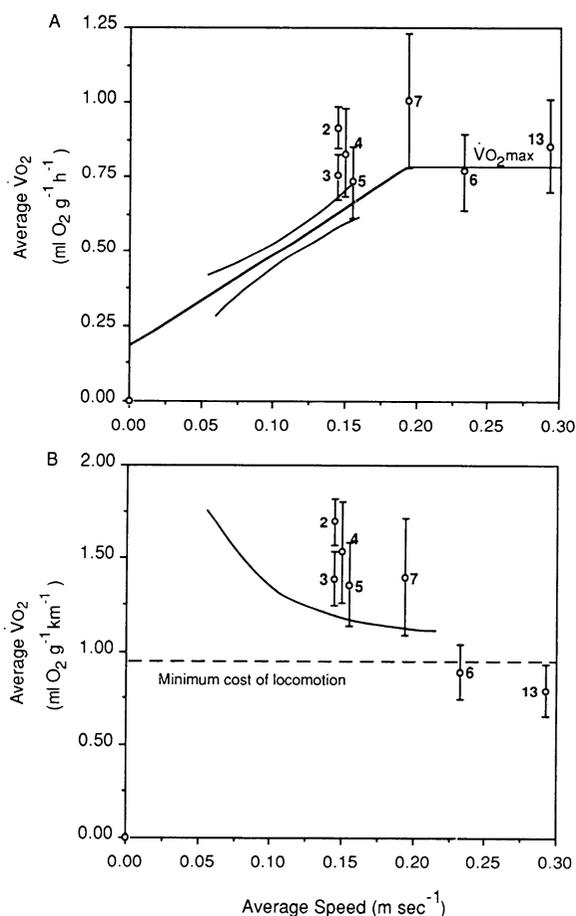


Fig. 3. A: average mass-specific $\dot{V}O_2$ or aerobic cost per time as a function of average speed. Values are means \pm SE. Solid line shows previous data for ghost crabs (mass 26.9 ± 0.7 g) exercising continuously (21). At submaximal speeds, 95% confidence intervals are included. Exercise protocols are indicated by numerals and correspond to protocols outlined in Table 1. Sample sizes are as follows: protocols 2 and 13, $n = 5$; protocols 6 and 7, $n = 4$; protocols 3 and 4, $n = 3$; and protocol 5, $n = 2$. $\dot{V}O_{2\text{ max}}$, maximal $\dot{V}O_2$. B: average mass-specific aerobic cost per distance as a function of average speed. Solid line represents aerobic cost of locomotion per distance for crabs exercising continuously (21). Exercise protocols and sample sizes as in A.

increases in $\dot{V}O_{2\text{ss}}$. The average aerobic cost per time of intermittent exercise was independent of the AvS ($F_{6,19} = 0.41$, $P = 0.87$; Fig. 3A) and was not significantly different from $\dot{V}O_{2\text{max}}$. The average aerobic cost per time for the 2-min E/2-min P protocol was significantly greater than the corresponding values for continuous exercise at the same AvS ($P < 0.05$) but was not significantly different from rates of other intermittent exercise protocols.

Aerobic cost per distance. The average aerobic cost per distance of intermittent exercise decreases as the speed of the exercise period increases and approaches the minimum cost of locomotion [C_{min} ; Fig. 3B (21)]. C_{min} is defined as the slope of the regression line from the plot of $\dot{V}O_{2\text{ss}}$ vs. speed (56) and represents the minimum amount of metabolic energy necessary to move 1 g of crab 1 km. The average aerobic cost per distance for the 2-min E/2-min P protocol was significantly greater than the corresponding values for continuous exercise at the same AvS ($P < 0.05$). The average aerobic cost per distance for other intermittent exercise protocols was not significantly different from the values corresponding to continuous exercise at the same AvS. At the highest AvS values tested (0.23 and 0.30 m/s), the aerobic cost per distance was not different from C_{min} .

Distance Capacity

Constant E-to-P ratio. Crabs exercising intermittently when E was 2 min (E/P = 1) traveled 5.8-fold farther before fatigue than those moving continuously at the same AbS (0.30 m/s, a supramaximal speed; $P < 0.001$) and 2.2-fold farther than those moving at the same AvS (0.15 m/s, a submaximal speed; $P < 0.05$; Fig. 4). When E was 3 min, the distance capacity was twofold greater than the total distance covered continuously before fatigue at the same AbS ($P < 0.005$) but was not different from the distance traveled continuously at the same AvS. When E was very short (30 s) or very long (4 or 5 min) and P was equal to E, the distance capacity was not statistically different from the total distance traveled continuously at the same AbS and was less than the total distance trav-

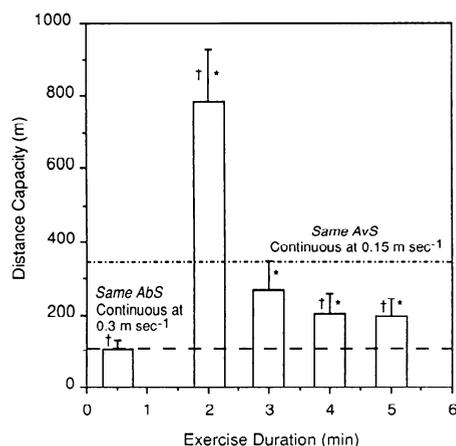


Fig. 4. Distance capacity as a function of exercise (and pause) duration. Exercise-to-pause duration ratio was kept constant (E/P = 1), and duration of exercise (and pause) period was varied. Values are means \pm SE; $n = 6$ for each protocol. Distance capacities are shown for comparison for crabs exercising continuously at same average speed (AvS; dot-dash line) or same absolute speed (AbS; dashed line). * Significantly different from distance capacity at same AbS ($P < 0.05$). † Significantly different from distance capacity at same AvS ($P < 0.05$).

eled before fatigue at the same AvS ($P < 0.05$).

Varied pause duration. When E was 120 s, a 30-, 60-, and 120-s pause period significantly increased the distance capacity compared with continuous exercise at both the same AbS ($P < 0.05$) and the same AvS ($P < 0.05$; Table 2). The longest pause duration (i.e., 120 s) resulted in the greatest distance capacity.

When E was 30 s, an increase in P did not increase distance capacity (Table 2). The total distance traveled intermittently was not significantly different from the distance traveled continuously at the same AvS when P was 5 and 15 s and was significantly less than the distance traveled continuously at the same AvS when P was 30 and 60 s ($P < 0.05$).

Varied speed. When the speed of the exercise interval was increased from 170 (0.30 m/s) to 220% MAS (0.40 m/s), the distance capacity decreased significantly ($P < 0.03$). The distance capacity for intermittent exercise in which the speed of the exercise period was 0.30 m/s (E = 5 s and P = 10 s) was not significantly different from the distance traveled continuously at the same AbS but was significantly less than the total distance traveled before fatigue for crabs exercising continuously at the same AvS ($P < 0.05$; Table 3). At the speed of 0.40 m/s during the exercise period, the distance capacity was significantly greater than the total distance traveled continuously at the same AbS ($P < 0.05$) but was significantly less than the distance capacity for crabs exercising continuously at the same AvS ($P < 0.05$).

Leg Muscle Lactate

Resting levels of lactate were $1.3 \pm 0.1 \mu\text{mol/g leg}$ (Table 4). Crabs that exercised continuously at 0.30 m/s fatigued after $7.5 \pm 0.9 \text{ min}$ ($n = 6$). Crabs in all exercise groups had significantly higher lactate levels than resting crabs ($P < 0.05$). No significant differences were found between the CE and IE groups or between the CE and IP groups. However, the lactate concentration in the IP group was significantly lower than that in the IE group ($P < 0.05$). In the IE group, the lactate levels were higher in the trailing leg than in the leading leg ($P < 0.01$).

DISCUSSION

Distance Capacity

Intermittent vs. continuous exercise. The limited aerobic capacities of ectotherms, relative to endotherms, per-

Table 2. Effect of pause duration on distance capacity

Exercise Period, s	Pause Period, s	Distance Capacity, m		
		Intermittent exercise	Continuous exercise at same AbS	Continuous exercise at same AvS
120	30	304.8 \pm 61.0*†	134.7 \pm 17.2	140
120	60	218.4 \pm 64.2*†	134.7 \pm 17.2	160
120	120	786.7 \pm 142.6*†	134.7 \pm 17.2	360
30	5	208.8 \pm 74.6	134.7 \pm 17.2	120
30	15	128.9 \pm 23.3	134.7 \pm 17.2	160
30	30	105.3 \pm 20.8†	134.7 \pm 17.2	360
30	60	123.6 \pm 36.3†	134.7 \pm 17.2	720

Values are means \pm SE; $n = 5$ for each intermittent exercise protocol. Values for distance capacity for continuous exercise at same AvS are estimated from data reported by Full (21). * Significantly different from distance capacity at same AbS ($P < 0.05$). † Significantly different from distance capacity at same AvS ($P < 0.05$).

Table 3. Effect of speed on distance capacity

Exercise Speed, m/s	Distance Capacity, m		
	Intermittent exercise	Continuous exercise at same AbS	Continuous exercise at same AvS
0.30	68.4±10.8*†	134.7±17.2	720
0.40	44.8±2.6*†	23.2±0.8	400

Values are means ± SE; $n = 5$ for each intermittent exercise protocol. Values for distance capacity for continuous exercise at same AvS are estimated from data reported by Full (21). * Significantly different from distance capacity at same AbS ($P < 0.05$). † Significantly different from distance capacity at same AvS ($P < 0.05$).

Table 4. Leg lactate concentration

	Leading Leg	Trailing Leg
Rest		1.3±0.1
Intermittent exercise	12.2±0.9	14.9±0.8
Intermittent pause	9.2±1.7	9.9±1.8
Continuous exercise	10.3±1.0	12.4±1.5

Values are means ± SE in $\mu\text{mol/g}$ leg; $n = 6$ for each condition.

mit only certain types of low-intensity continuous behaviors and preclude others that are of high intensity and demand stamina. The extent of these limitations has, thus far, only been determined for constant-speed steady-state exercise. The present results show that dynamic non-steady-state physiological adjustments can alter behavioral capacity and must be considered in ectotherms. Behaving intermittently can significantly alter the limitations on endurance determined during continuous activity.

The aerobic capacity of most ectotherms is an order of magnitude less than that of endotherms (6, 20, 24, 56). The highly active ghost crab is no exception (21). Because $\dot{V}O_{2\text{ss}}$ increases linearly with speed in most pedestrians, the low $\dot{V}O_{2\text{max}}$ of ectotherms is attained at speeds 1/7–1/10 that of endotherms (29). Ghost crabs change gait from a walk to a trot and a trot to a gallop at speeds similar to that of a mammal of the same body mass (22, 27). However, ghost crabs attain MAS at a slow walking speed, whereas a quadrupedal mammal can gallop before exceeding $\dot{V}O_{2\text{max}}$ (34). The speed at which $\dot{V}O_{2\text{max}}$ is attained is correlated with an inability to sustain continuous locomotion. Endurance of continuous steady-state exercise in the ghost crab decreases significantly at speeds approaching MAS (i.e., 0.18 m/s; Fig. 5).

Exercising intermittently can increase the distance capacity of ghost crabs significantly compared with continuous exercise and at the same time can permit behaviors of high intensity (i.e., supramaximal). With particular exercise and pause durations, crabs can walk further intermittently than they do continuously when compared at the same AbS or at the same AvS (Fig. 5). Not surprisingly, ghost crabs exercising intermittently (at 0.3 m/s; E = 2 min, P = 2 min) could walk 5.8-fold farther before fatigue than they could by exercising continuously at the same AbS. More importantly, however, a ghost crab could also walk twice as far intermittently at the same AvS used during continuous exercise (0.15 m/s) by walking twice as fast and resting half the time (Fig. 5). A ghost crab walking at speeds eliciting $\dot{V}O_{2\text{max}}$ (0.30 m/s) and resting half the time could still travel twice as far as

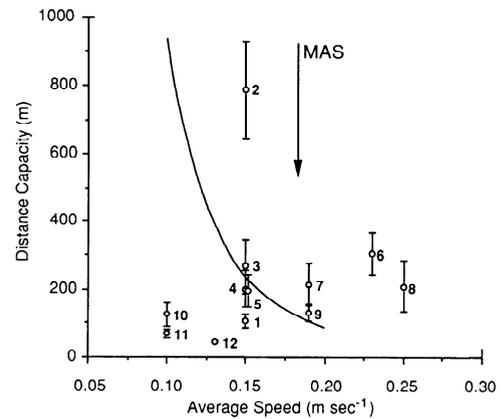


Fig. 5. Distance capacity as a function of average speed. Values are means ± SE; $n = 6$ for each protocol. Solid line represents distance capacity curve for continuous exercise (21). Experimental protocols are indicated by numerals and correspond to protocols outlined in Table 1. MAS, maximum aerobic speed.

a crab walking continuously at a speed that did not exceed $\dot{V}O_{2\text{max}}$ (0.15 m/s). By exercising intermittently ghost crabs were able to attain distance capacities more comparable to an animal walking continuously with a greater $\dot{V}O_{2\text{max}}$ and MAS. A speed that may demand 84% $\dot{V}O_{2\text{max}}$ when traveled continuously may only demand 73% if the crab moves intermittently.

Distance capacity is not increased simply by moving intermittently. The present results show that it may be reduced significantly relative to continuous exercise (Fig. 5). When ghost crabs alternate 30 s of exercise at 0.30 m/s with 30-s pause periods, distance capacity was decreased 3.4-fold compared with crabs exercising at the same AvS (0.15 m/s; Fig. 4 and Table 2). This example illustrates that behavior can be constrained if animals are forced to be active intermittently.

Effect of exercise and pause durations. Distance capacity of intermittent activity in ghost crabs is highly dependent on both the exercise and pause durations (Fig. 4 and Table 2). Studies of intermittent exercise on humans show that supramaximal exercise can be sustained if the exercise period is <30 s and the pause period is at least twice as long as the exercise period (19, 52). By contrast, the ghost crab fatigued quickly even when the duration of the pause period (e.g., 60 s) was twice that of the exercise period (e.g., 30 s). Using this protocol, crabs walked no further than during continuous exercise at the same AbS (Table 2). In general, longer durations of pause (i.e., >60 s) and exercise (i.e., >30 s) periods resulted in the greatest distance capacity of ghost crabs.

A pause of <30 s may be too short for ghost crabs to recover from the preceding exercise period before beginning the next exercise period (Fig. 4 and Table 2). The critical pause duration in ghost crabs appears to be between 30 and 120 s. When the pause periods were ≥ 30 s and the exercise period was 120 s, the total distance traveled by ghost crabs before fatigue was greater for intermittent exercise than for continuous exercise at the same AbS and AvS (Fig. 5 and Table 2). The longest pause duration (i.e., 120 s) resulted in a threefold increase in distance capacity compared with a pause duration of 60 s. Pause periods of <30 s did not result in distance

capacity that was greater than continuous exercise at the same AvS (Table 2).

In addition to a critical pause period, a critical exercise period of 120 s may be present in ghost crabs. Exercise periods ≤ 30 s did not result in distance capacity that was greater than continuous exercise at the same AvS, even when the pause period was twice the exercise period (Tables 2 and 3). Crabs actually traveled further with the same pause period (60 s) when the exercise duration was 120 s than when exercise lasted only 30 s. When exercise was sustained for >2 min (i.e., 3–5 min), fatigue occurred at speeds equal to or lower than those for continuous exercise at the same AvS (Fig. 4). At present, we cannot explain the benefit from a longer exercise duration.

The critical exercise and pause durations that maximize distance capacity in crabs are not the same as those in mammals. The physiological basis for this difference is likely to depend on a variety of rate processes that may enhance fuel mobilization and remove or delay the production of fatigue-causing agents.

Metabolic Response to Intermittent Exercise

It has been hypothesized that heavy intermittent exercise can be carried out indefinitely if the energy demand during activity is met primarily by high-energy phosphate splitting and oxygen store depletion as opposed to accelerated glycolysis (19, 44, 52). Restoration of high-energy phosphate levels and oxygen store repletion are relatively rapid, whereas recovery from H^+ accumulation in muscle, resulting from the dissociation of lactic acid, can be more prolonged and is often associated with fatigue (11). In human and rat studies, when the exercise period is <30 s and the pause period is at least twice as long as the exercise period, blood and muscle lactate levels remain near resting levels (16, 19, 44, 51). When the exercise period is longer and the pause period is shorter, lactate levels increase significantly above resting levels (16, 32, 41, 51). If renewal of stores is prevented by performing aerobic work rather than resting during the pause intervals, then greater lactate accumulation is seen in the next exercise interval (19). With respect to fuel, glycogen depletion can be similar in intense intermittent exercise and continuous exercise of the same average work output (17, 41). Glycolysis may be retarded and lipid utilization favored if exercise is performed intermittently. Evidence from intermittent exercise studies on humans suggests that lactate that is accumulated during the exercise period can be cleared during the pause period (16, 51).

The essence of the above hypotheses is that energy sources that are rapidly renewed during pause periods can be drawn from repeatedly during periods of intense exercise. Reliance on fatigue-producing processes, such as glycolysis, may be reduced or recovery from the end products generated by these processes may occur during pauses. This hypothesis may explain the increased distance capacity in ghost crabs, but data on lactate concentrations differ from those collected on humans and rats. Our results on intermittent exercise in the ghost crabs showed significant lactate accumulation even when exercise and pause durations were selected to maximize distance capacity (Table 4). There was, however, an overall net clearance of lactate from the leg during the 2-min

pause periods, but levels never returned to the concentrations at rest. Even though lactate did accumulate, the clearance results are in agreement with the performance measurements, which suggest that a critical pause duration of 60–120 s produced some recovery.

A relatively rapid net lactate clearance is consistent with rapid aerobic recovery observed in ghost crabs [half time to $\dot{V}O_{2\text{ rest}}$ ($t_{1/2\text{ off}}$) 2–6 min (21, 24)]. In general, other species of crabs require much longer periods to recover [$t_{1/2\text{ off}}$ 15–20 min (25, 33, 34)]. Elevated levels of $\dot{V}O_2$ may persist for 30 min to 6 h in other ectotherms, such as reptiles and amphibians (3). Rates of lactate removal tend to be slow in ectotherms relative to endotherms. Only 79% of the accumulated lactate is removed from a lizard after 2 h (31), whereas lactate removal is complete in rats within 30 min (9). These differences in lactate removal between endotherms and ectotherms may be related to the oxidative vs. gluconeogenic fate of lactate removal during recovery and the lower metabolic rates of ectotherms compared with that of an endotherm of comparable body size and body temperature. Tracer studies on lactate removal during recovery from exercise indicate that 60–75% of the lactate is oxidized in the white rat (10). By contrast, in a lizard (31) and an amphibian (59) $<10\%$ of the lactate is oxidized. Examination of the fate of lactate in ghost crabs may aid in explaining their ability to recover rapidly.

Another possible interpretation of the elevated lactate levels in ghost crabs at the end of the exercise period is that no one critical level of lactate exists that is correlated with fatigue. Removal of lactate per se may not be required to prolong intermittent locomotion. Anurans can be stimulated to 3 min of intense activity after fatiguing exercise, despite little decline in lactate of the hind-limb muscles (50). After the second bout of activity lactate levels rise further. It is likely that other physiological rate processes, such as restoration of stores or fuel mobilization, are more important in prolonging activity. It is possible that these recovery processes are aided by the elevated metabolism, circulation, and ventilation that occur during pause periods.

Cost of Intermittent Exercise

Intermittent exercise of ghost crabs was as or more expensive than continuous exercise both per time and per distance (Fig. 3). The aerobic cost estimates reported in the present study only include aerobic energy sources and exclude nonoxidative energy production. If the cost estimates included both aerobic and nonaerobic sources, the cost of intermittent locomotion would be even higher, because the crabs ran at supramaximal speeds during the exercise intervals.

The greater energy cost of intermittent locomotion is most likely a consequence of at least two different processes. First, the metabolic rate during the pause periods is always greater than the resting metabolic rate. If $\dot{V}O_{2\text{ ss}}$ was constant, an elevated $\dot{V}O_2$ during the pause would increase the average cost. Energy-demanding processes other than locomotion, such as circulation and ventilation, most likely do not return to resting rates during pauses. Second, the energy demand of the supramaximal speeds employed during the exercise intervals may require more energy than predicted from the linear

extrapolation of aerobic rates (26, 27). Stride frequency, and most likely the rate of muscle force development, will be greater during exercise intervals of intermittent locomotion compared with periods of continuous exercise at the same AvS. More-rapid rates of muscle force development are correlated with an increase in energy cost resulting from an increase in speed (28, 43). Muscles generating the same total amount of force require more energy as the number of contractions producing that force increases (7, 12, 54). The greater cost of intermittent stimulation is associated with the repeated cost of muscle activation and relaxation (e.g., Ca^{2+} movements). Intermittent locomotion could require more energy than continuous locomotion at the same AvS if the costs increase more than the increase in speed used during the exercise interval.

Implications for Future Study of Comparative Locomotor Physiology and Performance

The present study advances the previous exercise paradigm based on steady-state locomotion by including dynamic adjustments that can alter behavioral limitations. Future work should be directed in at least four areas. First, future laboratory studies on ghost crab intermittent locomotion should 1) further identify critical exercise and pause periods by testing intervals >30 and <120 s, 2) vary exercise intensity (%MAS) in terms of both the AbS and AvS, and 3) introduce low-intensity exercise during the pause period.

Second, physiological correlates of the change in performance seen during intermittent exercise (e.g., changes in high-energy phosphates, glycogen, lactic acid, pH, and oxygen stores) need to be established, so that additional mechanistic hypotheses can be proposed and tested. Searching for the mechanistic bases of differences in performance is likely to lead to a reevaluation of system function (e.g., muscular, circulatory, and respiratory systems).

Third, additional performance tests that include an intermittent protocol should be attempted in species that differ in activity pattern. The effect of intermittent behavior on performance is most probably general and not peculiar to ghost crabs, rats, and humans. Other ectotherms, such as lizards, often move at speeds above their MAS, but the locomotion is punctuated by pauses (30, 37). Seals taking many short dives, and relying on aerobic metabolism, can spend far more total time submerged than if they took a single lengthy dive (42).

Finally, measurements of individuals in the field are essential to establish where within the performance limits animals operate. O'Brien et al. (46) and McLaughlin (45) propose a continuum of locomotor patterns of foraging animals. The constant motion of cruising foragers (e.g., hawk) and the ambush tactics of sit-and-wait predators (e.g., lion) lie at the extremes of this continuum. Most animals occupy an intermediate position, displaying what O'Brien et al. (46) call a "saltatory" search mode. This intermittent foraging pattern is exhibited by a wide range of animals tested, including birds (15), lizards (38), and fish (18, 46, 47). In the future, studies that correlate behavior and ecology with steady-state continuous tests should also include an evaluation of intermittent performance. Examination of dynamic physiological adjustments, such as in intermittent exercise, are likely to

alter the performance limits established under a steady-state paradigm.

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