Performance Limits of Low-Temperature, Continuous Locomotion Are Exceeded When Locomotion Is Intermittent in the Ghost Crab

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ABSTRACT

Since a decline in temperature decreases aerobic capacity and slows the kinetics of exercise-to-rest transitions in ectotherms, we manipulated body temperature to better understand the performance limits of intermittent locomotion. Distance capacity (i.e., the total distance traveled before fatigue) of the ghost crab, *Ocypode quadrata,* was determined during acute exposure to 15°C inside a treadmill-respirometer. Instead of exacerbating the near-paralyzing effects of low body temperature resulting from the frequent transitions, intermittent locomotion allowed animals to exceed the performance limits measured during steady-state locomotion. At low temperature, distance capacity for continuous locomotion at 0.04 m s^{-1} (83% maximum aerobic speed) was 60 m. When 30 s of exercise at 0.08 m s^{-1} (166% maximum aerobic speed) was alternated with 30 s of rest, distance capacity increased to 271 m, 4.5-fold greater than continuous locomotion at the same average speed (83% maximum aerobic speed). A 30-s pause following a 30-s exercise period was sufficient for maintaining low lactate concentrations in muscle and for partial resynthesis of arginine phosphate. A greater dependency on nonoxidative metabolism due to slowed oxygen uptake kinetics at low temperature resulted in a decreased duration of the critical exercise period, which increased performance relative to that measured at higher temperatures (30 s at 15°C vs. 120 s at 24°C). Despite the ghost crab's limited aerobic capacity at 15°C, distance capacity during intermittent locomotion at low temperature can be comparable to that of a crab moving continuously at a body temperature 10°C warmer. While endurance capacity is generally correlated with maximum aerobic speed, we have demonstrated that both locomotor behavior

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and body temperature must be considered when characterizing performance limits.

Introduction

Moving intermittently can alter locomotor performance limits such as distance capacity (i.e., the total distance traveled before fatigue; Weinstein and Full 1992) substantially when compared to continuous locomotion at the same average speed. Changes in distance capacity depend on the exercise speed, exercise duration, and pause duration (Weinstein and Full 1992). Since intermittent locomotion involves frequent transitions from rest to exercise and exercise to rest, intermittent locomotor performance should be affected by factors that alter rates of dynamic processes, such as temperature.

In the present study, we manipulated body temperature (T_b) to better define the consequences of intermittent locomotion and begin to explore the mechanistic basis of intermittent locomotion. In ectothermic animals, T_b affects the rate processes of biological systems used during activity. A decrease in T_b increases the half-time to steady-state oxygen consumption ($\dot{V}_{O_2}$; Full 1987; Ishii et al. 1992), decreases steady-state $\dot{V}_{O_2}$ at a given speed, decreases the resting and maximal $\dot{V}_{O_2}$, decreases the speed at which maximal $\dot{V}_{O_2}$ is attained (i.e., the maximum aerobic speed), and decreases endurance capacity substantially (Herreid et al. 1981a; John-Alder and Bennett 1981; John-Alder et al. 1983; Full and Tullis 1990; Weinstein and Full 1994).

We predict that moving intermittently at low T_b will have several metabolic consequences. First, a decrease in T_b should decrease the total energetic cost of intermittent exercise because it will decrease the cost of steady-state locomotion at a given speed. Moreover, it should substantially reduce cost at the same relative workload (i.e., percentage of the maximum aerobic speed), because recruitment compression causes that same relative workload to be attained at a lower speed (Rome et al. 1984). Although cost is likely to be reduced at low temperature, we have demonstrated previously that the cost of intermittent locomotion is significantly greater than that of steady-state locomotion at a given speed (Weinstein and Full 1992). Second, if the kinetics of oxygen uptake are slowed during the rest-to-exercise transitions at low T_b, then the relative contribution from aerobic metabolism at the same percentage of maximum aerobic speed should be decreased. As a result, the relative contribution from nonoxidative metabolism (i.e., accelerated

glycolysis and high-energy phosphate breakdown) should be increased at low T_b. And finally, a slower rate of recovery during transitions from exercise to rest at low T_b should decrease the capacity of the system for subsequent rest-to-exercise transitions. Distance traveled before fatigue for intermittent locomotion at low temperatures is likely to depend on the interplay between the slowed kinetics on one hand and the reduced energy demand on the other. Intermittent locomotion could exacerbate the near-paralyzing effects of low T_b measured during steady-state locomotion. Conversely, it could release the animal from this constraint if intermittent locomotion at low T_b increases distance capacity relative to continuous locomotion, as it does at warmer temperatures (Weinstein and Full 1992).

For the present study, we selected the ghost crab, *Ocypode quadrata,* because of the wealth of data on energetics and locomotor performance that allows direct comparisons to published data (Full 1987; Full and Weinstein 1992; Weinstein and Full 1992, 1994; Weinstein et al. 1994). We measured two performance variables, aerobic cost and distance capacity, in the laboratory under controlled conditions during continuous and intermittent locomotion at low T_b. We had three main objectives. First, we examined whether making locomotion intermittent alters performance limits at low T_b (15°C). Second, we compared ghost crabs exercising continuously and intermittently at 15°C with those at 24°C (Weinstein and Full 1992) to examine the direction and magnitude of performance-limit alterations. Finally, we examined performance limits with respect to specific exercise and pause intervals as a function of T_b to investigate correlations between whole-animal performance and muscle-level biochemistry that may aid in discovering the mechanistic basis of intermittent-locomotion effects.

Material and Methods

Animals

Ghost crabs, *Ocypode quadrata* (28.3 ± 9.9 [SD] g; $n = 27$ individuals), were collected from the beaches surrounding the Duke University Marine Laboratory in Beaufort, North Carolina. 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 a 14L : 10D 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 30%–50% seawater 24 h after feeding. Only intermolt crabs were used. Individuals were kept in captivity less than 5 wk. Crabs were randomly assigned to exercise groups, and as a result, no individual exercised at all protocols. Each individual rested for at least 5 d between trials.

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

T_b. Prior observations, verified by thermocouple measurements, indicated that ghost crabs require 60 min of exposure to an ambient temperature of 15°C to attain a T_b of 15°C (Weinstein and Full 1994). This protocol was used in the present study to ensure ghost crab body temperatures of 15°C for all physiological measurements.

Resting $\dot{V}_{O_2}$. The crabs rested quietly in the treadmill-respirometer maintained at 15°C inside an incubator for 60 min to allow their T_b to reach 15°C (see Weinstein and Full 1994). After thermal equilibration, $\dot{V}_{O_2}$ was measured for 10 min before each exercise trial. The $\dot{V}_{O_2}$'s were averaged to obtain the resting mass-specific $\dot{V}_{O_2}$.

Continuous Exercise. The treadmill speeds selected for the present study were based on our previous investigation of *O. quadrata* during steady-state exercise (Weinstein and Full 1994). Crabs were exercised continuously to fatigue on a treadmill at 0.04, 0.08, or 0.12 m s⁻¹. A speed of 0.05 m s⁻¹ elicits the maximal $\dot{V}_{O_2}$ and is therefore the maximum aerobic speed at 15°C (Weinstein and Full 1994). Thus, the slowest speed selected for continuous exercise was submaximal (83% of the maximum aerobic speed), whereas the other two speeds were supramaximal (166% and 250% of the maximum aerobic speed). $\dot{V}_{O_2}$ measurements on crabs from the present study were not significantly different from those reported previously ($P > 0.05$, unpaired *t*-tests comparing steady-state $\dot{V}_{O_2}$ at 0.04 and 0.08 m s⁻¹ to previous data at the same speeds; Weinstein and Full 1994).

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

$\dot{V}_{O_2}$ Measurements

Crabs were exercised in a miniature treadmill-respirometer. The airflow rate was 150 mL min⁻¹. The relative humidity of the incurrent air was 40%–50%. Air leaving the treadmill chamber was dried with Drierite, and CO_2 was removed with Ascarite. The oxygen concentration was monitored at 10-s intervals with an O_2 analyzer (Ametek S3A), which was interfaced with a personal computer (Macintosh II) by data-acquisition hardware and software (LabView, National Instruments). The mass-specific $\dot{V}_{O_2}$ was calculated from the O_2 concentration (Withers 1977; Herreid et al. 1981*a*). Calculations of steady-state instantaneous $\dot{V}_{O_2}$ (Herreid et al. 1981*b*) were not significantly different from steady-state rates determined without the instantaneous correction (Withers 1977), as seen in our previ-

Table 1: Intermittent exercise protocols

Protocol	Exercise Period (s)	Pause Period (s)	Ratio	Absolute Speed (m s^{-1})	Average Speed (m s^{-1})
Constant ratio of exercise-to-pause duration:					
1	30	30	1	.08	.04
2	120	120	1	.08	.04
3	180	180	1	.08	.04
4	15	30	.5	.12	.04
5	30	60	.5	.12	.04
6	60	120	.5	.12	.04
Variable pause duration:					
2	120	120	1	.08	.04
7	120	60	2	.08	.05
8	120	30	4	.08	.07

Note. Ratio is expressed as the exercise-period duration divided by pause-period duration. The absolute speed is the speed during the exercise period. The average speed is calculated for a complete exercise/pause cycle. Sample size was $n = 5$ for each intermittent-exercise protocol. The maximum aerobic speed is 0.05 m s^{-1}.

ous studies on ghost crabs (Weinstein and Full 1992, 1994; Weinstein et al. 1994).

The time to attain 50% of the steady-state $\dot{V}_{O_2}$ ($t_{1/2on}$) was determined for crabs exercising continuously at 0.04 m s^{-1} (83% of the maximum aerobic speed). Calculations of $t_{1/2on}$ were made on data corrected for instantaneous $\dot{V}_{O_2}$. The effective volume of the treadmill chamber was 710 mL.

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) is expressed per unit time (mL O$_2$ g^{-1} h^{-1}).

Distance Capacity Measurements

Crabs were considered to be fatigued when they could no longer keep pace with the treadmill belt, even when prodded (Full 1987). 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 an index of endurance, instead of time to fatigue, because the term "endurance" usually connotes only continuous activity (Weinstein and Full 1992). 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 absolute speed used during the exercise intervals and with continuous exercise at the same average speed.

Muscle Metabolite Protocol and Measurements

After the $\dot{V}_{O_2}$ and distance capacity measurements were made, crabs were divided into four groups: (1) rest, (2) continuous-exercise, (3) intermittent-exercise, and (4) intermittent-pause groups (Fig. 1). All animals rested in the treadmill chamber for 60 min to allow their T_b's to reach 15°C. The rest group was frozen in liquid nitrogen following the 60-min thermal equilibration period. The continuous-exercise group of crabs was exercised continuously on the treadmill at 0.08 m s^{-1} (166% of the maximum aerobic speed) and then was immediately frozen. The exercise time for these crabs was 3 min. The intermittent-exercise protocol consisted of 30-s exercise periods (speed, 0.08 m s^{-1}; 166% of the maximum aerobic
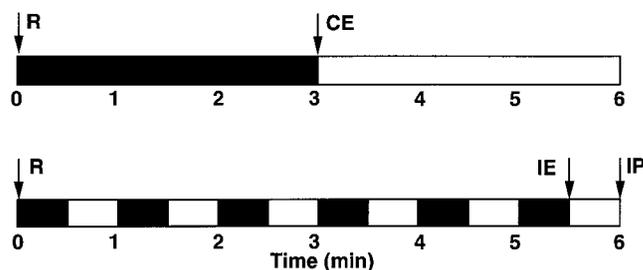

Figure 1. Muscle metabolite protocol. *Filled bars,* exercise periods; *open bars,* pause periods. Speed during the exercise intervals was 0.08 m s^{-1}. Samples were taken after 60 min of rest within the chamber (*R*), after continuous exercise to fatigue (*CE*), after the sixth intermittent-exercise period (*IE*), or after the sixth intermittent-pause period (*IP*), as indicated by arrows. Sample sizes were $n = 4$ crabs for the rest group and $n = 7$ crabs per group for continuous exercise, intermittent exercise, and intermittent pause.

speed) alternated with 30-s pause periods. The crabs in the intermittent-exercise group were frozen in liquid nitrogen upon completion of the sixth exercise period. Therefore, the crabs in the intermittent-exercise group traveled the same distance as the continuous-exercise group. Crabs in the intermittent-pause group were frozen at the end of the sixth pause period. Since crabs ran sideways on the treadmill, the primary direction of travel was recorded. Thus, muscles from leading and trailing legs could be analyzed separately.

Whole animals were frozen rapidly (<2 s) in liquid nitrogen and stored at $-80°$C for less than 1 wk before tissue preparation and analysis. The third walking legs, along with the associated extrinsic musculature (i.e., that within the body), were dissected free in a cold room ($-5°$C) and immediately homogenized in four volumes of 6% perchloric acid. After centrifugation, the resulting supernatant was neutralized with K_2CO_3. Leg lactate concentration was determined spectrophotometrically according to Full and Herreid (1984) with modifications from Prestwich (1988). Leg ATP (Lamprecht and Trautschold 1974) and arginine phosphate concentrations were determined spectrophotometrically (Lamprecht et al. 1974) as modified by Prestwich (1988).

Statistical Analysis

Unless noted, values are reported as means $\pm$ SE. We used a Mann-Whitney U-test to compare values for resting and maximal $\dot{V}O_2$ and $t_{1/2on}$ obtained in the present study with previous data. We performed ANOVA to determine the effects of experimental condition on $\dot{V}O_2$, distance capacity, and muscle metabolites. For comparisons between treatments, we used Scheffé's F-test.

Results

$\dot{V}O_2$

Resting $\dot{V}O_2$. The resting $\dot{V}O_2$ at 15°C was 0.05 ± 0.002 mL O_2 g^{-1} h^{-1} ($n = 51$ trials). This resting rate is not significantly different from previous data (Weinstein and Full 1994; Mann-Whitney U-test, $P > 0.05$).

Continuous Exercise. After the onset of exercise, $\dot{V}O_2$ increased to a steady state. The time to attain 50% of the steady-state $\dot{V}O_2$ ($t_{1/2on}$) at a treadmill speed of 0.04 m s^{-1} was 93.2 ± 12.6 s ($n = 5$), significantly longer (Mann-Whitney U-test, $P < 0.05$) than that measured at 24°C (28.7 ± 2.2 s; Full 1987).

Intermittent Exercise. For crabs exercising intermittently at 15°C, $\dot{V}O_2$ increased as the crab exercised and decreased as
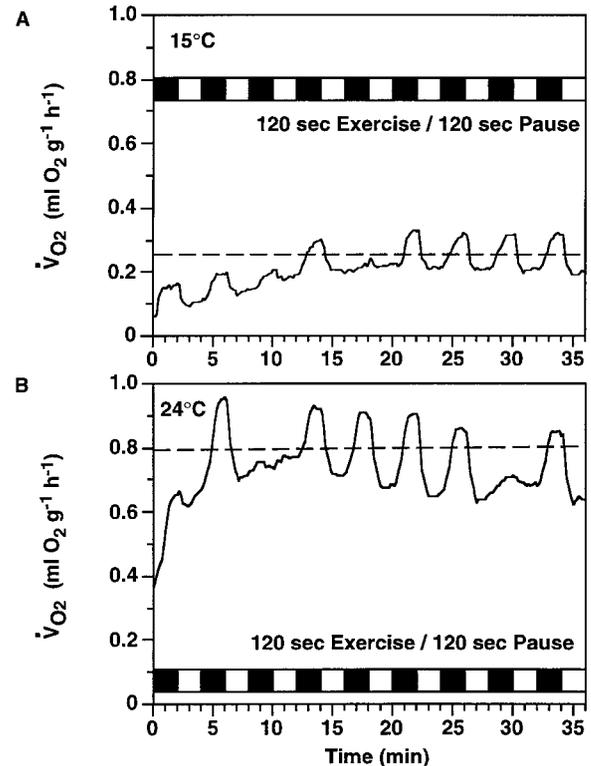

Figure 2. Instantaneous oxygen consumption kinetics for intermittent exercise at 15°C (*A*) and 24°C (*B*; Weinstein and Full 1992). Exercise protocol is indicated by exercise periods (*filled bars*) and pause periods (*open bars*). Exercise speed was 166% of the maximum aerobic speed in both examples. Dashed lines represent values for average aerobic cost. Body masses of crabs were 30.9 g (*A*) and 34.0 g (*B*).

the crab paused (Fig. 2*A*). Longer exercise and pause periods resulted in greater oscillations in instantaneous $\dot{V}O_2$ than shorter exercise and pause periods.

Aerobic Cost of Exercise

For continuous exercise at 15°C, steady-state $\dot{V}O_2$ increased linearly with speed (Fig. 3). At a treadmill speed of 0.05 m s^{-1}, the crab attained its maximal $\dot{V}O_2$, and further increases in speed did not result in subsequent increases in $\dot{V}O_2$. The aerobic cost of continuous exercise at a treadmill speed of 0.04 m s^{-1} and the maximal $\dot{V}O_2$ (determined at 0.08 m s^{-1}) were not significantly different from values measured previously at 15°C (Weinstein and Full 1994; Mann-Whitney U-test, $P > 0.05$). The crabs exercising continuously at 0.12 m s^{-1} (250% of the maximum aerobic speed) fatigued before attaining a steady state.

Comparisons of crabs exercising intermittently at 15°C to crabs exercising intermittently at 24°C with the same exercise duration, pause duration, and relative exercise speed (absolute
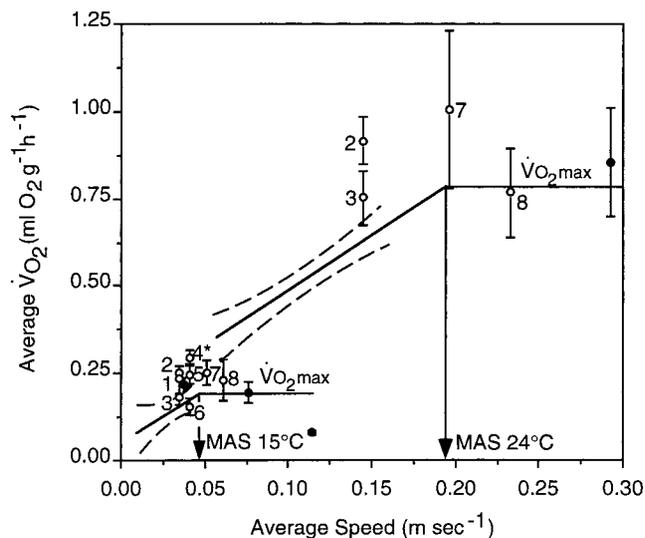
Figure 3. Average mass-specific $\dot{V}_{O_2}$ or aerobic cost per unit time as a function of average speed. Values are means ± SE. Solid lines show previous data for ghost crabs exercising continuously at 15°C (Weinstein and Full 1994) and 24°C (Full 1987). Dashed curves represent the 95% confidence intervals of the means. *Closed circles,* continuous exercise; *open circles,* intermittent exercise. Intermittent-exercise protocols are indicated by numerals and correspond to protocols outlined in Table 1. Sample sizes were $n = 5$ crabs for protocols 1, 2, 5–7, and continuous exercise at 0.04 m s^{-1} and $n = 4$ crabs for protocols 3, 4, and continuous exercise at 0.08 and 0.12 m s^{-1}. Crabs exercising continuously at 0.12 m s^{-1} fatigued before reaching a steady-state $\dot{V}_{O_2}$. Intermittent-exercise data for 24°C are from Weinstein and Full (1992). The exercise and pause durations for 24°C are the same as indicated for the 15°C protocols (Table 1). $\dot{V}_{O_{2max}}$, maximal $\dot{V}_{O_2}$; *MAS*, maximum aerobic speed. An asterisk indicates a significant difference from continuous exercise at the same average speed ($P < 0.05$).

speed = 166% of the maximum aerobic speed) illustrate an important effect of temperature. While $\dot{V}_{O_2}$ reached and oscillated around the average aerobic cost by the second exercise-pause cycle during intermittent exercise at 24°C (Fig. 2B), the average aerobic cost at 15°C was not attained and maintained until after several exercise/pause cycles (Fig. 2A).

The average aerobic cost per time of intermittent exercise at 15°C was independent of the average speed (ANOVA, $F_{2, 34} = 0.06$, $P > 0.05$; Fig. 3) and was not significantly different from maximal $\dot{V}_{O_2}$ ($P > 0.05$). The average aerobic cost per time for the 15-s exercise alternated with a 30-s pause (protocol 4, Table 1) was significantly greater than the corresponding value for continuous exercise at the same average speed ($P < 0.05$), but was not significantly different from other intermittent-exercise protocols.

Distance Capacity

Continuous Exercise. Crabs exercising continuously at a treadmill speed of 0.04 m s^{-1} (83% of the maximum aerobic speed)

fatigued after 24.1 ± 9.8 min, corresponding to a distance capacity of 60.1 ± 24.5 m. At a speed of 0.08 m s^{-1} (166% of the maximum aerobic speed), the crabs fatigued after 6.7 ± 1.3 min (33.5 ± 6.5 m). At the fastest speed, 0.12 m s^{-1} (250% of the maximum aerobic speed), the crabs fatigued after 4.1 ± 8.0 min (30.5 ± 8.0 m). The endurance capacity of these crabs is described by the equation: $t_{end} = 0.0178\ v^{-1.50}$, where t_{end} is endurance time (h) and v is velocity (km h^{-1}). This endurance relationship was not significantly different from previous data on ghost crabs at a T_b of 15°C (model for log-transformed data: $\dot{V}_{O_2}$ = constant + temperature + speed + [temperature × speed], $F_{1, 30} = 0.001$, $P > 0.05$; Weinstein and Full 1994).

Constant Exercise-to-Pause Ratio. Crabs exercising intermittently at a T_b of 15°C when the exercise duration was 30 s (exercise-to-pause ratio = 1) traveled 6.6 times farther before fatigue than those moving continuously at the same absolute speed (0.08 m s^{-1}, 166% of the maximum aerobic speed; $P < 0.05$) and 3.3 times farther than those moving continuously at the same average speed (0.04 m s^{-1}, 83% of the maximum aerobic speed; $P < 0.05$; Fig. 4A). When the exercise duration was 120 s (exercise-to-pause ratio = 1), the distance capacity was 3.8 times greater than the total distance traveled continuously before fatigue at the same absolute speed ($P < 0.05$) but was not different from the distance traveled continuously at the same average speed (Fig. 4A). When the exercise duration was 180 s (exercise-to-pause ratio = 1), the distance capacity was not statistically different from the total distance traveled continuously at the same absolute speed or average speed (Fig. 4A).

However, there appears to be a complex interaction between temperature, exercise (and pause) duration, and distance capacity (Fig. 4). While alternating 30 s of exercise with 30-s pause periods led to a significant increase in distance capacity compared with continuous locomotion at the same average speed at a T_b of 15°C (Fig. 4A), the same intermittent-exercise protocol led to a significant decline in distance capacity compared with continuous locomotion at the same average speed at a T_b of 24°C (Fig. 4B). By contrast, alternating 120 s of exercise with 120-s pause periods increased distance capacity compared with continuous locomotion at the same average speed at a T_b of 24°C (Fig. 4B) but not at 15°C (Fig. 4A).

Crabs exercising intermittently when the exercise duration was 15 s and the pause duration was 30 s (exercise-to-pause ratio = 0.5) traveled 8.1 times farther before fatigue than those moving continuously at the same absolute speed (0.12 m s^{-1}, 250% of the maximum aerobic speed; $P < 0.001$) and 4.5 times farther than those moving continuously at the same average speed (0.04 m s^{-1}, 83% of the maximum aerobic speed; $P < 0.001$; Table 2). When the exercise duration was longer than 15 s (exercise-to-pause ratio = 0.5), the distance capacity was not statistically different from the total distance traveled
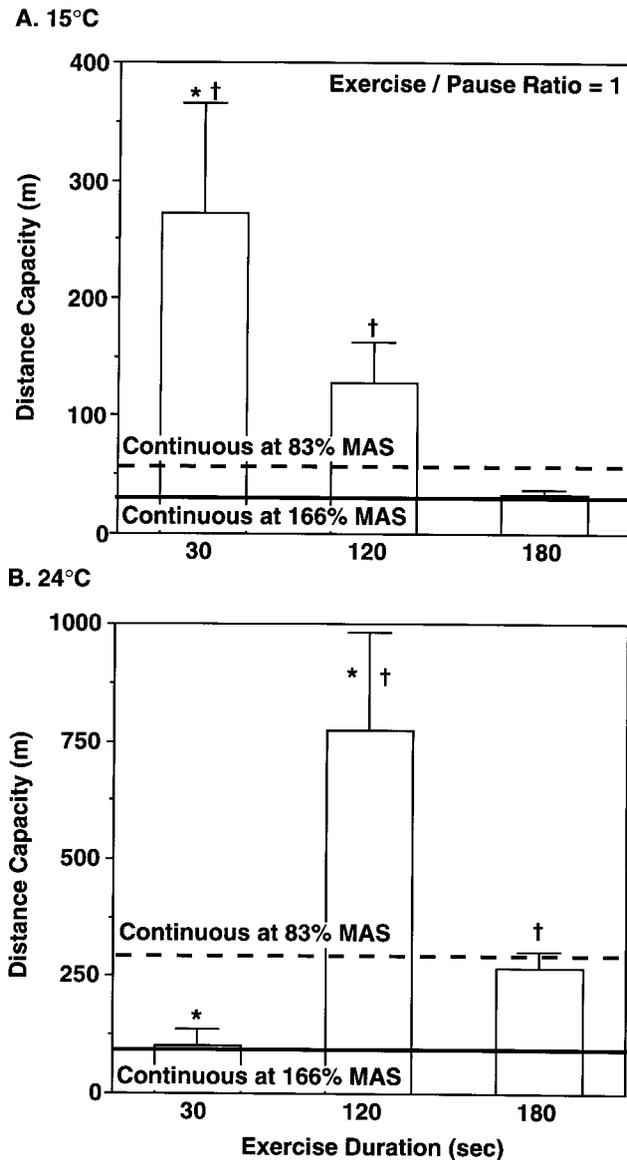
Figure 4. Distance capacity as a function of exercise (and pause) duration at 15°C (*A*) and 24°C (*B*). Exercise-to-pause duration ratio was kept constant (equal to 1), and exercise (and pause) period was varied. Values are means ± SE; $n = 5$ crabs for each protocol. For comparison, distance capacities are shown for crabs exercising continuously at the same average speed and absolute speed. The speed during the exercise period was 166% maximum aerobic speed (*MAS*) at both temperatures. The average speed was 83% of the maximum aerobic speed at both temperatures. An asterisk indicates a significant difference from continuous exercise at same average speed ($P < 0.05$). A dagger indicates a significant difference from continuous exercise at same absolute speed ($P < 0.05$).

continuously at the same absolute speed or average speed (Table 2).

Varied Pause Duration. When the exercise duration was 120 s, a 120-s pause period significantly increased distance capacity

compared with continuous locomotion at the same absolute speed but did not alter distance capacity compared to continuous locomotion at the same average speed. When the exercise duration was kept constant at 120 s, shorter pause durations (30 s and 60 s) did not alter distance capacity compared with continuous locomotion at the same absolute speed or average speed (Table 2).

Leg Muscle Metabolites

Lactate. Continuous exercise at 0.08 m s^{-1} (166% of the maximum aerobic speed) and intermittent exercise (intermittent exercise and intermittent pause groups) significantly increased leg lactate levels ($P < 0.001$; Table 3). Crabs in the intermittent-exercise and intermittent-pause groups had significantly lower leg lactate concentrations than crabs in the continuous-exercise group ($P < 0.01$). In the continuous-exercise and intermittent-exercise groups, lactate concentrations were significantly higher in the trailing legs (3.3 ± 0.3 and 2.4 ± 0.2 μmol g leg^{-1} in the continuous-exercise and intermittent-exercise groups, respectively) than leading legs (2.7 ± 0.3 and 1.7 ± 0.1 μmol g leg^{-1} in the continuous-exercise and intermittent-exercise groups, respectively; $P < 0.05$). Lactate concentrations were similar in leading and trailing legs for the intermittent-pause group.

ATP. Exercise did not significantly alter leg muscle ATP concentration (Table 3). ATP concentrations were similar in leading and trailing legs for all exercise groups.

Arginine Phosphate. Continuous and intermittent exercise significantly decreased leg muscle arginine phosphate levels below resting levels ($P < 0.001$; Table 3). Crabs in the intermittent-pause group had significantly higher arginine phosphate concentrations than those in the continuous-exercise group ($P < 0.05$). Arginine phosphate levels were similar in leading and trailing legs for all exercise groups.

Discussion

Intermittent versus Continuous Exercise Performance

Distance Capacity as a Function of T_b. Cold crabs exercising continuously at slow, sustainable speeds (below the maximum aerobic speed) had endurance as low as a warm crab sprinting. Cold crabs exercising intermittently exceeded this low-temperature limitation and had the endurance of an animal with a T_b almost 10°C warmer walking continuously at sustainable speeds. Specifically, crabs moving intermittently at an average speed of 0.04 m s^{-1} at a T_b of 15°C (protocol 1, Table 1) had a 4.5-fold increase in distance capacity and traveled distances similar to crabs moving continuously at 0.15 m s^{-1} at a T_b of 24°C (Fig. 5). This observation indicates that frequent dynamic

Table 2: Effect of intermittent locomotion on distance capacity

Exercise/Pause Period (s)	Distance Capacity (m)		
	Intermittent Exercise	Continuous Exercise at Same Average Speed	Continuous Exercise at Same Absolute Speed
15/30	199.9[a, b]	60.1	30.5
	($\pm$5.7)	($\pm$24.5)	($\pm$8.0)
30/60	104.5	60.1	30.5
	($\pm$37.3)	($\pm$24.5)	($\pm$8.0)
60/120	56.5	60.1	30.5
	($\pm$13.6)	($\pm$24.5)	($\pm$8.0)
120/120	128.7[b]	60.1	33.5
	($\pm$34.0)	($\pm$24.5)	($\pm$6.5)
120/60	82.7	39.6[c]	33.5
	($\pm$46.6)		($\pm$6.5)
120/30	43.0	36.2[c]	33.5
	($\pm$5.8)		($\pm$6.5)

Note. Values for distance capacity are means $\pm$ SE (in parentheses); $n = 5$ for each exercise protocol.

[a] Significantly different from distance capacity at the same average speed ($P < 0.05$).

[b] Significantly different from distance capacity at the same absolute speed ($P < 0.05$).

[c] Values for distance capacity for continuous exercise at the same average speed are estimated from the endurance curve calculated from continuous-exercise data in the present study.

adjustments can have a significant impact on an animal's performance at low temperature. While endurance capacity is generally correlated with the maximum aerobic speed, we have demonstrated that both locomotor behavior and T_b must be considered when characterizing performance limits.

The exercise and pause intervals that resulted in increased distance capacity appeared to be temperature sensitive (Figs. 4, 5); those yielding maximum capacity at a T_b of 15°C were not the same as those at a T_b of 24°C (Weinstein and Full 1992). At a T_b of 24°C, ghost crabs had a critical exercise period that increased distance capacity of 120 s, and exercise periods less than or equal to 30 s did not alter distance capacity compared with continuous locomotion at the same average speed (Figs. 4, 5; Weinstein and Full 1992). By contrast, when ghost crabs with a T_b of 15°C moved intermittently with an exercise period of 120 s, distance capacity was not altered compared with continuous locomotion at the same average speed (Figs. 4, 5; Table 2). The only exercise periods that increased distance capacity compared with continuous locomotion at the same average speed at a T_b of 15°C were less than or equal to 30 s (Figs. 4, 5). While the rate of recovery was slower at a T_b of 15°C than at 24°C, energy demand was also lower. These observations suggest that the lower metabolic rate and the slower kinetics at a T_b of 15°C favored a shorter critical exercise period, one that was approximately one-third the $t_{1/2on}$ response for aerobic metabolism. By contrast, the critical exercise period at a T_b of 24°C was four times longer than the $t_{1/2on}$ response (Full 1987; Weinstein and Full 1992).

Cost of Locomotion as a Function of T_b. Moving intermittently at a T_b of 15°C was at least as expensive as moving continuously at the same average speed per unit time (Fig. 3), even when only aerobic sources were compared. The aerobic cost estimates reported in the present and earlier studies exclude nonoxidative energy production from high-energy phosphates and accelerated glycolysis. If the cost estimates of intermittent exercise included both aerobic and nonoxidative sources, the cost of intermittent locomotion would probably be even higher compared with continuous locomotion at the same average speed, since the crabs exercised at speeds greater than the maximum aerobic speed during the exercise periods. The greater cost of intermittent locomotion, compared with continuous locomotion at the same average speed, was most likely a consequence of the high energy demand of the exercise period, where the exercise intensity is greater than the maximum aerobic speed, and the elevated metabolic rate during the pause period (i.e., above the resting metabolic rate; Weinstein and Full 1992).

To examine differences in energy supply and demand during intermittent exercise at 15° and 24°C, it was necessary to compare total metabolic cost and the relative contributions from various energy sources. The total energetic cost can be estimated from the sum of the ATP equivalents generated from aerobic metabolism (i.e., integrating the area under the steady-state $\dot{V}O_2$ curve and approximating O_2 store depletion), high-energy phosphate breakdown, and accelerated glycolysis (i.e., lactate accumulation; Di Prampero et al. 1970; Gaitanos et al. 1993; Trump et al. 1996). Our best estimate of total metabolic

Table 3: Leg muscle metabolite concentrations

Metabolite	Protocol			
	Rest	Continuous Exercise	Intermittent Exercise	Intermittent Pause
Lactate	1.1	3.0[a]	2.0[a, b]	2.1[a, b]
	(±.1)	(±.2)	(±.1)	(±.1)
ATP	2.5	3.2	2.6	2.8
	(±.2)	(±.3)	(±.2)	(±.3)
Arginine phosphate	13.8	6.7[a]	7.3[a]	8.9[a, b, c]
	(±.9)	(±.9)	(±.9)	(±.7)

Note. Values are means (μmol g leg^{-1}) ± SE. Sample size was $n = 5$ crabs for rest and $n = 7$ crabs per group for continuous exercise, intermittent exercise, and intermittent pause. Values for leading and trailing legs were averaged for individual crabs in continuous-exercise, intermittent-exercise, and intermittent-pause groups.

[a] Significantly different from rest ($P < 0.05$).
[b] Significantly different from continuous exercise ($P < 0.05$).
[c] Significantly different from intermittent exercise ($P < 0.05$).

cost at the onset of exercise at a T_b of 15°C was 7.2 μmol ATP g^{-1}, compared with 37.3 μmol ATP g^{-1} at a T_b of 24°C (Fig. 6; Table 3; Full and Weinstein 1992), despite similar relative workloads (i.e., ~170% of the maximum aerobic speed) and time periods (i.e., ~3 min). The total metabolic cost at the same relative workload was lower at a T_b of 15°C than at 24°C because the exercise speed was slower (0.08 m s^{-1} at 15°C vs. 0.3 m s^{-1} at 24°C; Weinstein and Full 1992) and the metabolic demand of exercise at a given speed decreases with T_b (John-Alder and Bennett 1981; John-Alder et al. 1983; Bennett et al. 1984; Full and Tullis 1990; Weinstein and Full 1994). More important, the relative energy contribution from nonoxidative metabolism to the total cost was higher at 15°C (83%) than at 24°C (68%). These estimates suggest that while the total energetic cost at the onset of exercise was reduced at low T_b, there was an increased reliance on nonoxidative metabolism.

Effect of T_b *on Metabolic Response to Intermittent Exercise*

The greater reliance on nonoxidative metabolism at the onset of exercise at low temperature provides us with a starting point for speculation concerning the mechanistic basis of the effect of temperature on intermittent locomotion. To increase performance at low temperature, we hypothesize that shorter exercise and pause cycles than those used at high temperature are necessary (Fig. 4) because oxygen uptake kinetics are slow at low temperatures. In the ghost crab, the $t_{1/2on}$ time for the rest-to-exercise transition was three times longer at a T_b of 15°C than at a T_b of 24°C (see Results; Full 1987).

A longer $t_{1/2on}$ is consistent with our estimated increased reliance on nonoxidative metabolism at 15°C compared with 24°C (Fig. 6). Relative energy contributions from accelerated glycolysis at 15° and 24°C were similar at the onset of continuous exercise (~42%; Fig. 6). Therefore, the major difference

in nonoxidative metabolism during intermittent locomotion at 15° and 24°C in ghost crabs appears to involve arginine phosphate. Although the absolute rate of arginine phosphate depletion at the onset of exercise was slower at 15°C (2.3 μmol min^{-1}; Table 3) than at 24°C (3.5 μmol min^{-1}; Full and Weinstein 1992), the relative contribution of high-energy phosphate breakdown to total cost was significantly greater at 15°C (43%) than at 24°C (23%; Fig. 6).

There may be a critical exercise duration related to the muscle's reliance on high-energy phosphate breakdown at the onset of each exercise bout. Our distance capacity data (Table 2) reveal that performance decreased at 15°C as the exercise interval was increased, despite the proportional lengthening of the pause period. Perhaps the time interval that enhances intermittent-exercise performance at 15°C was compressed relative to that at 24°C (Fig. 4) because longer exercise at 15°C requires a disproportionately greater reliance on nonoxidative metabolism, and high-energy phosphates in particular, even at the same relative workload (percentage of the maximum aerobic speed).

Recovery kinetics may also play a role at low temperature during intermittent locomotion. The kinetics of the recovery (i.e., the time to attain 50% of the decline in steady-state $\dot{V}O_2$) are clearly temperature sensitive in terrestrial ectotherms (see, e.g., Bennett 1972; Bennett and Gleeson 1976; Gleeson 1980; Wagner and Gleeson 1996), and the fate of metabolites is complex and still controversial (Full and Herreid 1984; Withers et al. 1988; Gleeson and Dalessio 1989; Lallier and Walsh 1991, 1992; Henry et al. 1994). Our distance capacity results suggest that there may be a critical pause duration necessary to increase intermittent-exercise performance relative to continuous exercise, and we suspect it is temperature sensitive. Distance capacity for intermittent locomotion at 15°C decreased as the pause period was decreased from 120 s to 30 s while keeping exercise
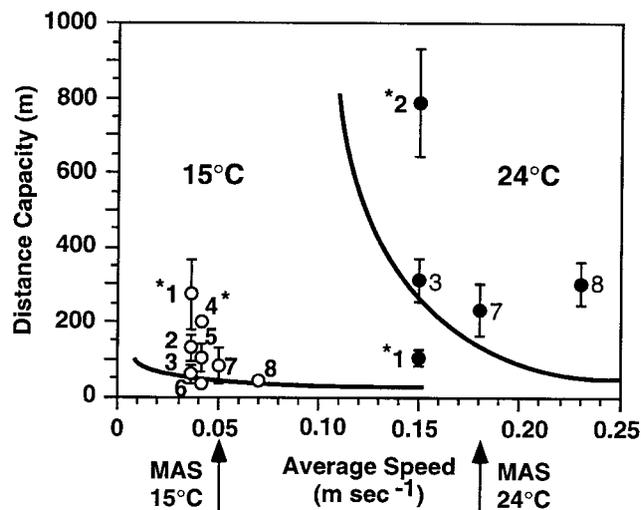
Figure 5. Distance capacity as a function of average speed at 15° and 24°C. Values are means ± SE. Solid lines represent distance capacity curves for continuous locomotion, and arrows represent maximum aerobic speed (*MAS*) at 15°C (Weinstein and Full 1994) and 24°C (Full 1987). Open symbols represent intermittent exercise at 15°C. Intermittent-exercise protocols are indicated by numerals and correspond to protocols outlined in Table 1. Closed symbols represent intermittent exercise at 24°C (Weinstein and Full 1992). Numerals next to symbols for 24°C also correspond to protocols in Table 1. An asterisk indicates a significant difference from continuous exercise at the same average speed ($P < 0.05$).

duration constant (i.e., at 120 s; Table 2). Ghost crabs exercising with the same exercise (i.e., 120 s) and pause intervals (i.e., 30–120 s) and relative exercise speed (170% of the maximum aerobic speed) at 24°C also suffered a decline in performance as the pause duration was shortened.

At 15°C, intermittent exercise with short exercise and pause intervals (i.e., 30 s) resulted in the greatest performance enhancement (Fig. 4). A 30-s pause period at 15°C was sufficient for a significant resynthesis of arginine phosphate, even though the resting concentration was not attained (Table 3). Although the 30-s pause period was not sufficient for the net clearance of lactate from the leg muscle, ghost crabs still had significantly lower lactate levels than crabs exercising continuously at the same absolute speed. In contrast to data at 15°C, intermittent exercise with 30-s exercise bouts alternated with 30-s pause periods at a T_b of 24°C resulted in a decrease in distance capacity, no net clearance of lactate from leg muscle, and no resynthesis of arginine phosphate during the pause period (Full and Weinstein 1992). At 24°C, ghost crabs exercising with longer exercise and pause periods (i.e., 120 s) showed the greatest performance enhancement (Fig. 4). During the 120-s pause period, there was a net clearance of lactate from leg muscle and a significant resynthesis of arginine phosphate (Weinstein and Full 1992). Perhaps at higher temperatures with rapid oxygen uptake kinetics, enhanced performance of intermittent

locomotion is more dependent on a sufficient pause or recovery period.

It has become apparent that when exercise-to-rest as well as rest-to-exercise transitions are made frequently, as in the case of intermittent exercise, there is a complex interaction of the metabolic processes and their temperature dependencies. In the present study, we demonstrated that intermittent locomotion can mitigate the performance limitations of low temperature. However, the exercise and pause durations that improve performance are temperature sensitive and, therefore, differ from those discovered to increase distance capacity at higher temperatures. We hope that our nascent attempt to correlate metabolites with known alterations in performance can guide future experiments and establish a framework for studies that examine the complex mechanisms that appear to function during intermittent locomotion. Causal mechanisms linking muscle metabolism and whole-animal performance remain elusive. In human subjects, dietary creatine supplementation, which increases intramuscular creatine phosphate stores, improves supramaximal intermittent-exercise performance when exercise periods are short (Balsom et al. 1993), but not when exercise periods are long (Febbraio et al. 1995). Although these results are consistent with our interpretations in the present study, other research suggests that exhaustion from submaximal, repeated, isometric exercise may be unrelated to changes in high-energy phosphate or muscle pH (Saugen et al. 1997).

Future studies need to explore further the relationship between behavioral, physiological, and metabolic mechanisms that alter whole-organism performance when activities are carried out intermittently rather than continuously. While chang-
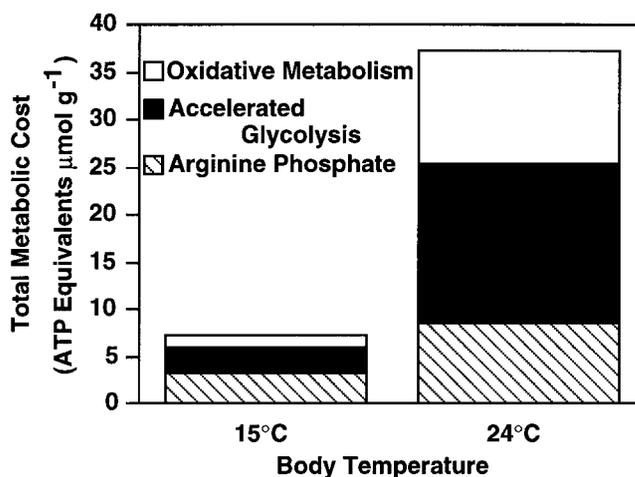

Figure 6. Estimated total metabolic cost of approximately 3 min of continuous exercise at 15° and 24°C. Total cost was calculated as the sum of ATP generated from oxidative metabolism, accelerated glycolysis, and arginine phosphate. Exercise speed was approximately 170% of the maximum aerobic speed at both temperatures. Data are taken from Table 3 and from Full and Weinstein (1992).

ing T_b provides a method to manipulate aerobic capacity and kinetics within a single individual or species, an equally valuable approach would include a comparison of continuous and intermittent performance in a range of species. The tremendous interspecific variation in aerobic capacity and kinetics could be exploited to further define limits of performance during intermittent locomotion. Moreover, we have not yet sufficiently defined design advantages and disadvantages as they relate to transitions rather than infrequently observed steady-state maxima. While current models of oxygen and energy transport are based on results from constant-speed, steady-state exercise, incorporating dynamic adjustments made during intermittent, non-steady-state exercise will provide testable hypotheses for differences in system function within and among species. Finally, the present study reveals the need to characterize the extent to which intermittent locomotion in the field alters proposed steady-state performance limitations (Weinstein 1995).

Acknowledgments

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