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The cost of incline locomotion in ghost crabs (*Ocypode quadrata*) of different sizes

Alexa Tullis · Scott C. Andrus

Abstract It is well established that the metabolic cost of horizontal locomotion decreases as a regular function of animal body mass, regardless of body form and phylogeny. How body size affects the cost of incline exercise remains much less clear. Studies on vertebrates have led to the hypotheses that the cost of vertical work is independent of body mass and that the added cost of locomoting on inclines is lower for small animals. Studies on vertebrates and a few invertebrates provide evidence both for and against these hypotheses. To gain further insight into the cost of incline exercise, we measured oxygen consumption of small (2.33 ± 0.07 g) and large (46.66 ± 5.33 g) ghost crabs (*Ocypode quadrata*) locomoting horizontally and up a 20° incline. The slope of the oxygen consumption versus speed relationship (= minimum cost of transport) was not significantly different for small crabs exercising horizontally and on an incline. However, the intercept for incline exercise was significantly higher, indicating that small crabs used more energy during incline exercise than during horizontal exercise. Incline had no effect on the slope or intercept of the oxygen consumption versus speed relationship for large crabs. Our results suggest that the cost of incline locomotion may be large for small animals and that the cost is not independent of body size. Our results add to the growing body of research indicating that body mass is but one factor that determines the cost of incline locomotion and efficiency of vertical work.

Introduction

Studies on mammals and birds have demonstrated that the metabolic cost of terrestrial locomotion decreases as a regular function of body mass (Fedak and Seeherman 1979; Taylor et al. 1970, 1982). In contrast, the mechanical work required to move the body forward remains relatively constant regardless of body size (Full 1989; Heglund et al. 1982). Combining these two relationships reveals that the efficiency of converting metabolic energy to mechanical work increases with body size during terrestrial locomotion (Alexander 2005; Full and Tu 1991). Recent studies on mammals confirm the general patterns described above, but provide evidence for a step-change in the scaling of metabolic costs, mechanical cost, and locomotor efficiency in small and large mammals rather than a smooth linear relationship (Nudds et al. 2009; Reilly et al. 2007). Authors of these studies hypothesize that postural differences in these two groups may help explain the discontinuity (Nudds et al. 2009; Reilly et al. 2007).

The relationships described above apply to horizontal locomotion. How body size affects costs when animals locomote on inclines remains unclear, and attempts to estimate energetic efficiency using data from incline studies have failed to yield consistent results. An early study comparing large and small mammals concluded that the additional cost of incline locomotion averaged 15.5 J kg^{-1} m^{-1} and was independent of body mass (Taylor et al. 1972). Therefore, because the cost of horizontal locomotion increases with decreasing body mass,

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the incremental increase in cost during incline locomotion should decrease with decreasing body mass (Taylor et al. 1972), and may help explain why small animals appear to effortlessly ascend inclines. Empirical tests, however, have failed to consistently support the hypotheses that the cost of vertical work is a constant 15.5 J kg^{-1} m^{-1} ("constant efficiency" hypothesis), or that the cost of incline locomotion is minimal in small animals. For example, a comparison of locomotory costs of several species of mammals ranging from 250 g to 250 kg led to the conclusion that the additional cost of incline locomotion was independent of body mass but was closer to 27 J kg^{-1} m^{-1} (Cohen et al. 1978). A recent study comparing the cost of incline locomotion in mammals and birds showed a larger increase in locomotory costs for the smaller species than for the larger, refuting both the constant efficiency hypothesis and the hypothesis that the cost of incline locomotion is relatively smaller for smaller animals (Snyder and Carello 2008).

Arthropods present an opportunity to further investigate how body size influences the cost and efficiency of locomotion because they extend the size range well below that of mammals and birds. Studies have shown that, despite major differences in body form, horizontally exercising crustaceans, insects, and myriapods fit the same general pattern that has been found for birds and mammals (Full 1987, 1989; Full et al. 1990; Lighton 1985). However, the few studies that have measured the metabolism of arthropods during incline exercise have only added to the ambiguity about how body size influences locomotory cost and efficiency. For example, 12 mg ants showed no change costs when walking up 30° and 60° inclines relative to when walking horizontally (Lipp et al. 2005), supporting the hypothesis that the added cost of incline locomotion decreases with decreasing body size. On the other hand, cockroaches averaging 0.8 g used substantially more energy when ascending 45° and 90° inclines than during horizontal locomotion (Full and Tullis 1990). Finally, larger 5 g cockroaches showed no increase in cost when locomoting up inclines ranging from 5°–25° (Herreid et al. 1981).

In the present study, we investigate the cost of incline locomotion in another species of arthropod, the ghost crab (*Ocypode quadrata*), a semi-terrestrial species with demonstrated running ability (Full 1987; Weinstein et al. 1994; Weinstein and Full 1994). Studying ghost crabs will add to the body of knowledge on the cost of incline locomotion in two ways. First, because ghost crabs can vary substantially in body mass, it is possible to compare small and large individuals with similar body form (Haley 1969). This is particularly relevant given recent work suggesting that postural differences may explain much of the differences in locomotory costs and efficiency between large and small terrestrial animals (Nudds et al. 2009; Reilly et al. 2007).

Second, to date there have been only three studies on the metabolic cost of incline locomotion in invertebrates, all of which were insects (Full and Tullis 1990; Herreid et al. 1981; Lipp et al. 2005). Thus, information from the present study may help reveal patterns in how incline influences the locomotory costs of invertebrates. If efficiency of vertical work is constant, then any increase in cost during incline locomotion should be less for small crabs than for large crabs. On the other hand, if efficiency decreases with decreased body size, as it does for horizontal exercise, then small crabs should show proportionally greater increases in cost during incline locomotion relative to large crabs.

Materials and methods

Animal care

Ghost crabs, *O. quadrata,* were purchased from Gulf Specimens, Inc. in Panacea, Florida, and shipped to the University of Puget Sound Biology Department. Small crabs ($N = 5$, 2.13–2.53 g, average 2.33 g $\pm$ 0.07 SE) and large crabs ($N = 4$, 34.76–58.74 g, average 46.66 g $\pm$ 5.33 SE) were housed individually in 30 $\times$ 15 $\times$ 9 cm and 46 $\times$ 30 $\times$ 15 cm plastic containers, respectively. The containers were filled with approximately 4–8 cm sand and 40–60% seawater (Instant Ocean^{TM}). Containers were tilted to provide crabs with access to terrestrial or aquatic environments and maintained at 21°C on a 12:12 light:dark cycle. Crabs were fed frozen commercial fish (silversides, *Menidia menidia*) or krill (*Euphasia* spp.) twice per week, and each container was thoroughly cleaned the following day. Individual crabs were tested on average twice per week and were fasted for at least 24 h before measuring oxygen consumption. Housing conditions and experiments were performed with the approval of the University of Puget Sound's Animal Care and Use Committee.

Measuring steady-state oxygen consumption

To measure oxygen consumption, individual crabs were placed on a treadmill housed in an airtight Lucite respirometer. The internal dimensions were 4 cm wide $\times$ 5.5 cm high $\times$ 9 cm long for the small crab respirometer and 7.5 cm wide $\times$ 10 cm high $\times$ 25 cm long for the large crab respirometer. The tread belts were constructed out of vinyl fabric that prevented the crabs from slipping during locomotion. To measure oxygen consumption during incline locomotion, the treadmill respirometer was tilted to an angle of 20°. The respirometer was placed within a temperature-controlled chamber to maintain ambient temperature at 24°C. To help prevent desiccation, the inside of the respirometer was sprinkled with a

small amount of water prior to introducing an animal. Crabs of both size classes were first exercised horizontally within 4 weeks. Immediately after these trials were complete, crabs were exercised on a 20° incline within the next 3 weeks. For both horizontal and incline trials, we tested the lower speeds first to minimize the chance of injury that would render an animal unable to complete more trials.

Oxygen consumption was measured using open-flow respirometry according to established procedures (Herreid et al. 1981; Full 1987). Room air was drawn through the respirometers with an air pump (model R-2, AEI Technologies, Pittsburgh, PA) at rates of 50 ml min^{-1} for small crabs and 300 ml min^{-1} for large crabs. Before entering the analyzer, air passed through filters containing Drierite (Acros Organics, Morris Plains, NJ) and soda lime (Fisher Chemicals, Fair Lawn, NJ) to remove water vapor and carbon dioxide, respectively. The oxygen content of the air was measured with dual-channel electrochemical oxygen analyzer (model S-3A/II, AEI Technologies), which was calibrated with ambient air prior to data collection. One channel of the analyzer measured oxygen content of ambient air and served as a reference while the other measured the oxygen content of the air exiting the respirometer. Data from the analyzer were collected at a sampling rate of 0.67 Hz with a PowerLab 4 s equipped with Chart v. 3.5.6/s and visualized on a PowerMac 6500 computer.

The basic protocol for measuring oxygen consumption followed the procedures of Full (1987). To begin each experiment, a crab was placed in the treadmill respirometer and given a 30-min rest period. Resting oxygen consumption ($\dot{V}_{O_2 \text{rest}}$, ml O$_2$ g^{-1} h^{-1}) was determined by averaging the readings over the last 5–10 min of this rest period when the animals were quiescent. Animals were then exercised for 20–25 min at a single speed. For small crabs, the speeds were 0.10, 0.16, 0.20, and 0.26 (incline) or 0.31 (level) km h^{-1}. For large crabs, the speeds were 0.20, 0.34, and 0.46 km h^{-1}. The maximum aerobic speeds during horizontal locomotion reported for this species are approximately 0.3 km h^{-1} for small crabs and 0.6 km h^{-1} for large crabs (Full 1987). In addition, the speeds chosen were 6–15% of the maximum running speed reported for small and large crabs (Perry et al. 2009). At the speeds we examined, the crabs' gait is considered a walk (Blickhan and Full 1987). Steady-state oxygen consumption ($\dot{V}_{O_2 \text{ss}}$, ml O$_2$ g^{-1} h^{-1}) was determined from the final 4–6 min of an exercise bout when the oxygen content of the air exiting the respirometer remained stable. Crabs were weighed to the nearest 0.01 g following each exercise bout.

The behavior of each crab was monitored during rest and exercise. If the crab exhibited excessive extraneous movements during exercise that interfered with continuous locomotion (e.g., clinging to the sides of the chambers or rapid and repeated switching of leads), the results were not included in the final analyses. Results were also not used if crabs could not sustain exercise long enough to reach $\dot{V}_{O_2 \text{ss}}$.

Data and statistical analysis

Mass-specific oxygen consumption, in units of ml O$_2$ g^{-1} h^{-1}, corrected to STPD, was calculated using the following equation:

$$\dot{V}_{O_2} = \left(\frac{\dot{V}_E \times \Delta F_{O_2}}{\left(1 - F_{I_{O_2}}\right) \times m} \right) \tag{1}$$

where $\dot{V}_E$ is the flow rate of air exiting the respirometer, ΔF_{O_2} is the difference in oxygen content of air entering and exiting the respirometer, $F_{I_{O_2}}$ is the oxygen content of the air entering the respirometer, and m is body mass (Withers 1977). The cost of transport at a given speed (COT, in ml O$_2$ g^{-1} km^{-1}), defined as the amount of energy an animal uses to travel a given distance, was calculated for each condition (horizontal and incline exercise) by dividing individual measurements of $\dot{V}_{O_2 \text{ss}}$ by the speed at which they were obtained. These values were used to calculate average values for the COT at a given speed and substrate angle.

The metabolic cost of lifting 1 kg of body mass 1 m vertically was calculated with the following equation:

$$\text{Cost of lifting} = \left(\frac{\text{MCOT}_{in} - \text{MCOT}_h}{\sin \theta} \right) \times 20.1 \tag{2}$$

where θ is the angle of incline (degrees), 20.1 is the factor to convert ml O$_2$ into Joules, and MCOT (ml O$_2$ g^{-1} km^{-1}) is the minimum cost of transport (i.e., the minimum amount of energy needed to move 1 g of animal 1 km) (Taylor et al. 1972). In this equation, MCOT$_{in}$ and MCOT$_h$ are the MCOT for incline and horizontal locomotion, respectively.

Whole-animal efficiency during vertical work was estimated from the following equation:

$$\text{Efficiency (\%)}$$
$$= \left(\frac{\text{mechanical work to lift 1 kg 1 m vertically}}{\text{metabolic energy to lift 1 kg 1 m vertically}} \right) \tag{3}$$

where the mechanical work to lift 1 kg 1 m vertically = 9.8 J kg^{-1} m^{-1}, and the metabolic energy to lift 1 kg 1 m vertically comes from equation (2) above (Taylor et al. 1972).

All statistics were performed using SPSS, version 13. A two-way ANOVA was used to determine if there were significant differences in the $\dot{V}_{O_2 \text{rest}}$ of small and large crabs resting horizontally and on an incline. Separately for each substrate angle and size class, polynomial regression

analyses were used to determine if a second order polynomial equation described the relationship between speed and $\dot{V}_{O_{2ss}}$ significantly better than a linear equation. In all cases, adding the next higher order coefficient did not explain significantly more of the variation (polynomial regression analysis: small horizontal, $F_{1,8} = 0.89$, $P = 0.37$; small incline, $F_{1,9} = 2.72$, $P = 0.13$; large level, $F_{1,9} = 0.25$, $P = 0.63$; large incline, $F_{1,8} = 0.01$, $P = 0.92$). Thus, all $\dot{V}_{O_{2ss}}$ versus speed data sets were treated as linear. Analyses of covariance (ANCOVA), with angle treated as categorical and speed as continuous, were used to determine if there was a significant difference in the relationships between speed and $\dot{V}_{O_{2ss}}$ during horizontal and incline exercise for small crabs and again for large crabs. If there was no significant interaction between angle and speed, the interaction term was removed from the model and the ANCOVA re-run to determine the influence of angle and speed on $\dot{V}_{O_{2ss}}$ (Engqvist 2005). Separately for each size class, the COT during horizontal and incline locomotion at a given speed were compared with two-sample t tests. Because of our criteria for acceptable measurements of oxygen consumption, as well as circumstances outside of our control (e.g., an animal losing a portion of an appendage), not all animals used in this study have values for every speed and incline combination, precluding us from using standard statistical analyses for repeated measurements. To avoid underestimating α due to pseudo-replication, differences were considered significant at $P < 0.01$ (Buck and Barnes 2000). Means are presented as ±standard errors.

Results

Resting oxygen consumption

The influence of substrate angle on the $\dot{V}_{O_{2rest}}$ did not differ between small and large crabs (two-way ANOVA, $F_{1,14} = 2.298$, $P = 0.15$); for crabs of both sizes, incline had no significant effect on $\dot{V}_{O_{2rest}}$ (two-way ANOVA, $F_{1,14} = 1.190$, $P = 0.29$). However, $\dot{V}_{O_{2rest}}$ of small crabs was significantly greater than that of large crabs (two-way ANOVA, $F_{1,14} = 46.438$, $P < 0.0001$; Table 1).

Oxygen consumption during exercise

Small crabs

The $\dot{V}_{O_{2ss}}$ of small crabs increased with speed up to 0.2 km h^{-1} when exercising horizontally and on a 20° incline (Fig. 1; Table 1). Above 0.2 km h^{-1}, $\dot{V}_{O_{2ss}}$ did not increase further suggesting that the crabs were running above their maximum aerobic speed. Over the range of speeds where $\dot{V}_{O_{2ss}}$ increased, there was no significant interaction between angle and speed on $\dot{V}_{O_{2ss}}$ (ANCOVA, angle × speed, $F_{1,19} = 0.005$, $P > 0.90$), indicating that speed influenced $\dot{V}_{O_{2ss}}$ similarly for both substrate angles. During both horizontal and incline exercise, small crab $\dot{V}_{O_{2ss}}$ increased significantly with speed (ANCOVA; speed, $F_{1,20} = 45.118$, $P < 0.001$). In addition, there was a significant effect of angle on $\dot{V}_{O_{2ss}}$ (ANCOVA; angle, $F_{1,20} = 16.382$, $P = 0.001$), such that the intercept for incline exercise was significantly higher than that for horizontal exercise (0.50 vs. 0.31 ml O_2 g^{-1} h^{-1}, respectively; Fig. 1; Table 1).

The average COT for small crabs exercising on an incline was 20–30% greater than that for level exercise (Fig. 2). This represented a significant increase at 0.2 km h^{-1} (t test; $t = -4.248$, $df = 7$, $P = 0.004$), but fell short of statistical significance at 0.1 (t test; $t = -2.208$, $df = 6$; $P = 0.069$), and was not significant at 0.16 km h^{-1} ($t = -1.533$, $df = 4$, $P = 0.20$).

Large crabs

As with the small crabs, the influence of speed on $\dot{V}_{O_{2ss}}$ did not differ between large crabs exercising horizontally and

Table 1 Oxygen consumption of small and large ghost crabs (*Ocypode quadrata*) while resting and exercising horizontally and on an 20° incline

Body mass (g)	Substrate angle (°)	$\dot{V}_{O_{2rest}}$(ml O_2 g^{-1} h^{-1})	$\dot{V}_{O_{2ss}}$(ml O_2 g^{-1} h^{-1}) versus speed (v, km h^{-1})
2.33 ± 0.07 (N = 5)	0	0.16 ± 0.019[a]	$\dot{V}_{O_{2ss}} = 3.76v + 0.31$ ($R^2 = 0.82$, $F_{1,9} = 41.36$, $P < 0.001$)
	20	0.20 ± 0.023[a]	$\dot{V}_{O_{2ss}} = 3.85v + 0.50$ ($R^2 = 0.60$, $F_{1,10} = 14.71$, $P = 0.003$)
46.66 ± 5.33 (N = 4)	0	0.062 ± 0.011[b]	$\dot{V}_{O_{2ss}} = 0.73v + 0.32$ ($R^2 = 0.40$, $F_{1,10} = 6.75$, $P = 0.027$)
	20	0.055 ± 0.006[b]	$\dot{V}_{O_{2ss}} = 0.51v + 0.39$ ($R^2 = 0.26$, $F_{1,9} = 3.11$, $P = 0.11$)

Means are ±standard error

Statistical results from regression analyses are presented after each regression equation. ANCOVA results comparing the relationship between speed and $\dot{V}_{O_{2ss}}$ for horizontal and incline locomotion within each size class are presented after each regression equation

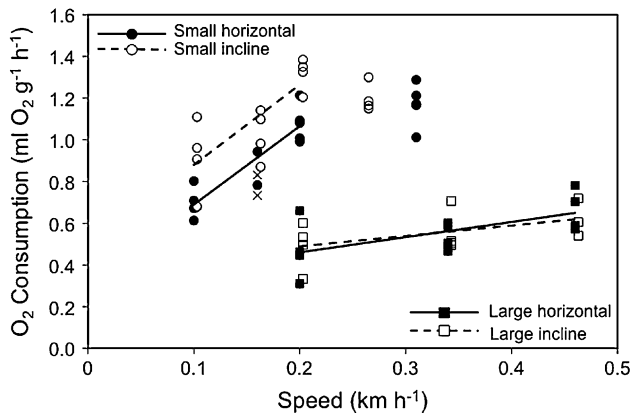Different letters indicate significant differences at $P < 0.01$

Fig. 1 Steady-state oxygen consumption ($\dot{V}_{O_{2ss}}$) versus speed for small and large ghost crabs (*Ocypode quadrata*) exercising horizontally and on a 20° incline. Each *symbol* represents the oxygen consumption of a single individual. *Filled and open circles* represent small crabs (2.33 ± 0.07 g; $N = 5$) exercising horizontally and on an incline, respectively. *Filled and open squares* are for large crabs (46.66 ± 5.33 g; $N = 4$) exercising horizontally and on an incline, respectively. *Lines* representing the linear regression of $\dot{V}_{O_{2ss}}$ and speed for crabs exercising horizontally are solid while those for crabs exercising on an incline are *dashed* (equations given in Table 1). Included with the small crab data are points representing oxygen consumption of animals running at speeds above their maximum aerobic speed (0.26 and 0.30 km h^{-1}). These points were not included in statistical analyses. Data points for two small crabs running horizontally at 0.16 km h^{-1} (*times symbol*) are shown for comparison but were not included in any statistical analyses because these individuals did not exercise consistently at the other conditions. Some data points have been slightly offset to facilitate comparison between incline and horizontal results

on a 20° incline (ANCOVA; angle × speed, $F_{1,19} = 0.289$, $P = 0.597$). During both horizontal and incline locomotion, $\dot{V}_{O_{2ss}}$ increased significantly with speed (ANCOVA; speed, $F_{1,20} = 10.043$, $P = 0.005$; Fig. 1; Table 1), but angle of locomotion had no influence on $\dot{V}_{O_{2ss}}$ (ANCOVA; angle, $F_{1,20} = 0.00$, $P = 0.99$).

The average COT was not significantly different for large crabs exercising horizontally and on an incline at any of the speeds tested (*t* test; 0.20 km h^{-1}, $t = -0.220$, $df = 6$, $P = 0.83$; 0.34 km h^{-1}; $t = -0.262$, $df = 6$, $P = 0.80$; 0.46 km h^{-1}, $t = 0.539$, $df = 5$, $P = 0.61$).

The cost of walking up inclines

Because our results for small ghost crabs suggested that locomotory costs increase when the animals walk up a 20° incline, we used these results to estimate the metabolic cost of vertical work using Eq. 2. Previous studies employing this equation have obtained values for $MCOT_h$ and $MCOT_{in}$ from the slopes of the $\dot{V}_{O_{2ss}}$ versus speed relationships for horizontal and incline locomotion, respectively (Cohen et al. 1978; Full and Tullis 1990; Snyder and Carello 2008). Because the slopes were not significantly
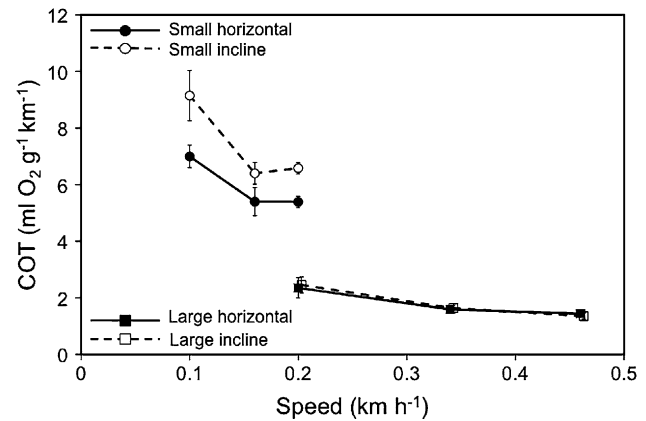

Fig. 2 Average cost of transport (COT $\pm$ SE) versus speed for small and large ghost crabs (*Ocypode quadrata*) exercising horizontally and on a 20° incline. Symbols and sample sizes are as in Fig. 1. Average COT was calculated by dividing individual values of $\dot{V}_{O_{2ss}}$ by the appropriate speed from the data shown in Fig. 1. Individual values were then averaged to obtain a group average for each condition

different for small crabs exercising horizontally and on an incline (Fig. 1; Table 1), we were unable to use these values to calculate the cost of lifting. Instead, we obtained values for $MCOT_h$ and $MCOT_{in}$ from the COT data presented in Fig. 2 (Wickler et al. 2000). From these results, the $MCOT_h$ of small crabs averaged 5.39 ± 0.44 ml O$_2$ g^{-1} km^{-1} while $MCOT_{in}$ averaged 6.40 ± 0.76 ml O$_2$ g^{-1} km^{-1} (Fig. 2). Using these values for $MCOT_h$ and $MCOT_{in}$ in Eq. 2 yielded a lifting cost for small ghost crabs of 59.4 J kg^{-1} m^{-1}. Using this value in Eq. 3 generated an efficiency of 16.5% for small crabs ascending a 20° incline.

Discussion

The effect of incline on the cost of locomotion in ghost crabs

Results from the present study do not support the hypothesis that the cost of vertical work is constant or that the cost of incline locomotion is proportionally smaller for smaller animals. Specifically, our results showed that the cost for small (2.33 g) ghost crabs exercising on a 20° incline increased by 20–30% relative to those running horizontally, while that of large (46.7 g) crabs remained unchanged. Elevated costs during incline locomotion have been demonstrated in birds, mammals, and an insect (Bamford and Maloiy 1980; Cohen et al. 1978; Ellerby et al. 2003; Full and Tullis 1990; Snyder and Carello 2008; Taylor et al. 1972). In these studies, the greatest effect of incline was to increase the slope of the line relating $\dot{V}_{O_{2ss}}$ and speed, which represents the MCOT (Taylor et al. 1970). In

contrast, our results for small crabs suggest that exercising on an incline can influence the cost of locomotion by elevating energy requirements to a similar degree at each speed while the MCOT remains constant (Fig. 1). The vertical shift of the $\dot{V}_{O_{2ss}}$ versus speed relationship with no change in slope translated into an additional energy requirement of approximately 0.20 ml O_2 g^{-1} h^{-1} at the speeds ranging from 0.1 to 0.2 km h^{-1}.

In contrast to the small crabs, our results revealed no increase in the energetic requirements of large ghost crabs walking up an incline. Similar results have been documented in mice (30 g), cockroaches (5.0 g), and ants (0.01 g) (Taylor et al. 1972; Herreid et al. 1981; Lipp et al. 2005, respectively). One explanation put forth for these results is that the already high locomotory costs of small animals made any additional increases during incline running relatively insignificant (Herreid et al. 1981; Lipp et al. 2005; Taylor et al. 1972). This explanation is not adequate to explain our results for the large crabs since the cost to walk up an incline did increase for the small crabs. Differences in body form between small and large crabs are not likely to explain our results since ghost crabs are geometrically similar in terms of carapace width and the leg length within the size range studied (Haley 1969). These points, combined with the fact that the large crabs were comparable in size or larger than animals that show increases in locomotory costs with incline (0.8 g cockroaches, 35 g mice, 43 g quail; Full and Tullis 1990; Snyder and Carello 2008), suggests that body mass, morphology, or posture alone cannot explain why large crabs showed no increase in cost during incline walking. It has been suggested that measuring only oxygen consumption could underestimate the actual locomotory costs if animals relied increasingly on anaerobic metabolism during incline exercise (Snyder and Carello 2008). Although we cannot rule this out, we believe that any contributions from anaerobic sources would have been minimal in the present study because the crabs were exercising at speeds below the maximum aerobic speed for horizontal locomotion determined for this species (Full 1987).

Uncovering the biological reasons for why small but not large crabs showed elevated cost during incline locomotion, and why the elevated cost was manifest in an upward shift in the $\dot{V}_{O_{2ss}}$ versus speed and not an increase in the MCOT, remains a topic for future studies (although different sized respirometers may have affected the absolute values of oxygen consumption measured, it is unlikely that this factor contributed to the different metabolic responses observed between small and large crabs during incline locomotion). Investigations focusing on the limb kinematics and/or motor unit activities as crabs locomote on various inclines (e.g., Full and Tullis 1990; Duch and

Pflüger 1995, respectively) would help address both of these questions. Extending this research to other species of crabs is also important for determining if our results apply only to this species or to crabs in general.

The cost and efficiency of incline locomotion

A seminal study investigating the energetics of small and large mammals locomoting on inclines led Taylor et al. (1972) to conclude that the cost to lift one unit body mass one vertical meter was 15.5 J kg^{-1} m^{-1}, regardless of body size. This led to the hypothesis that the efficiency of vertical work was constant and provided an explanation for why many small animals seemed to be able to effortlessly scale steep inclines; because the cost of horizontal locomotion increases with decreasing body size, the additional cost to scale inclines could be negligible in very small animals (Taylor et al. 1972). Subsequent studies on mammals, birds, and an insect have called into question both that the cost of lifting is a constant 15.5 J kg^{-1} m^{-1}, and that the cost of incline locomotion is low in small animals (Cohen et al. 1978; Full and Tullis 1990; Snyder and Carello 2008). Results for ghost crabs provide further evidence against both hypotheses. Not only did the smaller crabs show elevated locomotory costs during incline exercise, but the larger crabs showed no effect of incline on cost, a result opposite to that predicted if the cost of lifting was constant. Moreover, our calculations yield a lifting cost of 59.4 J kg^{-1} m^{-1}, well above the 15.5 and 27 J kg^{-1} m^{-1} that have been previously proposed (Taylor et al. 1972; Cohen et al. 1978, respectively). The estimated energy cost of lifting for small ghost crabs is roughly 1/4 that proposed for 0.8 g cockroaches locomoting up 45° and 90° inclines (223 and 290 J kg^{-1} m^{-1}, respectively), the only other invertebrate for which these calculations have been possible (Full and Tullis 1990). A recent study on small vertebrates locomoting up 10° inclines yield lifting costs of approximately 100 J kg^{-1} m^{-1} (43 g quail and 35 g mice; Snyder and Carello 2008). Recalculating the costs for the species mentioned above using $MCOT_h$ and $MCOT_{in}$ values determined in the same manner as was used for the ghost crabs (see Methods), yields very similar results; lifting costs of approximately 238 J kg^{-1} m^{-1} for cockroaches, and 137 and 68 J kg^{-1} m^{-1} for quail and mice, respectively, with an average of 103 J kg^{-1} m^{-1} for the vertebrates.

Our results do not support the hypothesis that the efficiency of vertical work is independent of body mass. We have estimated an efficiency of 16.5% for small ghost crabs ascending inclines. This value is substantially lower than the 36–60% efficiencies estimated from early studies on mammals (Cohen et al. 1978; Taylor et al. 1972), but more

in line with 10% efficiency estimated for small mammals and birds ascending 10° inclines (Snyder and Carello 2008; estimating efficiencies with the recalculated lifting costs cited above for mice and quail yield values of 14 and 7%, respectively). Comparison with invertebrates where efficiency was calculated in a similar manner is limited to one study on cockroaches which reported efficiencies of 4.4 and 3.4% for 0.8 g animals ascending 45° and 90° inclines, respectively (Full and Tullis 1990). These values are substantially lower than the 16.5% efficiency estimated for small ghost crabs. If the efficiency of vertical work was largely determined by body size, it would be expected that values calculated for the 2.3 g crabs would be closer to those for the 0.8 g insects than for the ∼40 g vertebrates.

It has been suggested that efficiency of vertical work may partly depend on substrate angle (Bamford and Maloiy 1980; Cohen et al. 1978; Snyder and Carello 2008). As a way to address this hypothesis, we calculated the net cost of incline exercise per degree incline for the crabs in the present study and a variety of animals from the literature. We did this by dividing the difference between $MCOT_{in}$ and $MCOT_h$ with substrate angle, a method similar to that employed by Cohen et al. (1978). This comparison, across animals ranging from less than 1 g to nearly 500 kg, and differing in body form, posture, and phylogeny, reveals that the net cost of incline locomotion per degree incline is relatively constant for animals weighing between 200 g and 500 kg, regardless of posture or leg number (Fig. 3). This result confirms and expands upon results from a study on mammals (Cohen et al. 1978), and lends support to the findings of Snyder and Carello (2008) that the efficiency of vertical work is relatively constant for birds and mammals between 1 and 500 kg. In contrast, the net cost of incline locomotion appears much more variable for animals <200 g. Although there are too few data points to draw any solid conclusions, postural differences among these animals (birds, mammals, and arthropods), as well as those between small and large
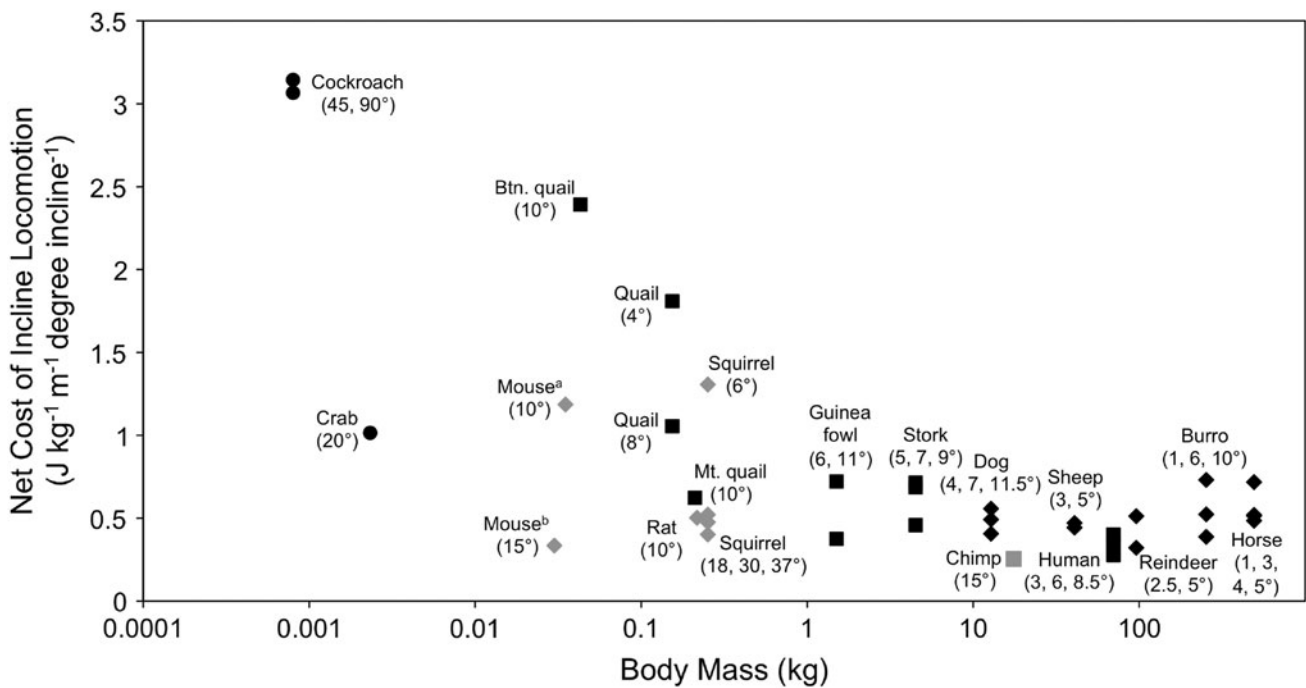

Fig. 3 The net cost of incline locomotion versus log body mass for various animals. Net cost was calculated by subtracting the $MCOT_h$ from $MCOT_{in}$ (J kg^{-1} m^{-1}) and dividing the difference by the substrate angle (°). Multiple data points at a single body mass represent values for animals exercised at more than one substrate angle. Substrate angles were rounded to the nearest 0.5° and are indicated below each animal. Symbols are as follows: *black diamonds* quadrapedal mammals with an upright posture, *grey diamonds* quadrapedal mammals with a crouched posture, *black squares* bipedal birds and mammals, *grey squares* "knuckle-walking" mammal, *black circles* hexapedal and octapedal arthropods. *Superscript letters* indicate values for the same species from different studies. Literature sources for values used in the calculations were: burro, *Equus asinus*, Yousef et al. (1972); chimpanzee, *Pan troglodytes*, Taylor et al. (1972); cockroach, *Periplaneta americana*, Full and Tullis (1990); dog, *Canis familiaris*, Raab et al. (1976); guinea fowl, *Numida meleagris*, Ellerby et al. (2003); horse, *Equus caballus*, Eaton et al. (1995); human, *Homo sapiens*, Margaria et al. (1963); mouse[a], *Mus musculus*, Taylor et al. (1972); mouse[b], *M. musculus*, Snyder and Carello (2008); button quail, *Coturnix chinensis*, Snyder and Carello (2008); mountain quail, *Oreortyx pictus*, Snyder and Carello (2008); quail, *Coturnix coturnix*, Warncke et al. (1988); rat, *Rattus norvegicus*, Snyder and Carello (2008); reindeer, *Rangifer tarandus groenlandicus*, White and Yousef (1977); sheep, *Orvis aries*, Clapperton (1964); squirrel, *Tamiasciurus hudsonicus*, Wunder and Morrison (1974); stork, *Leptoptilos crumeniferous*, Bamford and Maloiy (1980)

animals, may contribute to this variability. With regard to the two arthropods included in this comparison, crabs from the present study had costs within the range found for small birds and mammals, while the net costs for the cockroach were the highest of all the species included. Finally, the comparison in Fig. 3 shows that the range of net costs for a single species exercised on different inclines is comparable to that observed among the larger species examined, suggesting that incline does influence the efficiency of incline locomotion.

Overall, data from the present study add to the body of work suggesting that the cost of lifting can be substantial for small animals and is not independent of body size. Beyond this conclusion, there is still considerable variation in estimates of the cost of incline locomotion and the efficiency of vertical work. More studies on factors such as substrate angle, posture, as well as phylogenetic differences are needed to get a clearer picture of the factors that contribute to the cost and efficiency of locomoting up inclines.

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