The Journal of Experimental Biology 214, 2202-2214

doi:10.1242/jeb.054700

RESEARCH ARTICLE

Energetics of burrowing by the cirratulid polychaete *Cirriformia moorei*

Kelly M. Dorgan[1,*,†], Stephane Lefebvre[2], Jonathon H. Stillman[1,2] and M. A. R. Koehl[1]

[1]Department of Integrative Biology, University of California, Berkeley, CA 94720-3140, USA and [2]Romberg Tiburon Center for Environmental Studies, San Francisco State University, Tiburon, CA 94920, USA

*Present address: Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0202, USA

†Author for correspondence (kdorgan@ucsd.edu)

Accepted 18 March 2011

SUMMARY

Burrowing through marine sediments has been considered to be much more energetically expensive than other forms of locomotion, but previous studies were based solely on external work calculations and lacked an understanding of the mechanical responses of sediments to forces applied by burrowers. Muddy sediments are elastic solids through which worms extend crack-shaped burrows by fracture. Here we present data on energetics of burrowing by *Cirriformia moorei*. We calculated the external energy per distance traveled from the sum of the work to extend the burrow by fracture and the elastic work done to displace sediment as a worm moves into the newly formed burrow to be 9.7 J kg^{-1} m^{-1} in gelatin and 64 J kg^{-1} m^{-1} in sediment, much higher than for running or walking. However, because burrowing worms travel at slow speeds, the increase in metabolic rate due to burrowing is predicted to be small. We tested this prediction by measuring aerobic metabolism (oxygen consumption rates) and anaerobic metabolism (concentrations of the anaerobic metabolite tauropine and the energy-storage molecule phosphocreatine) of *C. moorei*. None of these components was significantly different between burrowing and resting worms, and the low increases in oxygen consumption rates or tauropine concentrations predicted from external work calculations were within the variability observed across individuals. This result suggests that the energy to burrow, which could come from aerobic or anaerobic sources, is not a substantial component of the total metabolic energy of a worm. Burrowing incurs a low cost per unit of time.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/214/13/2202/DC1

Key words: burrowing, energetics of locomotion, cost of transport, polychaete locomotion, aerobic metabolism, anaerobic metabolism, tauropine.

INTRODUCTION

Burrowing has been considered to be much more energetically expensive than other forms of locomotion, but it has been argued that the high cost of burrowing is justified because sediments provide habitat and a refuge from predators (Hunter and Elder, 1989). Trevor calculated high energetic cost of burrowing per distance traveled for several polychaetes and suggested that soft-bodied burrowers are less efficient than those with a rigid exoskeleton (e.g. *Emerita*) (Trevor, 1978). Crawling with a hydrostatic skeleton has been found by several authors to be costly (Denny, 1980; Casey, 1991; Berrigan and Lighton, 1993). Generalizing locomotory strategies across animals with hydrostatic skeletons is questionable, however, as the mechanics of burrowing in saturated sediments differ substantially from those of terrestrial crawling (Dorgan, 2010).

Whereas costs of transport for running, flying and swimming are measured from oxygen consumption rates, previous estimates for burrowing by polychaetes were based on external work converted to metabolic work, assuming an efficiency constant. This approach makes comparisons between burrowing and other modes of locomotion difficult. Moreover, these estimates of external work to burrow (Trevor, 1978; Hunter and Elder, 1989) implicitly assumed that sediment plastically deformed around the worm to create a burrow.

Recent work, however, has shown that marine muds on the short time and length scales relevant for burrowing worms are elastic solids (Johnson et al., 2002; Boudreau et al., 2005), and that worms extend burrows through muds by fracture (Dorgan et al., 2005; Dorgan et al., 2007; Dorgan et al., 2008; Che and Dorgan, 2010). For example, the polychaete *Nereis virens* Sars everts its pharynx to apply stress to the walls of its burrow, perpendicular to its direction of travel. This stress is amplified at the crack tip just in front of the worm, and when enough stress is applied to exceed the fracture toughness of the sediment, the burrow extends by fracture (Dorgan et al., 2005). This mechanism allows the worm to apply forces over relatively small distances to either extend the crack out in front of the pharynx or to simply extend the crack tip by moving forward in the burrow like a wedge (Dorgan et al., 2008). Intuitively, applying small forces that are amplified through mechanical advantage to extend a crack through the substratum should require less work than plastic deformation of the mud, suggesting that external work to burrow was probably overestimated. At the very least, previous estimates of the external work are clearly based on an inappropriate mechanical model and therefore should be re-evaluated.

A major limitation on measuring the energetic cost of burrowing has been that oxygen consumption measurements in muds are confounded by high abundances of bacteria and fauna that consume oxygen at rates that are difficult to quantify or control. Trevor measured oxygen consumption rates of burrowing polychaetes but pointed out that his measurements were probably inaccurate and instead used external work to calculate the energetic cost of burrowing (Trevor, 1978). That organisms in addition to the burrower were consuming oxygen during his experiments is suggested by the fact that his estimate of oxygen consumption of 6.9 mg O_2 (g wet mass)$^{-1}$ h^{-1}

is much higher than oxygen consumption rates for other active invertebrates [e.g. ~0.09 mg O_2 g^{-1} h^{-1} for *Urechis caupo* Fisher and MacGinitie 1928, irrigating its burrow (Julian et al., 2001); 0.3 mg O_2 g^{-1} h^{-1} for *Bullia digitalis* (Dillwyn 1817) in sand (Brown, 1979a); and 0.08 mg O_2 g^{-1} h^{-1} for crawling abalone, *Haliotis kamtschatkana* Jonas 1845 (Donovan and Carefoot, 1997)]. Gelatin as an analog for muddy sediments (Johnson et al., 2002; Boudreau et al., 2005) not only enables visualization of burrowing kinematics and measurement of forces using photoelastic stress analysis (Dorgan et al., 2005; Dorgan et al., 2007; Dorgan et al., 2008; Che and Dorgan, 2010) but also provides a medium with limited bacterial and no faunal oxygen consumption.

Polychaetes have a variety of anaerobic pathways and live in low-oxygen environments, therefore we measured anaerobic metabolism as well as aerobic metabolic rates in the study reported here. Anaerobic pathways can be classified into two main types: (1) pathways with high efficiency but low rates of energy production, such as the aspartate–succinate and glucose–succinate pathways, which are primarily used to survive anoxia, and (2) pathways with lower efficiency but faster rates of energy production, such as lactate and opine pathways, which are used for exercise-induced hypoxia (Livingstone, 1991). The cirratulid polychaete, *Cirriformia tentaculata* (Montagu 1808), has one muscle phosphagen, phosphocreatine, that can be used to phosphorylate ADP to make ATP (Bestwick et al., 1989). Phosphagens (type 2) have high rates of energy production and are generally used for short-term energy needs (Bestwick et al., 1989; Livingstone, 1991). Under anoxia, *C. tentaculata* used phosphocreatine and produced alanine and succinate but not lactate (Bestwick et al., 1989). In the study reported here, we assumed that alanine and succinate, which have low rates of energy production (type 1), would not be produced by burrowing worms during exercise, and thus we instead measured metabolites produced in higher-rate (type 2) pathways (Donovan et al., 1999), namely lactate dehydrogenase (LDH) and six opine dehydrogenases.

To calculate the energetic cost of burrowing, we measured oxygen consumption rates from overlying water as well as depletion of oxygen stored in hemoglobin by the cirratulid polychaete, *Cirriformia moorei* Blake 1996, burrowing in gelatin. We also measured concentrations of phosphocreatine, the principal phosphagen in these worms (Bestwick et al., 1989). Because the anaerobic metabolites produced by *C. moorei* had not been established, we measured first the opine dehydrogenase activities, then the anaerobic metabolite corresponding to the enzyme with highest activity, to quantify the anaerobic component of metabolism. External work was calculated from the work of fracture to extend the crack and the unrecovered elastic work needed to deform the sediment and create space for the worm's body. This paper gives the first estimates of the metabolic energy required to burrow based on measured aerobic and anaerobic components as well as the external work done calculated using mud material properties and fracture mechanics theory.

MATERIALS AND METHODS

Experimental set-up

Adult *C. moorei* (0.36±0.16 g wet mass.; mean ± s.d.) were collected from an intertidal flat at Inverness, CA, USA (38.09°N, 122.84°W), at low tide and kept in sediment under aerated seawater at 11°C until used in experiments. Burrowing experiments were done in glass aquaria (14×14×9 cm) filled with gelatin (28.35 g l^{-1}) made with artificial seawater of salinity ~30 (Instant Ocean, Aquarium Systems Inc., Mentor, OH, USA) following methods of Dorgan et al. (Dorgan et al., 2007).

Before pouring gelatin into an aquarium, a 15-mm diameter flat-bottomed vial was positioned to be submerged approximately 15 mm below the surface of the gelatin. The gelatin was left to set overnight, and the vial was gently removed before use to create a cylindrical hole of 2–3 ml volume in the surface of the gelatin. To prevent oxygen exchange with the overlying air, the surface of the gelatin was covered in overlapping pieces of Saran™ 8 polyvinylidene chloride barrier film, which has very low oxygen permeability. A crack was made in the bottom corner of the hole with forceps, and a worm was placed in the crack. The hole was filled with artificial seawater and covered with a piece of Saran in which two small holes were made. One was a slit through which the rotating end of a pager motor was inserted and then taped closed around the motor shaft to restrict exposure to air, and the other was a pinhole through which an oxygen probe (Unisense OXY50, Aarhus, Denmark) was inserted (Fig. 1). The pager motor stirred the water while oxygen concentration was measured.

Worms spent some time in the crack before beginning to burrow, during which measurements of 'resting' oxygen consumption and hemoglobin saturation were made. Once worms began to burrow, noted by extension of the crack, time was recorded and further rates of oxygen consumption were considered as 'burrowing'. If worms came out of the crack into the overlying water, the Saran, stirrer, and oxygen probe were removed, the worm was gently inserted back in the crack, water was exchanged, and Saran was replaced. Similarly, water was exchanged for fresh artificial seawater when its oxygen concentration dropped below 80% of saturation. While the worm was in the gelatin, hemoglobin saturation was measured using a T-stat ischemia monitoring system (Spectros Corporation, Portola Valley, CA, USA); a 1.5-mm diameter fiber-optic cable was inserted into the gelatin, oriented close to the worm and directed at the gills (described in detail below).

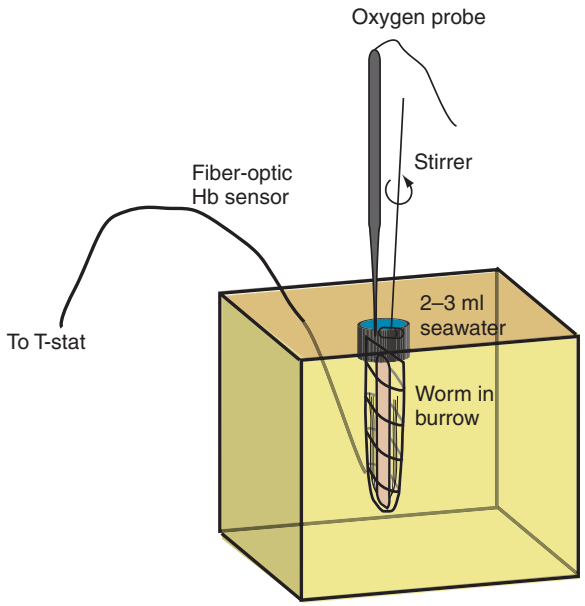

Fig. 1. Scheme of experimental setup showing worm in crack-shaped burrow (black outline) in gelatin (yellow). The small volume of water above the worm is shown with the oxygen probe and stirrer inserted from the top. The fiber-optic sensor for the T-stat ischemia monitoring system is inserted through the top of the gelatin, after lifting a small piece of Saran (not shown), and is pointed at the worm's gills (shown as thin lines on either side of the worm).

Once the worm stopped burrowing, often when encountering either a wall or the surface, it was removed from the gelatin and immediately frozen in liquid nitrogen and stored at –80°C for metabolite analysis.

Oxygen consumption rates

Oxygen concentration was measured in the overlying 2–3 ml water with a Clark-type microelectrode (Unisense OXY50) while worms were resting and burrowing. Care was taken to exclude air from the chamber and to mix water, by the pager motor, fast enough to maintain uniform oxygen concentration throughout the volume of water but not so fast as to disturb the worm (i.e. by lifting the gills up toward the motor). Oxygen consumed by the worm from water exchanged with the overlying water greatly exceeded diffusive flux of oxygen from the gelatin or advection of water into the burrow during burrow extension, so these terms were disregarded (Appendix). The microelectrode was connected to a picoammeter (Unisense P2000) and to a PC through a DAQ card (National Instruments 6036E with a BNC-2110 connector block, Austin, TX, USA). Data were recorded in Labview (v. 8.2, National Instruments). Before experiments, oxygen concentration (in volts) was measured at saturation (in artificial seawater aerated for at least 2 min) and in an anoxic solution of 0.1 mol l^{-1} sodium ascorbate and 0.1 mol l^{-1} NaOH. Volts were converted to oxygen concentration (mg O_2 l^{-1}) from these two endpoints, as the relationship between volts and oxygen concentration is linear from 0 to 100% saturation.

Oxygen consumption rate at 11°C was calculated from oxygen concentration as the difference in oxygen concentration over discrete time periods between exchanging water or reinserting the worm during resting periods. The volume of water in the overlying hole was measured before the worm was added and used to calculate total oxygen from concentration. Rates were normalized to wet mass of the worm (to obtain μg O_2 h^{-1} g^{-1}). Average oxygen consumption rate for each worm while resting or burrowing was calculated as a time-weighted mean of one to six discrete time periods.

Oxygen consumption from hemoglobin

While worms burrowed, spectrophotometric hemoglobin (Hb) saturation was determined using a T-stat monitor with a 1.5-mm fiber-optic endoscopic catheter (Spectros). This device is intended for human use and, similar to a pulse oximeter, calculates hemoglobin saturation from absorption at two wavelengths that differ in absorption characteristics for oxygenated and deoxygenated hemoglobin. Erythrocruorin from the related *Cirratulus grandis* Verrill 1873 [previously known as *Cirriformia grandis*, now a synonym of *Cirratulus grandis*; World Register of Marine Species (WoRMS), http://www.marinespecies.org/aphia.php?*P*=taxdetails&id=157297] has absorption spectra indistinguishable from that of human hemoglobin (Swaney and Klotz, 1971), which enabled the use of this medical device. Similarity in absorption spectra between annelid erythrocruorin and human hemoglobin is unsurprising as erythrocruorins are giant blood pigment complexes containing many hemoglobin subunits – erythrocruorin from the earthworm *Lumbricus terrestris*, for example, comprises 144 hemoglobin subunits held together by 36 linker subunits (Royer et al., 2000). Measurements of worms in oxygenated water showed that Hb saturation in the gills consistently exceeded 95%. Once worms were in the gelatin and measurement of oxygen concentration in overlying water had started, the fiber optic probe was inserted through the surface of the gelatin after lifting a small piece of Saran covering the surface. The probe was pointed at the gills and was moved occasionally as the worm burrowed to keep its tip near the gills. The instrument recorded an error message when Hb concentration was too low to obtain a good reading, and these points were removed. Change in Hb saturation over time was calculated from averages at the beginning of the resting time, when the worm started burrowing, and at the end of burrowing.

Total Hb concentration in worms was measured with standard methods and used to calculate oxygen consumption (μg O_2 g^{-1}) from measured percentage saturations. Worms were homogenized in 1.5 ml (g wet mass)$^{-1}$ Drabkin's solution, in which all forms of hemoglobin are oxidized to methemoglobin, which reacts with potassium cyanide to form cyanmethemoglobin. Absorbance of cyanmethemoglobin is measured spectrophotometrically at 540 nm and compared with a standard curve generated from known concentrations of bovine hemoglobin (Sigma-Aldrich H2500, St Louis, MO, USA). Hemoglobin concentration was measured for nine individual worms. Oxygen consumption from hemoglobin was calculated from the percentage saturation by multiplying by the concentration of hemoglobin and 1.36 ml O_2 (g Hb)$^{-1}$, the amount of oxygen stored in hemoglobin (Dijkhuizen et al., 1977).

Opine dehydrogenase activities

To assess the role of anaerobic metabolism in burrowing energetics, activities of pyruvate reductase enzymes were assayed to identify potential pathways of anaerobic metabolism. Activities of lactate dehydrogenase and six opine dehydrogenases were assayed. Individual worms were homogenized in 4 volumes (w/v) of 0.2 mol l^{-1} phosphate buffer with 5 mmol l^{-1} EDTA, 0.1% Triton X-100 and 0.3% polyvinylpyrrolidone (PVP) in a ground glass homogenizer. Homogenate was centrifuged at 4°C for 5 min at 10,000 ***g***, and the supernatant saved. Assays were performed by adding 5 μl of supernatant to 195 μl of assay buffer (0.1 mol l^{-1} sodium phosphate, pH 7.9, with 0.2 mmol l^{-1} NADH). The LDH reaction was initiated by adding 50 μl pyruvate (to produce a concentration of 3.2 mmol l^{-1}; total volume 250 μl). Octopine dehydrogenase (ODH), strombine dehydrogenase (SDH), alanopine dehydrogenase (ADH), tauropine dehydrogenase (TaDH) and lysopine dehydrogenase (LyDH) activities were determined following methods adapted from Schiedek (Schiedek, 1997). Reactions were initiated by adding 3.2 mmol l^{-1} pyruvate and an amino acid: 5.4 mmol l^{-1} arginine, 250 mmol l^{-1} glycine, 200 mmol l^{-1} alanine, 80 mmol l^{-1} taurine or 100 mmol l^{-1} lysine. Enzyme activities were determined by measuring the decline in absorption (A) at 340 nm (conversion of NADH to NAD^+) at 25°C using a Spectramax 340PC spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) and are reported as μmol min^{-1} (g wet mass)$^{-1}$. Activity was recorded for at least 5 min, and the slope of the linear decrease, ΔA (A min^{-1}) was used to calculate activity [μmol min^{-1} (g wet mass)$^{-1}$] as:

$$activity = \Delta A \times dil / \varepsilon \times L, \quad (1)$$

where dil is the dilution factor (=250, the product of the tissue dilution during homogenization, 5, and the dilution of the homogenate in the assay cocktail, 50), ε is the extinction coefficient (6.22 mmol l^{-1} cm^{-1}) for NADH at 340 nm, and L is the depth of the sample in a 96-well plate (0.674 cm). The activity of LDH was subtracted from the activities of the opine dehydrogenases, as the decrease in NADH results from the combination of LDH and the opine dehydrogenase being assayed. Enzyme activity was considered significant when means were higher than the control and significantly different at $P<0.05$ (ANOVA).

Tauropine and phosphocreatine assays

Tauropine and phosphocreatine concentrations were measured in worms that were immediately frozen in liquid nitrogen after burrowing, and stored at –80°C. Frozen worms were powdered using a mortar and pestle under liquid N_2. Frozen powder was thawed in 8 volumes (w/v) of ice-cold 6% perchloric acid (PCA). Samples were centrifuged at 10,000 ***g*** for 5 min at 4°C, and the supernatant was brought to pH>4.4 by addition of 5.2 mol l^{-1} potassium carbonate using Methyl Orange as a pH indicator, and held on ice for at least 15 min while the precipitate settled.

Phosphocreatine concentrations were determined using the hexokinase–glucose-6-phosphate dehydrogenase reaction according to standard methods (Heinz and Weisser, 1985).

Tauropine levels were determined enzymatically using recombinant tauropine dehydrogenase (TaDH, EC.1.5.1.23). Briefly, *tadh*-complete cDNA from *Arabella iricolor* (Kimura et al., 2004) (DDBJ/EMBL/GenBank accession number AB081841) was synthesized *in vitro* (GenScript, Piscataway, NJ, USA) and cloned into pUC57 plasmid. Subsequently, *tadh* was sub-cloned into pDONR207 and then pDEST17 expression vector (Gateway, Invitrogen, Carlsbad, CA, USA) in order to fuse a 6xHis tag in the TaDH N-terminal *Escherichia coli* (strain: Rosetta BL21 pLysS) transformed with pDEST17-*tadh* were cultured in a 3-l conical flask containing 21 LB medium plus 34 μg ml^{-1} chloramphenicol and 100 μg ml^{-1} ampicillin at 37°C, with vigorous shaking. Protein synthesis was induced when cell density ($A_{600\,nm}$) reached 0.3 by addition of 1 mmol l^{-1} IPTG for 2 h. Cells were harvested by centrifugation at 2500 ***g*** for 15 min at 4°C. Cells were lysed and TaDH purified using HisLink protein purification resin (Promega #V8823, Madison, WI, USA) according to the manufacturer's recommendations. Purified TaDH was dialyzed against 15 mmol l^{-1} potassium phosphate buffer, concentrated using Amicon modules (#UFC901024; Millipore, Billerica, MA, USA), and stored in 50% glycerol at –20°C until further use.

TaDH was used to convert tauropine to pyruvate and taurine in a buffer containing 0.62% hydrazine hydrate, 0.5 mol l^{-1} glycine, 6 mmol l^{-1} NAD^+ and 0.01 mol l^{-1} EDTA. Standard methods for measuring lactate concentrations (Gutmann and Wahlefeld, 1974) were adapted by adding EDTA to reduce baseline drift (Engel and Jones, 1978). Conversion of NAD^+ to NADH was measured at 340 nm. Tauropine concentration was determined from the change in absorbance using a standard curve from a dilution of a known concentration of tauropine. The tauropine standard was produced using perchloric acid to quench a tauropine dehydrogenase enzymatic conversion of pyruvate, NADH and taurine to tauropine and NAD^+. Tauropine concentration was assumed to be equimolar with NADH concentration.

RESULTS

Oxygen consumption rates

Oxygen consumption rates of *C. moorei* were highly variable (Fig. 2), both for resting and burrowing worms as well as from the two sources measured, overlying water (Fig. 2A) and hemoglobin (Fig. 2B). Mean oxygen consumption rates from overlying water, from hemoglobin and from the total of the two did not differ significantly between resting and burrowing worms (ANOVA, $P>0.05$), but a paired *t*-test showed a trend, although not statistically significant according to a two-sided test, of higher oxygen consumption by resting worms, 35±24 μg O_2 g^{-1} h^{-1}, compared with 30±21 μg O_2 g^{-1} h^{-1} for burrowing worms (Table 1; data for individual worms are presented in supplementary material Table S1). The 95% confidence interval of the difference between resting and burrowing (total) oxygen consumption rates is (–0.9, 32) μg O_2 g^{-1} h^{-1}, indicating that oxygen consumption while burrowing could be as much as 0.9 μg O_2 g^{-1} h^{-1} higher than while resting. However, one worm (see supplementary material Table S1, the worm with a mass of 0.231 g) showed a large increase in oxygen consumption both from water and hemoglobin while burrowing compared with resting; removal of that outlier resulted in significant differences in oxygen consumption between resting and burrowing worms (Table 1). [The sample size was too small to accurately test for normality, but the Wilcoxon signed rank test gave similar *P*-values (not shown) to those from the *t*-test.] Oxygen consumption from hemoglobin constitutes a considerable amount of the total oxygen consumption, 33±18 and 28±14% for resting and burrowing

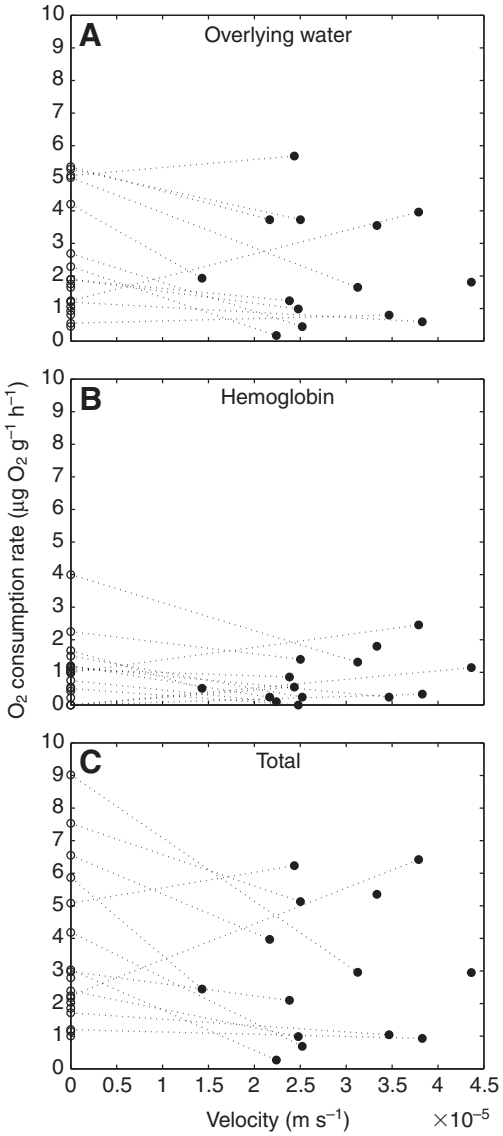

Fig. 2. Resting and burrowing oxygen consumption rates from overlying water (A), hemoglobin (B), and the combined total (C) for individual *Cirriformia moorei*. Resting rates (v=0) are shown as open circles on the y-axis and are connected to corresponding burrowing rates (filled circles) with a dotted line. Resting rates for worms that did not burrow are simply open circles with no connecting lines, and for worms that started burrowing too quickly for resting rates to be measured, only a solid circle is shown with no connecting line. Data are presented in supplementary material Table S1.

Table 1. Compiled oxygen consumption rates and metabolite concentrations

	Measured values			Calculated ATP production (μmol ATP g^{-1} h^{-1})		
	Resting	Burrowing	Seawater	Resting	Burrowing	Paired *t*-test (outlier removed)
Oxygen consumption rate (μg O_2 g^{-1} h^{-1})						
From overlying water	24.1±17.5	21.6±16.7		4.51±3.29	4.06±3.13	*P*=0.06 (0.004)
From hemoglobin	10.8±9.3	8.0±7.3		2.02±1.74	1.50±1.37	*P*=0.08 (0.015)
Total	34.9±23.7 (*N*=18)	29.6±21.3 (*N*=14)		6.54±4.43	5.56±4.00	*P*=0.06 (0.006)
Tauropine (μmol g^{-1})	4.41±1.03	4.47±1.39	4.58±1.19		0.13±4.75	
Phosphocreatine (μmol g^{-1})	0.56±0.18 (*N*=6)	0.52±0.20 (*N*=14)	0.96±0.18 (*N*=2)		0.065±0.587	
Total (μmol ATP g^{-1} h^{-1})				6.54±4.43	5.76±9.34	

Values are means ± s.d.

worms, respectively. Surprisingly, however, no relationship between oxygen consumption rates and burrowing velocity was observed (Fig. 2), nor was there a trend between oxygen consumption from water and from hemoglobin (Fig. 3).

Saturation of hemoglobin decreased both during resting and burrowing, reaching 0% saturation for some worms (Fig. 4). For worms in which hemoglobin saturation dropped to 0%, no behavioral changes were observed; worms generally stopped burrowing when they reached a wall rather than when hemoglobin saturation dropped below any specific values. Not all worms depleted the oxygen stored in hemoglobin. *C. moorei* has 5.26±0.81 mg Hb (g wet mass)$^{-1}$ (*N*=9), as measured using Drabkin's solution. Using conversion factors of 1.36 ml O_2 (g Hb)$^{-1}$ (Dijkhuizen et al., 1977) and 1.43 mg O_2 (ml O_2)$^{-1}$ (calculated from the mass of O_2 and that 1 mol of ideal gas at STP occupies 22.4 l), *C. moorei* can store 10±2 μg O_2 (g wet mass)$^{-1}$ in hemoglobin (Table 1).

Opine dehydrogenase activities

Activity of tauropine dehydrogenase was 9.1±3.8 μmol min^{-1}, approximately eightfold higher than any other pyruvate oxidoreductases. Much lower activities of lactate dehydrogenase, 0.6±0.5 μmol min^{-1} and alanopine dehydrogenase, 1.1±0.2 μmol min^{-1}, were found, and no activities of octopine, strombine and lysopine dehydrogenases were detected.

Tauropine concentrations

There was no statistically significant difference in tauropine concentrations between resting and burrowing worms in gelatin, 4.41±1.03 and 4.47±1.39 μmol (g wet mass)$^{-1}$, respectively (Table 1). In addition, no statistically significant differences in tauropine concentrations were found among worms that burrowed or rested in gelatin, worms that had been held in oxygenated seawater with a thin layer of sediment (~5 mm deep) for 2, 6 and 24 h, and worms collected from the field (Fig. 5). No statistically significant differences in tauropine concentrations were found even when the field-collected worms were separated into two groups: those found with gills near the surface, and those with gills well below the surface. Exposure to oxygen, either experimentally or in the field by holding the gills near the surface, does not seem to affect tauropine concentration. Rather, worms maintained ~4.5 μmol tauropine (g wet mass)$^{-1}$ across all activity levels (Fig. 5). Furthermore, no trend was observed between tauropine concentrations and oxygen consumption rates for resting (R^2=0.004, *P*=0.79) or burrowing (R^2=0.01, *P*=0.68) worms in gelatin (Fig. 6A).

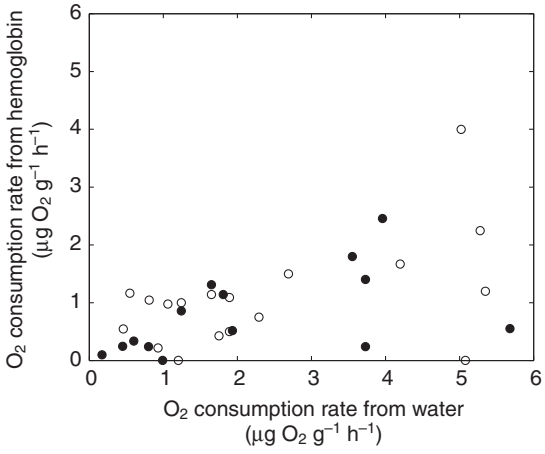

Fig. 3. No statistically significant correlation exists between oxygen consumption from hemoglobin and from overlying water, either for resting worms (open circles) or burrowing worms (filled circles).

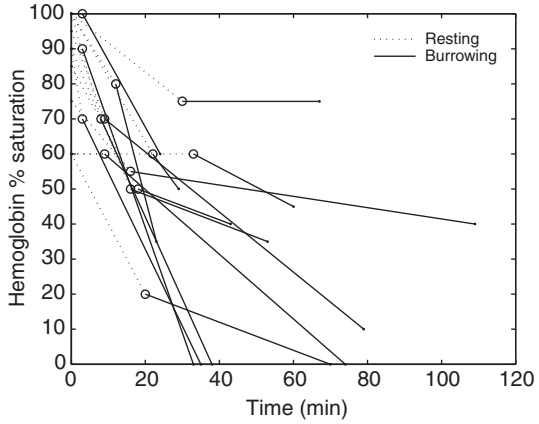

Fig. 4. Percentage saturation of hemoglobin of individual worms while resting (dotted) and burrowing (solid) over time. Open circles indicate the time at which the worm started burrowing, dashed lines originate at the starting saturation, and the solid lines terminate when the experiment ended.

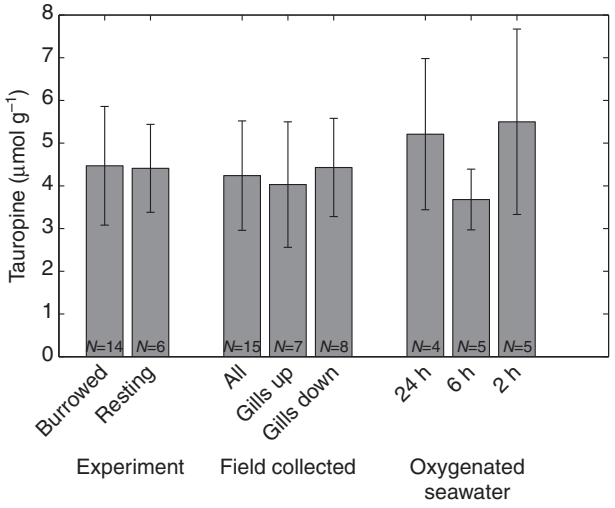

Fig. 5. Tauropine concentrations for worms that burrowed and resting worms compared with worms collected from the field, separated into those with gills near the sediment–water interface and those with gills well below the sediment–water interface, and worms held in a thin layer of sediment with oxygenated seawater for 24, 6 and 2 h. Error bars indicate standard deviations, and no significant difference was found among any treatments (ANOVA $P>0.05$).

Tauropine concentrations showed no correlation with worm mass, either for worms that burrowed or for resting worms, or with mean velocity for worms that burrowed (all $R^2<0.08$, $P>0.05$).

Phosphocreatine concentrations

There was no significant difference in phosphocreatine concentrations between resting and burrowing worms (Table 1). In addition, no trend was observed between phosphocreatine concentrations and oxygen consumption rates for resting ($R^2=0.08$, $P=0.25$) or burrowing ($R^2=0.18$, $P=0.13$) worms (Fig. 6B), or between phosphocreatine and tauropine concentrations for resting ($R^2=0.27$, $P=0.30$) or burrowing ($R^2=0.05$, $P=0.47$) worms. Phosphocreatine concentrations were, however, higher for worms that had been held in seawater than for those resting in gelatin or that had burrowed (Table 1).

DISCUSSION

Oxygen storage and consumption

Oxygen consumption rates, both from overlying water and from hemoglobin, were highly variable (Fig. 2). Paired comparison of resting and burrowing oxygen consumption rates, however, did show a difference (significant after removing one outlier), and oxygen consumption rates were fairly consistent for individuals over the period of the experiment while resting or burrowing. The observed higher inter-individual variability than intra-individual variability probably results from differences in physiological state, but we were unable to repeat the experiments many times on the same individual because worms were immediately killed for metabolite analyses. Another potential source of variability is microbial activity in the guts of the worms. All worms contained sediment in their guts, but the volume of sediment varied and was not measured. Microbial activities in deposit-feeder guts are high; most of the sediment in the gut is anoxic, and radial and longitudinal oxygen gradients indicate that oxygen is transported into the gut where it fuels microbial growth (Plante and Jumars, 1992).

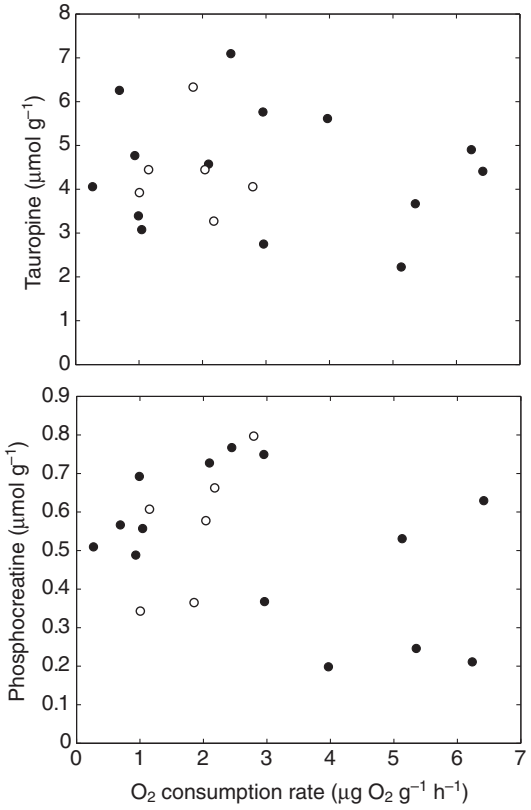

Fig. 6. Relationship of total oxygen consumption rate and tauropine (A) or phosphocreatine (B) concentrations. No statistically significant correlation exists either for resting worms (open circles) or burrowing worms (filled circles).

As a worm moves away from the surface water, its access to oxygen becomes more limited, and this isolation may contribute to lower oxygen consumption rates from overlying water for burrowing worms (Fig. 2A). We expected that use of stored oxygen would increase as worms moved away from the overlying water and that oxygen consumption from hemoglobin would be inversely proportional to that from the overlying water. However, no such relationship between oxygen consumption from hemoglobin and from water was found (Fig. 3). Instead, the use of stored oxygen varied widely among individual worms, not only in terms of rate of consumption, but also in how low the hemoglobin saturation dropped while the worm burrowed (Fig. 4). In some worms, oxygen saturation of hemoglobin approached an asymptote, whereas other individuals used all the oxygen stored in their hemoglobin quickly.

Blood pH can affect the oxygen affinity of blood pigments such as Hb. For example, erythrocruorin from the cirratulids *C. tentaculata* and *Cirratulus cirratus* Muller shows a negative Bohr shift, indicating decreased oxygen affinity at lower pH (Warren et al., 1981). Production of anaerobic metabolites decreases the pH, although opines (such a tauropine) are much less acidic than lactate (Zammit, 1978). Tauropine concentration in *C. moorei* was not correlated with either rate of oxygen consumption from hemoglobin ($R^2=0.07$, $P=0.37$) or percentage saturation of hemoglobin when the worms stopped burrowing ($R^2=0.004$, $P=0.83$). This result is unsurprising, as the tauropine concentration we measured did not correlate with the recent level of activity of a worm (i.e. either velocity or whether the worm had burrowed or was resting). We

did not measure pH of the blood so cannot determine whether use of oxygen stored in hemoglobin was related to pH in *C. moorei*.

C. moorei contains 5.26±0.81 mg Hb (g wet mass)$^{-1}$, higher than many invertebrates but not as high as the polychaete, *Euzonus mucronata* (Treadwell) or some vertebrates (Alyakrinskaya, 2002; Dangott and Terwilliger, 1986). *E. mucronata* has 42 mg Hb (g wet mass)$^{-1}$, although the worms in Dangott and Terwilliger's study had been kept in seawater until they voided the sediment from their guts (Dangott and Terwilliger, 1986), whereas our wet mass measurements included gut contents, consistent with our other measurements, but yielding lower estimated Hb concentration. Dales and Warren measured considerably lower Hb per body mass in *C. tentaculata*, but the variability in their estimates was very high (Dales and Warren, 1980). Based on our approximate measured resting or burrowing oxygen consumption rates of 35 μg O_2 g^{-1} h^{-1} (Table 1), the oxygen stored in the hemoglobin of *C. moorei* that we measured (10 μg O_2 g^{-1}) could theoretically allow the worm to respire for 18 min in subsurface sediments away from oxygenated water while using solely oxygen stored in hemoglobin. These values differ from those of Dales and Warren, who predicted that *C. tentaculata* could survive 5.8 h in anoxic conditions using only oxygen stored in hemoglobin (Dales and Warren, 1980). Their calculations, however, were based on oxygen consumption rates under low-oxygen conditions in which metabolic rates were substantially depressed, either through an overall reduction in metabolism or anaerobic metabolism. The oxygen consumption rates measured under these conditions were only 0.6 μg O_2 g^{-1} h^{-1} (Dales and Warren, 1980), orders of magnitude lower than our measurements for *C. moorei*, even without including our measurements of oxygen consumption from hemoglobin (Table 1). Their findings suggest that, by reducing its metabolic rate, *C. moorei* might survive on oxygen from hemoglobin much longer than our predicted 18 min.

Even though worms did not use their oxygen storage capacity as we expected, their high concentration of hemoglobin (per body mass) is probably beneficial in a low-oxygen environment. Thus, whether, and under what conditions, oxygen storage by hemoglobin is utilized by infaunal animals are intriguing questions. The T-stat ischemia system for monitoring hemoglobin oxygenation worked remarkably well for *C. moorei*, which has clearly visible blood in external gills. In addition to measuring hemoglobin saturation of worms in gelatin as presented here, we have measured hemoglobin saturation for several worms while in sediment in a thin aquarium when the gills were visible through the Plexiglas® wall, and as expected hemoglobin saturation decreased as worms burrowed below the sediment–water interface, then increased over time when the worms were resting near the surface with the gills exposed (K.M.D., unpublished data). This method of monitoring hemoglobin saturation *in situ* may be applicable to other organisms with relatively transparent body walls or exposed gills.

External work to burrow in gelatin

To move, organisms must do work against their environment to generate thrust and to overcome resistance, such as that from friction, and that external work done is the product of the force applied and the distance over which that force is exerted. External work to burrow in gelatin by fracture is the sum of the work of fracture to elongate the burrow ahead of the worm (W_{Cr}), and the work done against the elastic burrow wall to make space for the worm's body as it moves forward (W_{El}) (Dorgan et al., 2008).

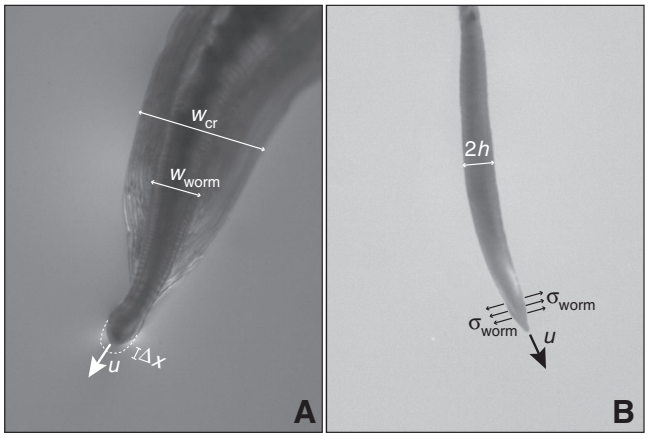

Fig. 7. Dorsal (A) and lateral (B) views of *C. moorei* burrowing in gelatin with the variables used to calculate external work indicated.

The work of fracture, W_{Cr} (in J), is the product of the fracture toughness, G_c (in J m^{-2}), and the area of new crack formed:

$$W_{Cr} = G_c\,(\Delta x)\,w_{Cr}, \quad (2)$$

where Δx (in m) is the distance the crack grows and w_{Cr} (in m) is the width of the crack (Dorgan et al., 2008) (Fig. 7). We calculated the rate of work of fracture, P_{Cr} (in J s^{-1}), as the worm burrows:

$$P_{Cr} = G_c\,u\,w_{Cr}, \quad (3)$$

where u is the average velocity of *C. moorei* burrowing in gelatin (u=5×10^{-5} m s^{-1}) (Che and Dorgan, 2010). We assumed a crack width, w_{Cr}, of 5 mm to represent the width of the body and gills of *C. moorei* in gelatin (Che and Dorgan, 2010). The fracture toughness, G_c, for gelatin is given by

$$G_c = [K_{Ic}\,(1-\nu^2)] / E, \quad (4)$$

where ν is Poisson's ratio (dimensionless), E is the material stiffness and K_{Ic} is the critical stress intensity (Anderson, 2005). Using the measured E for gelatin in the aquaria used here (E=4130 Pa) (Murphy and Dorgan, 2011), and K_{Ic} for gelatin (K_{Ic}=58 Pa m$^{0.5}$) (Dorgan et al., 2008), we found G_c to be 0.6 J m^{-2}. ν was assumed to be 0.5. Total rate of work of fracture was therefore calculated to be P_{Cr}=1.5×10^{-7} J s^{-1}.

Elastic work done (W_{El}) to open the crack as the worm moves forward is the product of the force exerted by the worm and the distance over which that force is applied. Forces exerted by *N. virens* have been measured using photoelastic stress analysis (Dorgan et al., 2007). For this method to be used, however, the force must be large and localized enough to appear as a clearly discernible light region against the darker background. Forces applied by *C. moorei* were small, and stress was spread along much of the anterior region of the worm. We therefore used finite element modeling (FRANC2D, Cornell Fracture Group; www.cfg.cornell.edu) to estimate from the stiffness of the gelatin the amount of stress necessary to deform the gelatin around a crack to obtain the shape of the worm, adapting methods of Dorgan et al. (Dorgan et al., 2007). Because the anterior of the worm was slightly thicker than the posterior and more stress is needed to displace the crack wall close to the crack tip, stress applied along the crack wall needed to be larger near the crack tip than around the posterior of the worm. Linearly increasing stress from 40 Pa at the tail of the worm to 100 Pa at the crack tip yielded deformations that closely matched the shape

of *C. moorei* in gelatin [data from lateral view images analyzed by Che and Dorgan (Che and Dorgan, 2010)]. To calculate the rate of work done against elasticity, P_{El} (in $J\,s^{-1}$):

$$P_{El} = 2\sigma_{worm} w_{worm} u h, \quad (5)$$

where σ_{worm} is the stress applied at the anterior of the worm (100 Pa), w_{worm} is the width of the worm, (here 0.0024 m), u is the velocity of the worm ($5\times10^{-5}\,m\,s^{-1}$) and h is the half-thickness of the worm near the anterior (measured as 0.0005 m) (Che and Dorgan, 2010). Because there are two burrow walls, a factor of 2 is included in the equation. In reality, the elastic work is done incrementally: as the worm moves forward a small Δx, it displaces the gelatin along the length of the sloped region of the anterior a small distance that is a fraction of the body thickness, $2h$ (Fig. 7). Our equation essentially sums all of these small increments to give the total work to deform the gelatin from a closed crack to the thickness of the worm. The rate of elastic work done by the worm as it burrows is $P_{El}=1.2\times10^{-8}\,J\,s^{-1}$, an order of magnitude lower than the rate of work of fracture.

Total rate of external work to burrow is calculated here as the sum of the rates of work to fracture and elastic work, $P_{tot}=1.6\times10^{-7}\,J\,s^{-1}$, although the two terms are not completely independent. When a crack grows, energy stored as elastic potential energy in the material being cracked is released, which means there may be some overlap between our calculated elastic work (stored as potential energy) and our calculated work of fracture (release of stored energy). Fracture occurs when enough stored energy, G, is available to exceed the fracture toughness, or energy release rate, G_c, of the material (Anderson, 2005). During a burrowing cycle, the worm does work to expand its body, storing elastic energy in the surrounding sediment (or gelatin), much of which is released when the crack extends by fracture. Our calculated rate of work done against elasticity, P_{El}, includes only the work done by the anterior part of the worm as it moves forward into the crack, which is why P_{El} is much smaller than P_{Cr}. In fact, the work done by the worm is elastic work as it expands the anterior of its body, but we have quantified it as the release of stored elastic energy through fracture. Actual elastic work done over the entire length of the worm is difficult to measure because it involves small changes in body thickness during peristalsis. Although the elastic work done by the worm to move forward is at least partially included in our calculations of work of fracture, we have included both terms to produce a more conservative estimate.

Although metabolic energy is used to pressurize the hydrostatic skeleton and maintain body shape against the elastic restoring force of the sediment, no external work is done when shape does not change. Stored elastic energy can be dissipated, resulting in plastic deformation, which probably occurs in natural sediments but is much reduced in gelatin (cf. Dorgan et al., 2007). It is also possible that worms may regain some stored elastic energy from the gelatin around them, for example, to supplement or replace the contraction of circumferential muscles in elongating the body, although this suggestion has not been tested. Our calculations of elastic work do not take potential storage of elastic energy or dissipation through plastic deformation into account, but rather include only the work to open the burrow.

The external energy to burrow, E_{ext}, normalized to body mass and distance traveled, was calculated from the rate of work, P_{tot}, as:

$$E_{ext} = P_{tot} / m_w u, \quad (6)$$

where $P_{tot}=1.6\times10^{-7}\,J\,s^{-1}$, m_w is the mean wet mass of the worms (0.33 g) and u is the mean velocity ($5\times10^{-5}\,m\,s^{-1}$) (Che and Dorgan, 2010).

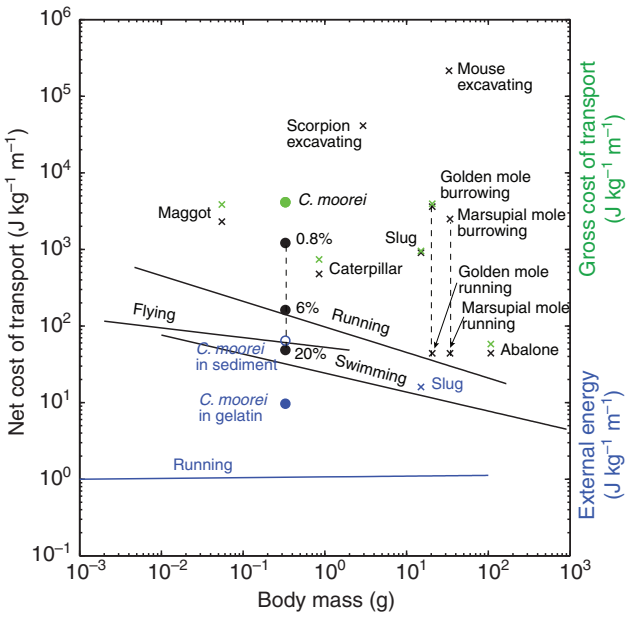

Fig. 8. External work for locomotion (blue), net cost of transport (black) and gross cost of transport (green). External work to burrow by *C. moorei* in gelatin (blue filled circle) and sediment (blue open circle) are higher than external work for runners, but comparable with that for terrestrial slugs (blue cross). Gross cost of transport for *C. moorei* (green filled circle) compared with calculated gross costs of transport for maggots (Berrigan and Lighton, 1993), caterpillars (Casey, 1991), slugs (Denny, 1980), abalone (Donovan et al., 1999) and desert moles (Seymour et al., 1998) (green crosses). Net cost of transport (*NCT*) for *C. moorei* calculated from external work assuming efficiencies of 0.8%, 6% and 20% (filled circles), in contrast to the *NCT* for crawlers and terrestrial burrowers, including a marsupial mole burrowing in desert sands (Withers et al., 2000), a hopping mouse while excavating a burrow (White et al., 2006) and a scorpion excavating a permanent burrow (White, 2001) (black crosses) and correlations for running, flying and swimming (black lines) calculated from metabolic rates. Correlations for *NCT* for running, flying and swimming are from Full (Full, 1997) and for external work of running are from Full (Full, 1991).

Using the procedures described above, we calculated that the external energy for burrowing per body mass per distance traveled, E_{ext}, for *C. moorei* in gelatin is $9.7\,J\,kg^{-1}\,m^{-1}$. This external energy for burrowing is roughly ten times larger than the $\sim1\,J\,kg^{-1}\,m^{-1}$ reported for various forms of terrestrial locomotion (e.g. walking, running, crawling) by animals across a large range of body sizes (Full, 1991) (Fig. 8).

External work to burrow in sediments *versus* in gelatin

Our calculation of external work, although clearly dependent on the kinematics of the worm, depends strongly on the mechanical properties of sediments. Specifically, the work to extend a crack depends directly on the fracture toughness, and the elastic work depends directly on the stiffness (as the internal pressure of the worm, σ_{worm}, is calculated from the body thickness and material stiffness). Assuming a fracture toughness for sediments of $385\,Pa\,m^{0.5}$ (Johnson et al., 2002), compared with $58\,Pa\,m^{0.5}$ for gelatin, the work would be 6.6× greater in sediments. Similarly, an elastic modulus of 27 kPa for sediments compared with 4130 Pa for gelatin would increase elastic work by a factor of 6.5. This would increase the total external work in sediments to $\sim64\,J\,kg^{-1}\,m^{-1}$, much higher than values for terrestrial walking (Fig. 8). However, we

calculated external work for *Nereis diversicolor* from Trevor's data, in which sediments were implicitly assumed to deform plastically around burrowers, to be much higher, 259 J kg^{-1} m^{-1} (Trevor, 1978).

Both fracture toughness and stiffness of sediments in the field are highly variable, and measurements have been limited to only a few sites. Even within one location, in Cole Harbor, NS, Canada, K_{Ic} varies from 280 to 490 Pa m$^{0.5}$ (Johnson et al., 2002), and recent *in situ* measurements have shown that K_{Ic} varies from close to 0 at the sediment–water interface to over 1300 Pa m$^{0.5}$ in deeper sediments (below those inhabited by burrowers) (B. D. Johnson, M. A. Barry, B. P. Boudreau, P. A. Jumars and K. M. Dorgan, in review). Stiffness measurements range from 27±10 kPa (Dorgan et al., 2007) for surface sediments from Lowes Cove, ME, USA to 139 kPa (Johnson et al., 2002) for subsurface sediments from Cole Harbor. Burrowing behaviors of *N. virens* depend on the ratio of fracture toughness to stiffness, with thicker and blunter body shape attained by pharynx eversion in relatively tough materials (Dorgan et al., 2008). As the work of fracture dominates the external work term, the absolute fracture toughness, rather than the ratio K_{Ic}/E, is important in determining work to burrow. Fracture toughness of muddy sediments have only recently begun to be measured (Johnson et al., 2002), and their spatial and temporal variabilities are not well understood. Small-scale heterogeneity in fracture toughness specifically is challenging to measure, but the work required to burrow may be reduced if there is considerable heterogeneity in toughness on the scale of burrowers that allows cracks to extend in the direction of least resistance rather than being steered by the burrower.

Calculations of cost of transport

The net cost of transport, *NCT*, calculated from oxygen consumption rates is:

$$NCT = (\dot{V}_{O_2\ \text{burrowing}} - \dot{V}_{O_2\ \text{resting}}) / mu, \quad (7)$$

where $\dot{V}_{O_2\ \text{burrowing}}$ is the oxygen consumption rate while burrowing, $\dot{V}_{O_2\ \text{resting}}$ is the oxygen consumption rate while resting, m is the body mass, and u is the velocity. Calculation of net cost of transport either from oxygen consumption or from the combined ATP from aerobic metabolism and anaerobic fermentation (Table 1) yields an obviously unrealistic negative cost of transport, and the variability in all components measured is very high. From the 95% confidence interval of the difference between resting and burrowing oxygen consumption rates, we can calculate a maximum *NCT* (the minimum is a large negative number) from aerobic sources of 136 J kg^{-1} m^{-1}, but because animals were killed for metabolite analyses, we have no similar estimate for maximum contribution from tauropine and phosphocreatine. Moreover, the high variability in resting oxygen consumption rates and the observation that oxygen consumption rates decreased (rather than increased) when *C. moorei* started moving suggest that the worms might have been reducing other components of metabolism when they started burrowing.

We had hypothesized that energy from anaerobic sources would compensate for the decreased oxygen consumption rate but found no significant difference in either phosphocreatine or tauropine concentrations between resting and burrowing worms. We did find low activities of two other dehydrogenase enzymes, but did not measure lactate or alanopine concentrations in worms after burrowing for several reasons. In preliminary experiments, no detectable lactate was found in *C. moorei*, consistent with an absence of lactate in the related *C. tentaculata* (Bestwick et al., 1989). In invertebrates in which activities of more than one dehydrogenase enzyme were found, the opine corresponding to the enzyme with highest activity accumulated at much higher levels than the other opines, whereas under environmental hypoxia the opine accumulated depended instead on the concentration of the corresponding amino acid in the animal tissue (Kreutzer et al., 1989). Because the activity of tauropine dehydrogenase was approximately eight times greater than that of alanopine dehydrogenase, it seems unlikely that alanopine would be produced during burrowing. It does appear that phosphocreatine is used when a worm is in the burrow away from surface water, as evidenced by our measurements of significantly lower phosphocreatine concentrations for worms in burrows (whether moving or resting) than for worms in oxygenated water. However, this decrease in phosphocreatine concentration was not higher when worms were burrowing than resting (Table 1).

We calculated the gross cost of transport, *GCT*, rather than a net cost (for which resting rate is subtracted from the burrowing rate) as:

$$GCT = \dot{V}_{O_2\ \text{burrowing}} / mu. \quad (8)$$

The calculated *GCT*, 4115±3011 J kg^{-1} m^{-1}, is not comparable with the more widely measured net cost of transport (cf. Schmidt-Nielsen, 1972) or minimum cost of transport for other forms of locomotion [minimum cost of transport is calculated as the slope of the oxygen consumption rate plotted as a function of velocity and is often smaller than net cost of transport because it excludes the increase in cost from resting to moving slowly, e.g. postural costs (Full, 1991)]. Our calculated *GCT* can, however, be compared with the *GCT* for maggots, calculated from Berrigan and Lighton (Berrigan and Lighton, 1993), and that for burrowing by desert moles in sand (Seymour et al., 1998) and the values are very similar (Fig. 8). Although not comparable to many values in the literature for other species, *GCT* provides an upper bound for the metabolic cost of transport. If we make the extreme assumption that all other aspects of metabolism are shut off during burrowing, the *GCT* suggests an extremely high cost of transport.

Nonetheless, because we found that net metabolic cost of burrowing gave negative values, it appears that some reduction of other metabolic processes does occur during burrowing. We had originally intended to measure the net energetic cost of burrowing from aerobic and anaerobic components, calculate the external work done by the worm to burrow, and then determine the efficiency of locomotion as the ratio of the two. That efficiency, assuming the gross cost of transport, is 0.2%, lower than any calculated efficiency of locomotion of which we are aware.

Although we were unable to calculate net cost of transport from metabolic energy, we can assume an efficiency and calculate the expected net energetic cost of transport from external work, as was done previously for burrowing (Trevor, 1978; Hunter and Elder, 1989). The efficiency of locomotion for a 0.33 g animal walking on land is approximately 0.8% [see fig. 7.9 in Full (Full, 1991)]. This value is calculated from the ratio of the mechanical energy to the minimum metabolic cost of transport for terrestrial locomotion. This low efficiency for small animals has been attributed to the cost of turning muscles on and off as smaller runners generally have higher rates of force production, as well as to the greater mechanical advantage of longer limbs (Full, 1991). Burrowing worms move much more slowly than runners and, obviously, lack limbs, so these arguments do not necessarily apply, and it seems quite possible that the efficiency of burrowing is higher than those for runners of comparable size. Using efficiency measurements for a different mode of locomotion introduces a high degree of uncertainty, but for lack of better data we consider 0.8% to be reasonable, though probably an underestimate. In contrast, Trevor assumed an efficiency of 20% to calculate net cost of transport from external work of

burrowing for polychaetes (Trevor, 1978). Efficiency of burrowing by the whelk *Bullia digitalis* in sandy sediments was found to be considerably lower, ~6% (Brown, 1979b). Brown suggests that 6% is a more accurate estimate of burrowing efficiency than the 20% used by Trevor (Brown, 1979b; Trevor, 1978). Brown compared oxygen consumption by whelks burrowing into sand with work measurements calculated from forces measured for whelks as they burrowed, whereas Trevor used an estimate of muscular efficiency, which is higher than *in vivo* efficiency of locomotion (Brown, 1979b; Trevor, 1978).

Assuming efficiencies of 0.8% for legged terrestrial locomotion (Full, 1991), 6% for burrowing whelks (Brown, 1979b) and 20% as previously assumed for burrowing worms (Trevor, 1978), from our calculated external energy, E_{ext}=9.7 J kg^{-1} m^{-1}, cost of transport COT=1212, 162 and 49 J kg^{-1} m^{-1}, respectively. The costs of transport calculated from these three efficiencies still span a broad range but seem to indicate that burrowing may be more energetically costly per distance traveled than other forms of locomotion (Fig. 8). This result is consistent with direct comparisons showing much higher costs of burrowing through desert sands than running for the Namib desert golden mole (Seymour et al., 1998) and the northwestern marsupial mole (Withers et al., 2000) (Fig. 8). The cost of burrowing by *C. moorei* is comparable to the cost per distance of burrowing through desert sands by moles and less than that of burrowing by mice (White et al., 2006) or scorpions (White, 2001) (Fig. 8).

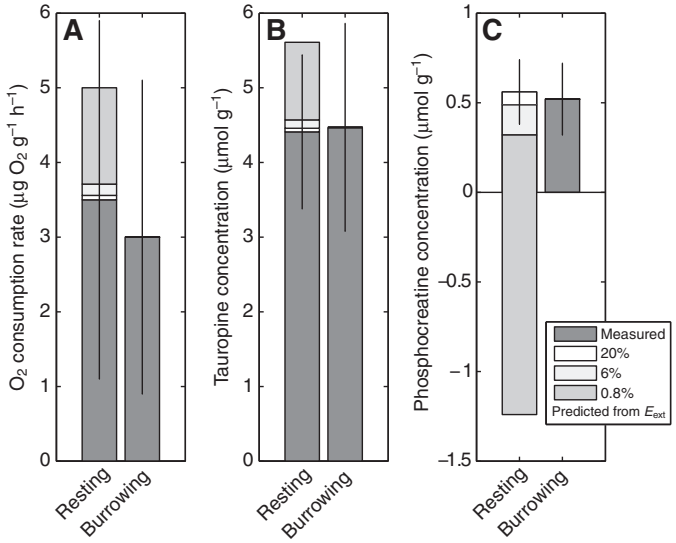

Fig. 9. Increases in oxygen consumption rate (A), tauropine concentration (B) and phosphocreatine concentration (C) predicted from external work (E_{ext}) assuming efficiencies of 0.8, 6 and 20%. Predicted increases assume all energy comes from that source. (Predictions for 20 and 6% overlie those for 0.8%.)

Predictions from external work calculations

If the metabolic cost of worm burrowing (predicted from external work and an assumed efficiency) is high relative to other forms of locomotion, it is surprising that no differences in oxygen consumption rate or tauropine or phosphocreatine concentrations were found between resting and burrowing worms. To better understand this discrepancy, we used the calculated value for the external work to burrow to predict the increase in oxygen consumption rate due to burrowing if the worms relied entirely on aerobic respiration for moving through the sediment. By assuming an oxycaloric coefficient of 450 J (mmol O_2)$^{-1}$ (Dejours, 1981), we calculated for efficiencies of 0.8, 6 and 20% that the increase in oxygen consumption due to burrowing would be 15.5, 2.1 and 0.6 μg O_2 g^{-1} h^{-1}, respectively (Fig. 9). Similarly, we assumed absence of metabolite regeneration and that all the energy for burrowing came from anaerobic metabolism, and we predicted the amount of tauropine that would be produced if that was the only anaerobic pathway used to support the external work needed to burrow. We did a similar calculation to predict the amount of phosphocreatine used if that was the only source of energy. For these calculations, we assumed conversions of 6 mmol ATP (mmol O_2)$^{-1}$, 1.5 mmol ATP (mmol tauropine)$^{-1}$ and 1 mmol ATP (mmol phosphocreatine)$^{-1}$ (Donovan et al., 1999). For efficiencies of 0.8, 6 and 20%, we calculated predicted increases of 1.9, 0.25 and 0.076 μmol tauropine g^{-1} h^{-1} and decreases of 2.9, 0.39 and 0.12 μmol phosphocreatine g^{-1} h^{-1}, respectively. Given the average burrowing time of 38 min in our experiments, the worms that burrowed would be predicted to have 1.2, 0.16 or 0.048 μmol g^{-1} higher tauropine concentrations and 1.8, 0.24 or 0.072 μmol g^{-1} lower phosphocreatine concentrations than resting worms (Fig. 9).

Both the increases in oxygen consumption rates and in tauropine concentrations predicted from external work are within the variability found in our experiments, which indicates that either oxygen consumption or tauropine production alone could fuel burrowing and yet be undetectable in our experiments. The increase in oxygen consumption rate while burrowing predicted from external work is less than the standard deviation for either burrowing or resting worms of 21.3 and 23.7 μg O_2 g^{-1} h^{-1}, respectively (Table 1). The difference between burrowing and resting oxygen consumption rates calculated for individual worms is –12.1±27.7 μg O_2 g^{-1} h^{-1}. The predicted increase in tauropine concentration is also close to the error in tauropine measured for the lowest efficiency, 0.8%, and much smaller than the error for predicted values assuming higher efficiencies (Table 1). Moreover, our predictions are based on the assumption that all energy comes from oxygen consumption or tauropine production, but it is also possible that a combination of aerobic and anaerobic metabolism may be used during burrowing. In this case, increases in oxygen consumption rates and tauropine concentrations would be lower than our predictions and even less likely to be detectable given the high variability among individuals.

The decrease in phosphocreatine predicted from external work is larger than or comparable to the measured values of phosphocreatine concentration (Table 1), suggesting that unless burrowing efficiency is very high (close to 20%), phosphocreatine alone does not fuel burrowing. This finding, as well as the decrease in phosphocreatine concentration for resting and burrowing worms relative to worms in water (Table 1) is consistent with the use of phosphagens as a short-term, high energy source (Livingstone, 1991). Phosphocreatine may be used over a short period immediately after the worms are placed in the gelatin with reduced access to oxygen, but does not fuel burrowing activities over the longer periods of the experiment.

Tauropine concentrations

Tauropine concentrations for *C. moorei* were much higher than those found in muscle tissue of the abalone, *H. kamtschatkana*, which were 0.3 μmol (g wet mass)$^{-1}$ for resting abalone foot muscle, 1.5 μmol (g wet mass)$^{-1}$ for abalone that had crawled quickly, and in the adductor reached 3.8 μmol (g wet mass)$^{-1}$ only after 16 h of air exposure (Donovan et al., 1999). Compared with concentrations in mollusk tissue, our concentrations are underestimated because not

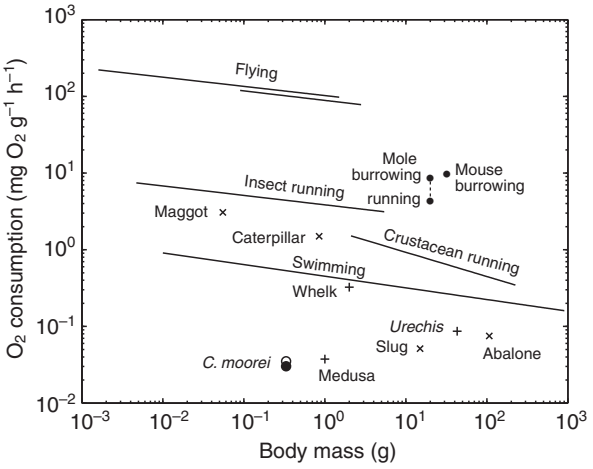

Fig. 10. Oxygen consumption rates for *C. moorei* while resting (open circle) and burrowing (closed circle) compared with invertebrate runners, flyers and swimmers (lines) and crawlers (crosses). Also shown (plus signs) are oxygen consumption rates for whelks burrowing in sand (Brown, 1979a), *Urechis* irrigating its burrow (Julian et al., 2001) and medusa jetting (Daniel, 1985). Correlations are from Full (Full, 1997). Also shown are oxygen consumption rates for terrestrial burrowers (small closed circles), the golden mole (Seymour et al., 1998) and a hopping mouse excavating a burrow (White et al., 2006).

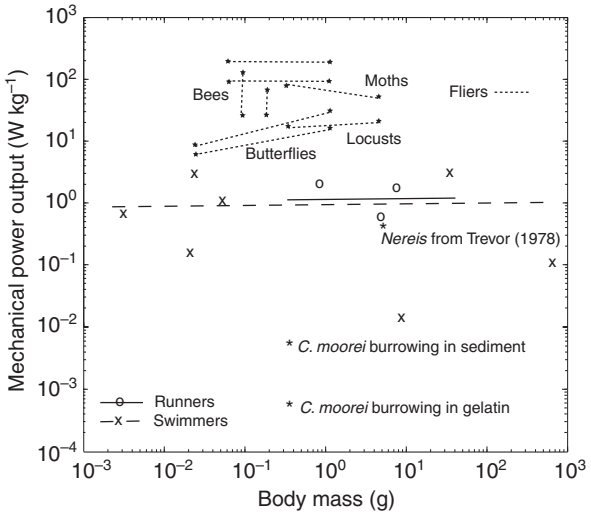

Fig. 11. Mechanical power output ($W\,kg^{-1}$), calculated as the product of E_{ext} and u, for *C. moorei* burrowing (*) compared with runners (o), swimmers (x), and fliers (star). Trevor's estimate for *Nereis*, based on the assumption that sediments deform plastically around the worm, is shown (*) to be much higher than our calculations for *C. moorei* (Trevor, 1978). Figure adapted from Full (Full, 1997).

only was tauropine normalized to total mass of the worm rather than muscle mass, but the mass of the worms included the mass of the sediment in the gut. Tauropine concentrations in muscle tissue of *C. moorei* was therefore probably much higher than our measurements indicate.

Despite the high concentrations of tauropine found in the worms, our predictions of tauropine produced based on external work were small. Even if the tauropine found in our worms was produced by glycolysis during burrowing before our experiment started, comparison of our highest predicted tauropine concentration of $1.2\,\mu mol\,g^{-1}$ to our measured concentration of $4.46\,\mu mol\,g^{-1}$ in worms that had burrowed 5.9±2.7 cm suggests that worms would have needed to burrow at least 22 cm prior to experiments without metabolizing or excreting tauropine. If the tauropine was produced under environmental hypoxia rather than during exercise-induced hypoxia, we would expect the tauropine concentrations to be lower for the worms kept in oxic conditions for 24 h than for the worms from the field or those kept in oxic conditions for shorter periods, but no differences were found (Fig. 5).

The question of why tauropine concentrations were high in all worms sampled and independent of treatment is intriguing. It may simply be that tauropine is relatively inert (e.g. less acidic than lactate) and that worms metabolize it slowly or in response to cues other than oxygen availability. Whereas metabolites such as propionate, acetate and succinate are excreted by annelids under anoxic conditions, no evidence of excretion of nitrogen-containing end products such as opines has been found (Ellington, 1983). Tauropine is produced from pyruvate and taurine. Thus, tauropine production might provide a mechanism for storing pyruvate, and that tauropine might be converted back to pyruvate under conditions in which food is limited. A related suggestion is that conversion of taurine to tauropine regulates taurine concentrations. Taurine was found to be a strong phagodepressant in three species of deposit-feeding spionid polychaetes (Ferner and Jumars, 1999), although its effect as a chemical cue on cirratulids has not been tested.

Energetics of burrowing

Our external energy calculations indicate that burrowing worms have higher external work costs per body mass per distance traveled than those for other forms of locomotion (Fig. 8). This result is unsurprising, as creating space in a solid in which to move can be reasonably expected to require more work than moving over land. The work to extend a crack through either gelatin or sediment is an order of magnitude larger than the calculated rate of elastic work to make room for the body as the worm moves forward. Burrowing worms, however, cover much smaller distances and move much slower than most other motile animals (Full, 1991).

Oxygen consumption rates of burrowing worms are lower than those of runners, flyers and swimmers, comparable only to a few slowly moving crawlers and jetters (Full, 1997) (Fig. 10). Oxygen consumption rates are a measure of energy (converted to energy using an oxycaloric coefficient) normalized to body mass and time, in contrast to the net cost of burrowing, for which energy is normalized to body mass and distance. The difference in oxygen consumption rates between burrowing and resting worms was not detectable in our experiments, as predicted from our external work calculations. Lower metabolic rates of deep-sea cephalopods than those in shallower waters have been attributed to a decreased reliance on fast locomotion in predator–prey interactions when visibility is limited (Seibel et al., 1997). Most burrowing worms rely on their opaque environment rather than rapid escape responses to avoid predation, and instead move slowly over small distances to find patches of food. Similarly, jetting medusae, which also have low oxygen consumption rates (Daniel, 1985) (Fig. 10), move slowly and rely on camouflage rather than rapid movements.

Perhaps more interesting, when the external work is normalized to time rather than distance, in other words, multiplied by velocity to yield 4.8×10^{-4} and $3.3\times10^{-3}\,J\,kg^{-1}\,s^{-1}$ for burrowing in gelatin and sediment, respectively, the mechanical power of burrowing

is much lower than for other forms of locomotion (Full, 1997) (Fig. 11). We suggest that the slow speeds at which burrowing worms travel enables them to exert enough work to extend the burrow without significantly increasing their metabolic rates above resting.

The low metabolic cost of burrowing by *C. moorei* is consistent with a low cost of feeding by another deposit-feeding polychaete, *Abarenicola pacifica* (Taghon, 1988). The cost of mechanical feeding by *A. pacifica*, not including digestive costs, was undetectable either algebraically through an energy balance of growth and other metabolic costs or by comparing mass loss of starved worms feeding on clean sand without organic material to starved worms that were not feeding (Taghon, 1988). Taghon also calculated that the energy for *A. pacifica* to burrow, based on the high estimate for cost of transport for the closely related *Arenicola marina* (Trevor, 1978), added to the energy required to transport sediment from the feeding depth to the surface where it is defecated, is still <1% of the standard metabolic rate for *A. pacifica* (Taghon, 1988). Although Trevor's calculation of the cost of transport for burrowing was questionable, his result is relatively close to our estimate based on external work calculations and an estimated efficiency of 0.8% (Trevor, 1978).

Comparing burrowing to other forms of locomotion depends on the metric used to measure performance. External work required to move a unit of distance is very high and largely attributable to the work of extending the burrow by fracture. The increase in metabolic energy per unit of time, i.e. in power required to burrow, however, is very small and undetectable in our experiments. From a mechanical perspective and cost per unit of distance moved, burrowing is costly, but from an ecological perspective and cost per unit of time, this cost is negligible.

APPENDIX

Assessment of potential oxygen sources

Preliminary experiments were done to calculate the relative importance of potential sources of oxygen to the worms from advection and diffusion. Consider the crack-shaped burrow as a control volume in which oxygen consumption can be calculated as the difference between oxygen flux into and out of the burrow (Fig. A1). Flux across the burrow walls (J_{wall}) is diffusive (labeled 1 in Fig. A1) and was calculated using a concentration gradient ($\partial C/\partial z$) from an oxygen profile measured as the probe was moved closer to the worm's gills. The worm was moving horizontally (x-axis) in the tank and the probe was lowered from above (z-axis). The flux J_{wall} occurs across two walls and is the product of diffusive flux from Fick's law and the surface area:

$$J_{wall} = \int 2(-D\frac{\partial C}{\partial z}(x))w_{Cr}(x)\partial x \ , \qquad (A1)$$

where D is the diffusion coefficient and w_{Cr} is the crack width. Assuming that the concentration gradient and the crack width are constant along the length of the worm and discretizing the equation, then

$$J_{wall} = -2D\,(\Delta C / \Delta z)\, w_{Cr}\, L \, . \qquad (A2)$$

Preliminary experiments showed a slightly varying concentration gradient that decreased toward the posterior of the worm, and a concentration gradient of $350\,mg\,O_2\,L^{-1}\,m^{-1}$ was measured over approximately the anterior third of the worm. Using this concentration gradient and $D=2\times10^{-9}\,m^2\,s^{-1}$ for oxygen in seawater, and assuming a crack width of 0.008 m, worm length of 0.04 m, and worm mass of 0.3 g, yields a total flux of

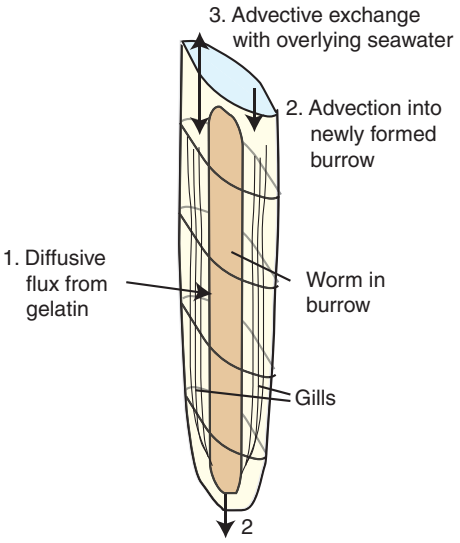

Fig. A1. A worm burrowing in gelatin could potentially get oxygen from (1) diffusion from the gelatin across the burrow wall, (2) advection of overlying water into the burrow as the worm extends the crack and expands the volume of the burrow, or (3) advective exchange with the overlying water, which is measurable as a drop in O_2 concentration in the overlying water.

$0.05\,\mu g\,O_2\,h^{-1}\,g^{-1}$, several orders of magnitude lower than the oxygen consumption rates measured from the overlying water. This term was therefore ignored. Our use of gelatin, which as normally prepared has much higher oxygen concentration than sediment, as an analog was dependent on the contribution from diffusion being small.

Flux of oxygen from the overlying water to the worm across the opening of the burrow is primarily advective, and preliminary observations of oxygen concentrations along the burrow showed fluctuations consistent with the assumption that increases in oxygen in the burrow come in bursts from advective pumping. Advection can be divided into two components: flux of water into the burrow to fill the space opened by the worm as it burrows (labeled 3 in Fig. A1) and exchange of burrow water with overlying water (labeled 2 in Fig. A1). The increase in burrow volume (dV_{Cr}/dt) was approximated as:

$$\frac{dV_{Cr}}{dt} = u(\pi\frac{w_{Cr}}{2}h) \ , \qquad (A3)$$

assuming the burrow is an elliptic cylinder with the half-width ($w_{Cr}/2$) and half-thickness (h) as the radii and u is the velocity of the worm. Making the conservative assumption that the inflowing water has the same concentration as the overlying water, C_0, and that all oxygen is consumed by the worm, the flux is therefore:

$$J = dV_{Cr}/dtC_0 = u\,[\pi\,(w_{Cr}/2)\,h]\,C_0 \, , \qquad (A4)$$

and contributed $0.009\,\mu g\,O_2\,h^{-1}\,g^{-1}$, again small enough to neglect.

Finally, we made the assumption that the concentration of oxygen around the worm did not vary over time and therefore the term dC/dt in the advection–diffusion equation could be neglected. Monitoring of oxygen concentrations at several points as the worm moved past showed similar concentrations close to the worm at different times as the worm burrowed, supporting this assumption.

LIST OF SYMBOLS AND ABBREVIATIONS

C	concentration of oxygen ($mg\,O_2\,l^{-1}$)
C_0	concentration of oxygen in the overlying water ($mg\,O_2\,l^{-1}$)
E	stiffness / Young's modulus (Pa)
E_{ext}	external work per body mass per distance traveled ($J\,kg^{-1}\,m^{-1}$)
G_c	fracture toughness ($J\,m^{-2}$)
GCT	gross cost of transport ($J\,kg^{-1}\,m^{-1}$)
h	half-thickness of worm (m)
Hb	hemoglobin
J_{wall}	oxygen flux across the burrow wall ($mg\,O_2\,m^{-2}$)
K_{Ic}	critical stress intensity factor/fracture toughness ($Pa\,m^{0.5}$)
L	length of worm
m_w	mass of worm (g)
NCT	net cost of transport ($J\,kg^{-1}\,m^{-1}$)
P_{Cr}	rate of work to extend the burrow by fracture ($J\,s^{-1}$)
P_{El}	rate of elastic work ($J\,s^{-1}$)
P_{tot}	total rate of work ($J\,s^{-1}$)
u	velocity of worm ($m\,s^{-1}$)
V_{Cr}	volume of the crack-shaped burrow
$\dot{V}_{O_2}$	rate of O_2 consumption
w_{Cr}	width of crack (m)
W_{Cr}	work to extend the burrow by fracture (J)
W_{El}	elastic work (J)
w_{worm}	width of worm (m)
Δx	increment of crack growth (m)
ν	Poisson's ratio (dimensionless)
σ_{worm}	stress applied by the anterior of the worm (Pa)

ACKNOWLEDGEMENTS

The authors appreciate helpful advice on anaerobic metabolism from George Brooks, Rajaa Hussien, Mike Horning, Matt Johnson and Chien-Ting Liu, and on hemoglobin measurements from Sean Gross. George Brooks also generously provided lab space and supplies for anaerobic metabolism assays. Michael Fierro at Spectros assisted with methods for measuring hemoglobin saturation. James Che, Elizabeth Murphy, Eve Robinson, Lindsay Waldrop and Samantha Zeman helped collect worms. We thank Pete Jumars, Dennis Evangelista and an anonymous reviewer for helpful comments on the manuscript. This project was funded by NSF IOS grant #0642249 to M.A.R.K.

REFERENCES

Alyakrinskaya, I. O. (2002). Physiological and biochemical adaptations to respiration of hemoglobin-containing hydrobionts. *Biol. Bull.* **29**, 268-283.
Anderson, T. L. (2005). *Fracture Mechanics: Fundamentals and Applications*. Boca Raton, FL: CRC Press.
Berrigan, D. and Lighton, J. R. (1993). Bioenergetic and kinematic consequences of limblessness in larval Diptera. *J. Exp. Biol*, **179**, 245-259.
Bestwick, B. W., Robbins, I. J. and Warren, L. M. (1989). Metabolic adaptations of the intertidal polychaete *Cirriformia tentaculata* to life in an oxygen-sink environment. *J. Exp. Mar. Biol. Ecol.* **125**, 193-202.
Boudreau, B. P., Algar, C., Johnson, B. D., Croudace, I., Reed, A., Furukawa, Y., Dorgan, K. M., Jumars, P. A., Grader, A. S. and Gardiner, B. S. (2005). Bubble growth and rise in soft sediments. *Geology* **33**, 517-520.
Brown, A. (1979a). Oxygen consumption of the sandy-beach whelk Bullia digitalis meuschen at different levels of activity. *Comp. Biochem. Physiol.* **62A**, 673-675.
Brown, A. (1979b). The energy cost and efficiency of burrowing in the sandy-beach whelk *Bullia digitalis* (Dillwyn) (Nassariidae). *J. Exp. Mar. Biol. Ecol.* **40**, 149-154.
Casey, T. M. (1991). Energetics of caterpillar locomotion: biomechanical constraints of a hydraulic skeleton. *Science* **252**, 112-114.
Che, J. and Dorgan, K. M. (2010). It's tough to be small: dependence of burrowing kinematics on body size. *J. Exp. Biol.* **213**, 1241-1250.
Dales, R. P. and Warren, L. M. (1980). Survival of hypoxic conditions by the polychaete *Cirriformia tentaculata*. *J. Mar. Biol. Assoc. UK* **60**, 509-516.
Dangott, L. J. and Terwilliger, R. C. (1986). The role of extracellular hemoglobins in the oxygen consumption of the burrowing polychaete, *Euzonusmucronata* (Treadwell). *J. Exp. Mar. Biol. Ecol.* **97**, 193-204.
Daniel, T. L. (1985). Cost of locomotion: unsteady medusan swimming. *J. Exp. Biol.* **119**, 149-164.
Dejours, P. (1981). *Principles of Comparative Respiratory Physiology*. Amsterdam: Elsevier-North-Holland Biomedical Press.
Denny, M. (1980). Locomotion: the cost of gastropod crawling. *Science* **208**, 1288-1290.
Dijkhuizen, P., Buursma, A., Fongers, T. M. E., Gerding, A. M., Oeseburg, B. and Zijlstra, W. G. (1977). The oxygen binding capacity of human haemoglobin. *Pflugers Arch.* **369**, 223-231.
Donovan, D. and Carefoot, T. (1997). Locomotion in the abalone Haliotis kamtschatkana: pedal morphology and cost of transport. *J. Exp. Biol.* **200**, 1145-1153.
Donovan, D., Baldwin, J. and Carefoot, T. (1999). The contribution of anaerobic energy to gastropod crawling and a re-estimation of minimum cost of transport in the abalone, *Haliotis kamtschatkana* (Jonas). *J. Exp. Mar. Biol. Ecol.* **235**, 273-284.
Dorgan, K. M. (2010). Environmental constraints on the mechanics of crawling and burrowing using hydrostatic skeletons. *Exp. Mech.* **50**, 1373-1381.
Dorgan, K. M., Jumars, P. A., Johnson, B., Boudreau, B. P. and Landis, E. (2005). Burrowing mechanics: burrow extension by crack propagation. *Nature* **433**, 475.
Dorgan, K. M., Arwade, S. R. and Jumars, P. A. (2007). Burrowing in marine muds by crack propagation: kinematics and forces. *J. Exp. Biol.* **210**, 4198-4212.
Dorgan, K. M., Arwade, S. R. and Jumars, P. A. (2008). Worms as wedges: effects of sediment mechanics on burrowing behavior. *J. Mar. Res.* **66**, 219-254.
Ellington, W. R. (1983). The recovery from anaerobic metabolism in invertebrates. *J. Exp. Zool.* **228**, 431-444.
Engel, P. C. and Jones, J. B. (1978). Causes and elimination of erratic blanks in enzymatic metabolite assays involving the use of NAD+ in alkaline hydrazine buffers: Improved conditions for the assay of -glutamate, -lactate, and other metabolites. *Anal. Biochem.* **88**, 475-484.
Ferner, M. C. and Jumars, P. A. (1999). Responses of deposit-feeding spionid polychaetes to dissolved chemical cues. *J. Exp. Mar. Biol. Ecol.* **236**, 89-106.
Full, R. J. (1991). The concepts of efficiency and economy in land locomotion. In *Efficiency and Economy in Animal Physiology* (ed. R. W. Blake), pp. 97-132. Cambridge: Cambridge University Press.
Full, R. J. (1997). Invertebrate locomotor systems. In *The Handbook of Comparative Physiology* (ed. W. Dantzler), pp. 853-930. Oxford: Oxford University Press.
Gutmann, I. and Wahlefeld, A. W. (1974). L-(+)-Lactate: determination with lactate dehydrogenase and NAD. In *Methods of Enzymatic Analysis* (ed. H. Bergmeyer and K. Gawehn), pp. 1464-1468. San Diego, CA: Academic Press.
Heinz, F. and Weisser, H. (1985). Creatine phosphate. In *Methods of Enzymatic Analysis* (ed. H. Bergmeyer and K. Gawehn), pp. 507-514. San Diego, CA: Academic Press.
Hunter, R. D. and Elder, H. Y. (1989). Burrowing dynamics and energy cost of transport in the soft-bodied marine invertebrates *Polyphysia crassa* and *Priapulus caudatus*. *J. Zool.* **218**, 209-222.
Johnson, B. D., Boudreau, B. P., Gardiner, B. S. and Maass, R. (2002). Mechanical response of sediments to bubble growth. *Mar. Geol.* **187**, 347-364.
Julian, D., Chang, M., Judd, J. and Arp, A. (2001). Influence of environmental factors on burrow irrigation and oxygen consumption in the mudflat invertebrate Urechis caupo. *Mar. Biol.* **139**, 163-173.
Kimura, T., Nakano, T., Yamaguchi, T., Sato, M., Ogawa, T., Muramoto, K., Yokoyama, T., Kan-no, N., Nagahisa, E., Janssen, F. et al. (2004). Complementary DNA cloning and molecular evolution of opine dehydrogenases in some marine invertebrates. *Mar. Biotechnol.* **6**, 493-502.
Kreutzer, U., Siegmund, B. R. and Grieshaber, M. K. (1989). Parameters controlling opine formation during muscular activity and environmental hypoxia. *J. Comp. Physiol. B* **159**, 617-628.
Livingstone, D. R. (1991). Origins and evolution of pathways of anaerobic metabolism in the animal kingdom. *Am. Zool.* **31**, 522-534.
Murphy, E. A. K. and Dorgan, K. M. (2011). Burrow extension with a proboscis: mechanics of burrowing by the glycerid, *Hemipodus simplex*. *J. Exp. Biol.* **214**, 1017-1027.
Plante, C. and Jumars, P. (1992). The microbial environment of marine deposit-feeder guts characterized via microelectrodes. *Microb. Ecol.* **23**, 257-277.
Royer, W. E., Jr, Strand, K., van Heel, M. and Hendrickson, W. A. (2000). Structural hierarchy in erythrocruorin, the giant respiratory assemblage of annelids. *Proc. Natl. Acad. Sci. USA* **97**, 7107-7111.
Schiedek, D. (1997). *Marenzelleria viridis* (Verrill, 1873) (Polychaeta), a new benthic species within European coastal waters: Some metabolic features. *J. Exp. Mar. Biol. Ecol.* **211**, 85-101.
Schmidt-Nielsen, K. (1972). Locomotion: energy cost of swimming, flying, and running. *Science* **177**, 222-228.
Seibel, B. A., Thuesen, E. V., Childress, J. J. and Gorodezky, L. A. (1997). Decline in pelagic cephalopod metabolism with habitat depth reflects differences in locomotory efficiency. *Biol. Bull.* **192**, 262-278.
Seymour, R. S., Withers, P. C. and Weathers, W. W. (1998). Energetics of burrowing, running, and free-living in the Namib Desert golden mole (*Eremitalpa namibensis*). *J. Zool.* **244**, 107-117.
Swaney, J. B. and Klotz, I. M. (1971). Properties of erythrocruorin from *Cirraformia grandis*. *Arch. Biochem. Biophys.* **147**, 475-486.
Taghon, G. L. (1988). The benefits and costs of deposit feeding in the polychaete *Abarenicola pacifica*. *Limnol. Oceanogr.* **33**, 1166-1175.
Trevor, J. H. (1978). The dynamics and mechanical energy expenditure of the polychaetes Nephtys cirrosa, Nereis diversicolor and Arenicola marina during burrowing. *Estuar. Coast. Mar. Sci.* **6**, 605-619.
Warren, L. M., Wells, R. M. G. and Weber, R. E. (1981). Erythrocruorins (extracellular haemoglobins) from the cirratulid polychaetes *Cirriformia tentaculata* (Montagu) and *Cirratulus cirratus* (Müller) with special reference to saturation dependent characteristics of the oxygen equilibria. *J. Exp. Mar. Biol. Ecol.* **55**, 11-24.
White, C. R. (2001). The energetics of burrow excavation by the inland robust scorpion, *Urodacus yaschenkoi* (Birula 1903). *Aust. J. Zool.* **49**, 663-674.
White, C. R., Matthews, P. G. D. and Seymour, R. S. (2006). Balancing the competing requirements of saltatorial and fossorial specialisation: burrowing costs in the spinifex hopping mouse, *Notomys alexis*. *J. Exp. Biol.* **209**, 2103-2113.
Withers, P. C., Thompson, G. G. and Seymour, R. S. (2000). Metabolic physiology of the north-western marsupial mole, *Notoryctes caurinus* (Marsupialia: Notoryctidae). *Aust. J. Zool.* **48**, 241-258.
Zammit, V. A. (1978). Possible relationship between energy metabolism of muscle and oxygen binding characteristics of haemocyanin of cephalopods. *J. Mar. Biol. Assoc. UK* **58**, 421-424.