Locomotor and energetic consequences of behavioral thermoregulation in the sanguivorous leech Hirudo verbana

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ABSTRACT

Medicinal leeches (*Hirudo verbana*) thermoregulate with respect to their sanguivorous feeding behavior. Immediate postprandial preferences are for warmer than their initial acclimation temperature (T_a, 21 °C, Petersen et al. 2011), while unfed leeches have a lower preferred temperature (T_{pref}, 12.5 °C). This may reduce energy expenditure and defer starvation if feeding opportunities are limited. Energetic benefits may have an associated cost if low temperatures reduce mobility and the ability to locate further hosts. These costs could be limited if mobility is unimpaired at low temperatures, or if acclimation can restore locomotor performance to the levels at T_a. The transition from T_a to the unfed T_{pref} significantly reduced speed and propulsive cycle frequency during swimming, and extension and retraction rates during crawling. Aerobic metabolic rate was also reduced from 0.20 ± 0.03 W kg^{-1} at T_a to 0.10 ± 0.03 W kg^{-1} at T_{pref}. The Q_{10} values of 1.7–2.9 for energetic and swimming parameters indicate a substantial temperature effect, although part of the decline in swimming performance can be attributed to temperature-related changes in water viscosity. 6 weeks at T_a resulted in no detectable acclimation in locomotor performance or aerobic metabolism. The energetic savings associated with a lower T_{pref} in unfed leeches effectively doubled the estimated time until depletion of energy reserves. Given that some mobility is still retained at T_{pref}, and that acclimation is in itself costly, the energetic benefits of selecting cooler temperatures between feedings may outweigh the costs associated with reduced locomotor performance.

1. Introduction

Effective locomotion is essential to the fitness of many animals. Locomotor performance in ectotherms is profoundly affected by changes in environmental temperature (Angilletta et al., 2002). This is primarily a consequence of effects on muscle contractile velocity and energy metabolism, and in some cases changes in the physical environment, particularly in organisms moving through fluids (Fuiman and Batty, 1997; James, 2013; Johnston and Temple, 2002). Temperature effects on locomotor performance have direct links to organismal fitness through impacts on foraging ability, predator evasion and other mobility dependent behaviors (Angilletta et al., 2002; Pörtner et al., 2006). An appreciation of temperature effects on performance, and the extent and time course of any acclimation, is therefore vital for understanding the physiological ecology of any ectotherm (Precht, 1958; Angilletta et al., 2006).

The relationships between temperature, energy metabolism and mobility in many ectotherms are further complicated by the physiolo-

gical and physical demands associated with feeding and the management of energy reserves. For example, some ectotherms respond to food deprivation by reducing their preferred temperatures, lowering their metabolic rate and slowing the depletion of stored energy (Lillywhite et al., 1973; Abe, 1995; van Dijk et al., 2002; Brown and Griffin, 2005; Bicego et al., 2007; McCue, 2010). Feeding may also promote adjustment in preferred temperature. Digestion can have substantial energetic costs that divert energy from other physiological functions and limit locomotor performance (Secor, 2009; Thorarensen and Farrell, 2006). Some ectotherms increase their preferred temperature after feeding to raise their metabolic rate and shorten the time course of these postprandial processes (Gatten et al., 1974; Lang, 1979; Slip and Shine, 1988; Petersen et al., 2011). These energetic challenges are most acute where meal sizes are large and feeding opportunities are rare.

This pattern is carried to an extreme in sanguivorous leeches, where feeding may increase their pre-feeding mass by an order of magnitude (Lent et al., 1988; Claflin et al., 2009), and further feeding opportu-

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nities may not arise for many months (Mann, 1955). This pattern shapes many aspects of their physiology and behavior. Rare feeding opportunities are maximally exploited through suppression of behaviors that might prematurely terminate feeding (Gaudry et al., 2010), and the mechanical properties of the body musculature accommodate large meals and allow recovery of mobility after feeding (Claflin et al., 2009; Gerry and Ellerby, 2011). Behavioral thermoregulation also occurs (Petersen et al., 2011). Immediately after feeding, temperatures higher than the initial acclimation temperature (T_a) are preferred, increasing metabolic rate and reducing the time course of initial meal processing (Petersen et al., 2011). Leeches that have been unfed for several months select temperatures cooler than T_a. This likely reduces their metabolic rate and preserves energy reserves. Successful feeding, however, requires detection of, movement towards, and attachment to a host animal (Dickinson and Lent, 1984; Harley et al., 2013; Young et al., 1981). If the low preferred temperature reduces mobility, this could impose a cost associated with reduced foraging success.

Some ectotherms maintain maximal locomotor performance across a relatively wide temperature range (Ben-Ezra et al., 2008; Block et al., 2013), although to some extent this may be associated with a decoupling of temperature sensitive physiological processes from performance by mechanical factors. Given the relatively direct relationship between muscle contractile velocity, locomotor cycle frequency and speed in leeches (Gerry et al., 2012), a decline in performance at low temperatures is more likely in this case. Hirudine leech habitats show both spatial and seasonal, temporal variation in temperature (Elliott and Kutschera, 2011; Elliott and Tullett, 1986). The relatively short-lived postprandial preference for warmer temperatures and infrequency of feeding means that there is potential for long-term exposure to low temperatures. Under these circumstances, how do leeches remain able to exploit their next feeding opportunity? Under long term cold exposure, many ectothermic vertebrates mitigate the effects of low temperatures on locomotor performance through compensatory changes in muscle contractile properties and metabolic enzyme activities (Johnston and Temple, 2002; James, 2013). Acclimation in locomotor capacity (Precht, 1958) is therefore based on tissue level phenotypic plasticity (Wilson and Franklin, 2002). Given the importance of mobility to their foraging success, we hypothesized that leeches would exhibit performance acclimation to maintain performance at low temperatures similar to that commonly observed in vertebrate ectotherms.

In order to test this, locomotor performance data were collected for unfed medicinal leeches (*Hirudo verbana*) during their two primary modes of locomotion, swimming and crawling. Data were collected from leeches at their initial acclimation temperature (T_a, 21 °C), at their first exposure to their preferred temperature (T_{pref}, 12.5 °C), and periodically throughout a 6-week period being held at T_{pref}. Temperature changes can also affect locomotion through effects on the physical environment. At relatively small scales, the mechanics of movement through water are dominated by the effects of viscosity (Vogel, 1996) which varies with temperature (Kestin et al., 1978). Temperature effects on swimming performance in small organisms may therefore combine physiological and hydrodynamic factors. The size and velocity of leeches places them within the range of conditions under which the viscous effects of changing temperature are a potential confounding factor (Ellerby, 2009; Fuiman and Batty, 1997). In order to partition the effects temperature and viscosity changes on swimming we manipulated water viscosity in combination with temperature, maintaining viscosity as a constant while changing water temperature from T_a to T_{pref}. Metabolic rate data were also collected at T_a and T_{pref} to determine the potential energy saving associated with a low temperature when unfed.

Although there is a wealth of data concerning temperature effects on vertebrate locomotor performance (Johnston and Temple, 2002), few comparable data are available for invertebrates (Bailey and Johnston, 2005). Previous performance studies have largely focused

on developmental effects and cross-generational selection in arthropods (Alford et al., 2012; Clusella-Trullas et al., 2010; Crill et al., 1996; Gibert et al., 2001; Niehaus et al., 2012), rather than plasticity that modifies performance in the shorter term. There are few data concerning the acclimation of locomotor performance in annelids (Schröer et al., 2009; 2011), where temperature acclimation data are primarily restricted to tissue level responses to cold exposure that indicate plasticity in energy metabolism and respiratory function (Crockett et al., 2001; Holmstrup et al., 2007; Kristensen, 1983; Overgaard et al., 2009). The present study will therefore provide new insight into the effects of changing temperature on annelid locomotor performance, and whether functional plasticity can support compensatory performance changes at low temperatures.

2. Materials and methods

2.1. Animals

Medicinal leeches (*Hirudo verbana*, Siddall et al., 2007) were obtained from Leeches USA (Westbury, NY, USA). They were unfed and maintained on a 12: 12 light: dark cycle at an initial acclimation temperature (T_a) of 21 °C for a three to four months prior to experimentation. Before experimentation leeches were housed in groups of up to 20 in 8 l aquaria. The aquarium water was amended with $0.75 \, \mathrm{g \, l^{-1}}$ aquarium salt (Doc Wellfish, Chalfont, PA, USA). During experimentation individual leeches were housed in 180 ml screw-cap specimen storage containers with the lids perforated to allow gas exchange (Thermo Scientific 4 A 0180, Waltham, MA, USA). Mean leech masses were $1.75 \pm 0.22 \, \mathrm{g}$ (mean ± SD, N = 5, swimming and crawling performance), $1.80 \pm 0.24 \, \mathrm{g}$ (mean ± SD, N = 6, viscosity and temperature manipulations, and initial thermal preference measurements), and $1.71 \pm 0.25 \, \mathrm{g}$ (mean ± SD, N =6, metabolic rate measurements).

2.2. Temperature gradient exposure

Unfed leeches were exposed to a temperature gradient ranging from 10 to 30 °C. This consisted of an elongated (190 cm long x 6 cm deep x 8 cm wide), recirculating water tank with a cold water inflow at one end and a series of external heaters to raise the temperature of the water as it flowed through the tank with an approximate volume flow rate of $10 \, \mathrm{ml \, min^{-1}}$ (Petersen et al., 2011). Exiting water was chilled to 10 °C before re-entering the tank. This created a uniform temperature gradient. The tank was subdivided into 27 numbered sections and a starting location number for each leech chosen using a random number generated for the specified integer range (www.random.org). Temperature at the final location was recorded after 2 h. This was sufficient time to enable the leeches to complete initial exploration of the tank and settle in a chosen location and preferred temperature (T_{pref}). For subsequent experiments, leeches were exposed to the mean T_{pref}.

2.3. Locomotor performance analyses

Video recordings were obtained as leeches swam or crawled along a 5 cm wide x 76 cm long lane created by a plastic divider along the side of a glass 20 gallon aquarium tank. Video was recorded at a 60 Hz frame rate using an AOS X-PRI camera (AOS Technologies, Baden Daettwil, Switzerland) and downloaded to a PC using AOS Digital Imaging software. The camera was placed perpendicular to the plane of motion, 3 m from the mid-point of the aquarium tank. Length was calibrated from a 30 cm ruler placed along the center of the divided section of the aquarium. Video sequences were analysed in ImageJ (Abramoff et al., 2004) to determine swimming velocity, swimming cycle frequency, swimming stride length (the distance travelled per swimming cycle), and the extension and retraction velocities during