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Activity and positioning of eurythermal hydrothermal vent sulphide worms in a variable thermal environment

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ABSTRACT

Here we investigate behavioral responses to fine-scale spatial and temporal temperature gradients in a heat tolerant hydrothermal vent worm (*Paralvinella sulfincola*). While this species has been a model organism for understanding physiological adaptations to extreme environments, lacking are corroborative in situ experiments and characterization of temperature-related behaviors representing the lower thermal tolerance range of this species. To address this knowledge gap, we first quantified the upper thermal limit for this species using a rapid heating protocol executed remotely on the seafloor. Second, we used a combination of in situ observations and shipboard experiments to test for temperature-dependent patterns in activity and behavior. We confirm that *P. sulfincola* is remarkably eurythermal and demonstrates a thermal niche breadth exceeding 45 °C. We further show that the activity and positioning of worms relate to temperatures within its lower preferred range (i.e., between 4 and 20 °C). Worms tended to remain closer to their tube openings and held their branchial crown in a consistent location when fluids were relatively warm. By contrast, when fluids were cooler, both the distance worms were observed from their tubes and positioning of their branchial crown were more variable. A Bayesian hidden Markov model classified each worm at each time interval as being in a high or low activity state according to the magnitude of change in their orientation and how far they moved between successive time lapse images. We found that the transition between active and inactive states at any time period is related to fluid temperature. Our observations indicate that the behavior of the worms is temperature-dependent, which may in turn reflect temperature-related variables such as the delivery of food particles, dissolved oxygen concentration, or relative environmental variability. Our findings demonstrate that this species can respond behaviorally to very fine-scale environmental variability in a manner not simply predicted by models of increasing activity with temperature.

1. Introduction

In the ocean, hydrothermal vents create a uniquely variable thermal environment (e.g., Bates et al., 2010; Johnson et al., 1986; Tivey et al., 2002). Along mid-ocean ridges and in other geological settings where hot, toxic hydrothermal fluids exit at the seafloor and mix with cold, oxygen-rich bottom seawater, steep and fluctuating thermal gradients are maintained at small spatial scales (±5 °C changes over centimeters and minutes: Bates et al., 2010). Thus hydrothermal vent species that occupy a wide thermal niche present the opportunity to investigate adaptations and responses to different environmental regimes. In particular, a suite of abiotic and biotic factors co-vary with temperature, such as dissolved oxygen and sulphide, pH, pCO_2, and availability of particulate food (Johnson et al., 1986; Levesque et al., 2006).

High heat tolerance has been documented for several polychaetes living in the warmest habitat near hydrothermal outflows (e.g., *Hesiolyra bergi*: Shillito et al., 2001; *Alvinella pompejana*: Ravaux et al., 2013; Chevaldonné et al., 2000). Here we focus on the Juan de Fuca Ridge sulphide worm (*Paralvinella sulfincola*), known to withstand prolonged exposure to temperatures up to 55 °C in shipboard aquaria, the current record for the highest temperature tolerated by an aquatic metazoan (Girguis and Lee, 2006; Lee, 2003). This tube-dwelling species colonizes extreme hydrothermal vent habitats where substratum temperatures have been measured ranging from 20 to 80 °C (Juniper et al., 1992). These warm habitats are where most studies on the sulphide worm have focused (e.g., Grelon et al., 2006; Sarrazin and Juniper, 1999; Sarrazin et al., 1997). For instance, to characterize the

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behavior of sulphide worms in the challenging setting of the deep sea, Grelon et al. (2006) used video to record and classify the behaviors of multiple worms over 10 minute intervals and found that worms spend about half their time in an inactive state near their tube opening. In the remainder of their time budgets, worms were either withdrawn in their tubes or were active, displaying behaviors interpreted as tube maintenance, deposit feeding, and territorial strikes against conspecifics (Grelon et al., 2006).

Even so, sulphide worms are also found in cooler habitats and occasionally select temperatures as low as 5 °C in thermal gradient chambers (Bates et al., 2010). This raises the question of whether worms inhabiting cooler habitat fluids will display different levels of activity, or behaviors typical of worms in an inactive state. For instance, animal activity is predicted to be more rapid at higher temperatures on the basis of thermal reaction kinetics (Gillooly et al., 2001), whereby the mean kinetic energy of molecules influences the rate of biochemical reactions. While the sulphide worm may therefore represent one of the most eurythermal, ectothermic animals, the consistency of its activity and behavior across the entire breadth of its thermal niche is unknown.

This study explores body positioning by sulphide worms along steep environmental gradients in a hydrothermal vent system. We first confirmed the upper threshold for the activity of *P. sulfincola* in a novel seafloor experiment. Next, to assess whether the sulphide worm is eurythermal, we tested whether worms remain active at the lower end of their preferred thermal window in laboratory heat stress experiments, and using time-lapse observations executed on the seafloor. Last, we investigated if worms adjust the positioning of their branchial crown with changes in the fluid temperature. We first questioned whether worms could exhibit activity at the lower end of their thermal niche. We predicted that worms would be relatively inactive in cold fluids and would display behaviors more typical of active states at warmer temperatures.

2. Materials and methods

2.1. In situ critical thermal maximum

For in situ (seafloor) confirmation of the heat tolerance of *P. sulfincola* reported from shipboard studies, specimens were collected from Axial Volcano of the Juan de Fuca Ridge (JdF) at ~1500 m in depth using the manipulator on the submersible, *Alvin*, and immediately placed in a temperature-controlled chamber (Fig. 1). As electrically powered heating devices would potentially draw too much current and shorten the duration of the *Alvin* dive, hot vent fluids were used to heat a water jacketed stainless steel holding chamber to raise the fluid temperature within the chamber to >60 °C. As a control for animal survival in the chamber itself, on two independent occasions, worms were exposed to sublethal temperatures (maximum of 26 and 42 °C). Warm fluids were collected using a titanium wand held by the *Alvin* manipulator. Suction was provided by the *Alvin* pelagic pump. The walls of the device were constructed of 2.54 cm thick polypropylene, which provided insulation and did not significantly affect the payload weight in water (since polypropylene has near neutral buoyancy). To determine how much hot water to pump into the device, the temperature inside the water jacket was monitored using either the *Alvin* temperature probe, or an Omega temperature indicating label (RLC-50 Series) with a resolution of 5 °C. The indicating label was sealed in epoxy and could be monitored using the *Alvin* video system. Effects of hydrostatic pressure on indicating strip function were not evident in laboratory tests.

In the experiment, oxygen consumption of worms probably increased. Even though the experiment was short in duration, it is likely that oxygen conditions were low in the chambers. However, paralvinellids can survive without oxygen for over 24 h at moderate temperatures (R. Lee, unpublished data), suggesting that temperature was the dominant variable in the experiment.

Individual worms (n = 5 to 7) were placed on a flat surface and brushed into the stainless steel holding chamber. A clear polycarbonate lid was then secured over the chamber. Observations of animal movements within the holding chamber were made using the *Alvin* video system. Temperature inside the holding chamber was recorded using a DS1921 Thermochron ibutton (±0.5 °C) enclosed in a titanium pressure housing with polycarbonate endcaps (Bates et al., 2010; Rinke and Lee, 2009). To quantify an upper temperature limit, the chamber temperature was raised from 30 to 70 °C in 20 min (rapid increases in temperature occur when worms migrate to new locations or following abrupt changes in fluid discharge), following the heating method described above. Although heating was linear, ~2 °C min^{-1}, we cannot rule out the possibility that the temperature experienced by each worm differed slightly due to gradients within the experimental chamber. Simultaneous video of animal behavior with increasing temperature was used to determine the critical thermal maximum, indicated by cessation of movement, and onset of muscular spasms. We used logistic regression and the function "p.dose" from the package MASS (Venables and Ripley, 2002) in R (R Development Core Team, 2011) to back-calculate the median temperature at which experimental animals ceased movement and displayed spasms.

2.2. Temperature-dependent activity

To quantify temperature dependence in the activity of *P. sulfincola*, worms attached to pieces of sulphide chimney were collected using

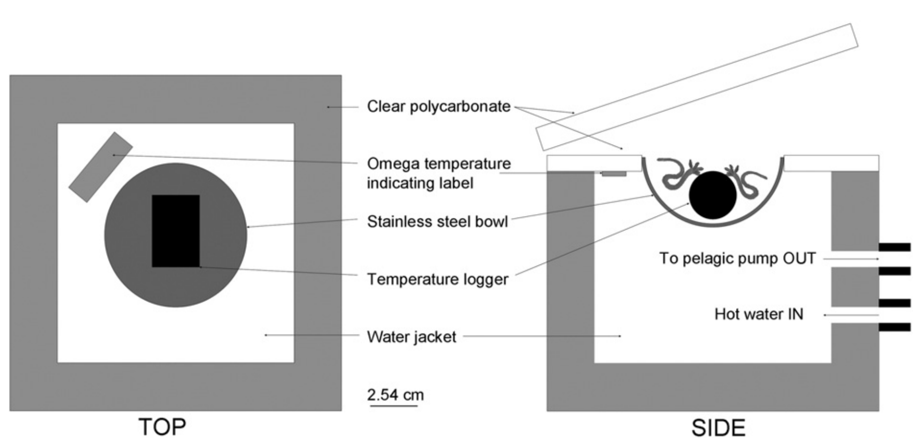

Fig 1. In situ heated chamber design and dimensions. Hot water from the vent environment was pumped into the water jacket of the stainless steel holding chamber. Chamber temperature was monitored with an Omega temperature label in the water jacket. Holding chamber temperature was recorded using a Thermochron ibutton logger (DS1921, ±0.5 °C) in a pressure housing.