

THERMAL PREFERENCE IN THE SMOOTHSKIN OCTOPUS
(MUUSOCTOPUS LEIODERMA)

by

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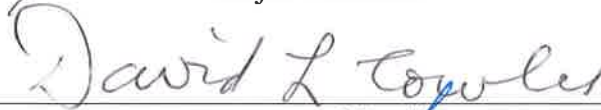
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Thermal Preference in the Smoothskinned Octopus (*Benthoctopus leioderma*)

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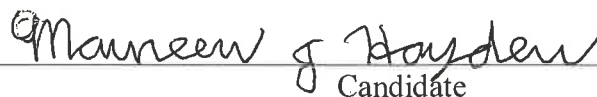


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ABSTRACT

The smoothskin octopus (*Muusoctopus leioderma*), typically a deep water species found between 90-500 m, has recently been found at depths of 13 m (Hochberg, 1998; Kore et al., in review). Collections of a deep water octopus in Burrows Bay, Washington have raised questions about the known distribution of this species. Temperature at the collection location (13°C) is warmer than temperatures at the upper portion of their known depth range (8°C). I hypothesized that *M. leioderma* is a recent immigrant to shallow water. Therefore, I predicted that *M. leioderma* would prefer temperatures cooler than 13°C, consistent with acclimation to deeper depths.

Total movement was quantified at temperatures between 4 and 13°C in a shallow, temperature controlled enclosure. Oxygen consumption of octopuses was also measured by open respirometry system at temperatures between 8°C and 13°C and movement rate changed as a function of temperature more than metabolic rate as a function of temperature suggesting behavioral avoidance of these temperatures. Temperature preference of 15 *Muusoctopus leioderma* was determined using a thermal gradient. Results from the temperature preference trials demonstrated that *M. leioderma* preferred temperatures cooler than the collection location. These data are consistent with the hypothesis that the Burrows Bay population of *M. leioderma* may be recent immigrants to shallow water.

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Introduction

During the summer of 2014, burrowing octopuses called smoothskin octopus (*Muusoctopus leioderma*) were observed in Burrows Bay, Washington (Kore, et al., in review). *Muusoctopus leioderma* is known from depths ranging from 90 to 1400 m and is most common between 450 to 600 m (Conners et al., 2014; Hochberg, 1998). Why is a putative deep water species of octopus being observed in shallow water?

Collections of *M. leioderma* from trawls and rare encounters by deep ROV dives reveal that this species is benthic. It has been observed along muddy bottoms, including observations in which they have emerged from bottom sediment (Cosgrove & McDaniel, 2009). They are found in the North Pacific Ocean spanning from California to the Sea of Okhotsk (Conners et al., 2014; Jorgensen, 2009). The closest documented occurrence of *M. leioderma* near Burrows Bay was 40 miles away at a depth of 70 m in the Satellite Channel, Vancouver Island (Hochberg, 1998). This octopus species has relatively large eyes, lacks the ability to change skin texture, has a distinct keel-like ridge around the lateral margin of the mantle, and lacks an ink sac (Norman, 2000).

Population expansion of many cephalopod species has been observed over the past six decades (Doubleday et al., 2016). In addition to the population expansion of cephalopods is geographic range expansion (Ramos et al., 2014a; Zeidberg & Robison, 2007). Collections of this deep water octopus in shallow water may suggest a depth range expansion, which would agree with these global trends. In order to determine if there is a recently arrived population of *M. leioderma* in Burrows Bay, temperature can be used as a proxy. In marine environments temperature tends to decrease and pressure increase with depth. Thus, deep water populations of *M. leioderma* would be exposed to cooler

temperatures and greater pressure than that of the Burrows Bay population. The simplest depth related parameter to test is temperature, as temperature differences can be easily generated in the lab and used to replicate temperatures similar to those found in deep water.

Physiological processes of an organism are influenced by temperature on both a molecular and whole organismal level. Marine organisms have a range of temperatures that they can tolerate, resulting in physiological limitations. While these limitations are important for many marine organisms, they are of particular interest in ectotherms, whose body temperature is dependent on the temperature of the environment. Changing temperatures can inhibit physiological processes by affecting the rate of enzymatic reactions and the degree of membrane fluidity (Martin et al., 1976; Schmidt-Nielsen, 2001). An increase in temperature can result in accelerated enzymatic reactions and increased membrane fluidity. Additionally, if the temperature is too high enzymes will denature, and if the temperature gets too low, enzymatic reaction rates could slow below useful rates (Clarke, 2003). The effect of temperature on metabolism is usually expressed in terms of Q_{10} (temperature coefficient), which is defined as the proportion of change of a biological function with a temperature change of 10°C (Schmidt-Nielsen, 2001). In general, organisms have a Q_{10} of 2 to 3 (Schmidt-Nielsen, 2001), with adult cephalopod Q_{10} values falling within this range (O'Dor & Wells, 1987; Rosa & Seibel, 2010; Rosa & Seibel, 2008).

Organisms tend to be physiologically adapted to their temperature of their normal environment. For example, thermal windows, or the temperature that bivalve molluscs could survive in, narrowed and skewed colder in arctic bivalves than in tropical or

temperate bivalves (Peck & Conway, 2000). Larval *Octopus maya* were shown to have an increase in thermal tolerance with increasing acclimation temperature (Noyola et al., 2013).

Also, preferred temperature, defined as the temperature range in which an individual spends most of its time if given a choice (Reynolds & Casterlin, 1979), generally approximate physiological optimal temperatures. For example, *Micropterus salmoides* prefers temperatures that maximize the proportion of metabolism available for growth (Kelsch, 1996). In addition, *Lepomis macrochirus* have their highest critical swimming speeds at preferred temperatures (Kelsch, 1996). Furthermore, Australian skinks have optimal sprinting speeds at preferred temperatures (Huey & Bennett, 1987).

Preferred temperature will often approximate “normal” environment temperatures. In aquatic organisms from thermally stable habitats with slow temperature oscillations, such as temperate and deep water marine habitats, preferred temperature closely resembles environmental temperature (Johnson & Kelsch, 1998). For example, juvenile *Oncorhynchus kisutch* that came from streams with lower and less variable temperatures had a lower thermal preference than those that came from streams with warmer and more variable temperatures (Konecki et al., 1995). In another example, Juvenile *Micropterus salmoides* were kept at 26°C for 3 months after which they were placed into one of five acclimation temperature treatments for 30 days and showed a close match between acclimation temperature and preferred temperature (Díaz et al., 2007). Also, Juvenile *Octopus maya* were reared at 25°C for 30 days after which they were placed into one of four acclimation temperature treatments for 20 days. Larval *O.*

maya aslo showed a close match between acclimation temperature and preferred temperature (Noyola et al., 2013).

Thus, the *a priori* expectation would be that preferred temperature matches environmental temperature and a deviation from this expectation suggests a violation of the assumptions stated above. A mismatch may be caused by an organism responding to biotic factors such as prey abundance, reduction of predators, or reduced competition, or to abiotic factors such as changes in temperature, pH, salinity, or oxygen.

Little is known about the ability of cephalopods to sense temperature. In vertebrates the ability to sense changes in temperature is partially due to temperature activated transient receptor potential (TRP) ion channels. There are at least six temperature activated TRP ion channels and each has unique characteristics (Dhaka et. al, 2006). The gene Ocbimv22010114m.g has been found in *Octopus bimaculoides* for a protein that is homologous to heat sensation in vertebrates, but the function is still unknown (JGI Metazome Database).

A mismatch in temperature between preferred temperature and environmental temperature may also influence an organism's behavior. These mismatches may be addressed using behavioral thermoregulation, the process by which an animal uses behavioral means to modify or control their body temperature (Reynolds & Casterlin, 1979; Weiss & Laties, 1961). If a marine invertebrate is at non-ideal temperatures, it may change its behavior such as increase its activity as it goes in search of more ideal temperatures. For example, larval *Octopus huttoni* showed avoidance of temperatures that were either above or below sea surface temperatures measured at coastal sites over the entire range of *O. huttoni* in New Zealand (Higgins et al., 2012). By avoiding these

harmful temperatures, these octopuses maintain a more ideal body temperature and thus demonstrate behavioral thermoregulation. Additionally, in populations of polynoids, fewer individuals have been observed in temperatures near their thermal limit, suggesting avoidance (Robert et al, 2012).

I hypothesized that *M. leioderma* in Burrows Bay are recent immigrants to shallow water and have been there less than a generation. Therefore, I predicted that they would be acclimated to, and thus prefer cooler temperatures than those measured in Burrows Bay. The measured temperature of Burrow Bay during the summer by dive computers is 13°C. The average temperature of the Juan de Fuca Strait near Bowman's Bay at depth of 113 m is 7.4°C (Ocean Network Canada Data Archive, 2014). Furthermore, I predicted that movement as a function of temperature would change greater than can be expected by change in metabolic rate as a function of temperature.

Materials and Methods

Octopus Collection and Handling

Seventeen *Muusoctopus leioderma* were collected from Burrows Bay, Washington (48°28'N, 122°42'W) by SCUBA between depths of 12-18 meters at 13°C as measured by temperature sensors in the dive computers of those collecting the octopuses. Octopuses were transported to Rosario Beach Marine Laboratory where they were housed in individual seawater tanks and provided with a constant flow of new seawater from Rosario Bay using the flow through system in the seawater hallway at 13°C. Smaller octopuses (3.0-4.6 g) were housed in small, plastic aquaria (0.23 x 0.15 x 0.18 m) and larger octopuses (12.7-44.2 g) were housed in clear Sterilite EZ carry large

storage containers (0.36 x 0.24 x 0.31 m). Octopuses were allowed 24 h to acclimate prior to any experiments as well as a minimum of 24 h of rest in between experiments as this was shown to be an appropriate amount of time for other octopus species (Onthank, 2008). Octopuses were kept in captivity for a minimum of 7 days and maximum of 30 days. Octopuses were fed daily and were offered small amphipods, isopods, or purple shore crabs (*Hemigrapsus nudus*) but only a few octopuses actively fed.

Movement Rate

Movement rate of octopuses was measured by quantifying movement in a temperature controlled enclosure (Figure 1). Three enclosures were used; one was created from a Rubbermaid 31 quart bin (0.70 x 0.40 x 0.48 m) and two others were created from Sterilite 34 quart bins (0.67 x 0.41 x 0.17 m). Bulkhead fittings were attached to opposite ends of the enclosures and hose barb fittings attached to plumb water through the walls of the bins. Polyvinyl chloride (3/4" PVP) tubing was used to connect each enclosure to a 93 W Hydrofarm ActiveAqua chiller. A pump was connected to each chiller with a valve to control the flow rate of seawater. Rapid water exchange helped to keep temperatures uniform throughout the enclosures.

Movement rate trials lasted for a total of 10 hours. Octopuses were exposed to targeted temperatures (13, 11, 9, 7, and 4°C) in either ascending or descending order. Octopuses were randomly assigned to experience temperatures in ascending or descending order. At the beginning of each trial an octopus was placed in the middle of the enclosure and allowed 5 min to acclimate. Octopuses were exposed to each temperature in the series for a total of 2 hours. Lights were kept on during the trials,

despite the *M. leidurma* being nocturnal. Trials utilizing infrared lights to illuminate the enclosures and track the octopuses were initially attempted, but were not successful, having image quality that was too poor for analysis.

The temperature in the enclosure was recorded with a Vernier stainless steel temperature probe. Temperature was recorded 10 times per minute using the Vernier Logger Lite software. The movement of the octopus in the enclosure was tracked using an overhead camera (Logitech Quickcam or Webcam 170) mounted to a curtain rod which allowed for a full overhead view of the enclosure(s). Images were captured from the Logitech Quickcam/Webcam 170 every 20 seconds using FFmpeg. Octopus movement was measured from time lapse images using the MTrack2 plugin in FIJI and calibrated with a 10 cm scale in each frame.

Metabolic Rate

Metabolic rates of octopuses at a range of temperatures between 13 and 4°C using open respirometry (Figure 2). Respirometer jackets were connected to a VWR recirculating chiller to control temperature. The stir bar in the holding chamber assisted in the mixing of seawater. A peristaltic pump was used to circulate seawater through the respirometry system. PyroScience Firesting oxygen optode flow through cells were placed at both the inflow and the outflow of each respirometer. Gas equilibration columns which were placed on the inflow of the respirometers to reoxygenate water returning to the respirometers.

Respirometry trials lasted for a total of 13 h with four target temperature treatments (12.6, 10.7, 8.8, and 6.9°C). Octopuses were placed into the respirometer with

filtered seawater and allowed 2 h to acclimate at 13°C. Previous studies with *Octopus rubescens* found that octopuses' metabolic rates no longer declined after 2 hrs in the respirometers, indicating a return to routine metabolic rates (Onthank, 2008). Over the course of 8 h after the acclimation period, octopuses were exposed to each temperature treatment for 2 h in descending order. In order to target temperature treatments of 13, 10, 7, and 4°C, the recirculating chiller was set to temperatures of 9, 7, 3, and 0°C because prior experience with this setup showed that the internal respirometer temperature was approximately 4°C greater than the chiller temperature. After the lowest temperature exposure was completed, octopuses were given a 1 hr recovery period with the chiller set to 9°C as metabolic rate measurement continued. Octopuses were removed from the respirometers at the conclusion of the recovery period and a background respirometry measurement was taken for 2 h with the chiller set to 9°C in order to account for respiration from microorganisms. Oxygen concentration of inflow and outflow was recorded once per minute. Flow rate ranged from 100 mL per minute to 150 mL per minute depending on the size of the octopus. Mass specific metabolic rate was calculated using

$$\frac{\{([\mu\text{molO}_2]_{in} - [\mu\text{molO}_2]_{out}) - ([\text{backgroundO}_2]_{in} - [\text{backgroundO}_2]_{out})\} * \text{flow } L \text{ min}^{-1} * (60 \text{ min } hr^{-1})}{\text{mass octopus } (g)}$$

The pre-trial acclimation period and the post-trial recovery period were trimmed from the data and calculation for mass specific metabolic rate was calculated in R.

Thermal Preference

Thermal preference was measured using a custom made thermal gradient aquarium (Figure 3). The thermal gradient had three main components: the holding tank and two thermally modified reservoirs. The two thermally modified reservoirs (Coleman 100 quart Xtreme 5 Wheeled Cooler) consisted of one cold water and one warm water unit. The cold reservoir was kept cool by being connected to three Hydrofarm ActiveAqua chillers (one 186 W and two 93 W) in a series. The warm reservoir was supplied with constant flowing seawater from Rosario Bay at 13°C and the water was warmed further using three in-water heaters. The reservoirs were fitted with a second outflow to prevent overflow and keep the water depth consistent in each tank. The reservoirs were kept on an upper level at about 0.61 m above the holding tank.

The main acrylic holding tank in which the thermal gradient was established measured 1.83 x 0.13 x 0.13m. The top panel of the tank was hinged clear acrylic and opened to allow easy access to organisms and equipment. The inflow of water from each reservoir was gravity fed to allow for constant water pressure. The inflow ports were equally distributed along the length of the tank and positioned at the bottom of the tank. The inflow ports were connected to one hose from the warm water reservoir and one from the cold water reservoir. The temperature of inflowing seawater was controlled by adjusting the flow rate by using valves on each line contributing water. Each inflow had a baffle to allow for more even distribution of incoming seawater. An outflow was placed directly across from each inflow. Seven outflow ports were placed at the top of the thermal gradient directly opposite of the seven inflow ports. The three outflow ports at the warm end of the gradient and four outflow port at the cold end each converged into a

common warm water and cold water outflow lines. Warm water outflow was discarded down the drain. Cold water outflow emptied into a return bucket. The return bucket had a pump, operated by a float switch in it which returned water to the cold reservoir.

The thermal gradient was established for 6 h prior to trials. Thermal preference trials lasted for 3 h. Octopuses were placed into the middle of the holding tank at the beginning of the trial at the median temperature. Six Vernier stainless steel temperature probes were placed equidistantly throughout the lid of the holding tank with three probes on either end of the tank connected to one of two LabQuest Mini multiple sensor interfaces. Both LabQuest Mini Ports were connected to a wireless USB hub connected to a laptop computer outside of the testing room. Temperature was recorded 10 times per minute using Vernier Logger Lite. Position of octopuses in the holding tank was tracked using an overhead Airlink 101 350k USB 2.0 webcam connected to a laptop computer and mounted to a curtain rod which allowed for a full overhead view of the holding tank. Images were captured from the Airlink 101 350k USB 2.0 webcam every 20 s using FFmpeg. The lighting for thermal preference trials was the same as during movement rate trials to allow for accurate tracking of the octopuses within the gradient by web cam. The position of the octopus was determined using time-lapse photos and the MTrack2 plugin in FiJI. Linear interpolation was used to estimate the temperature of the water the octopus selected using data from the temperature probes and the position of the octopus.

Data Analysis and Statistics

Movement rate data were categorized by temperature. Temperature was placed into data bins in intervals of 0.1°C from the minimum temperature to the maximum

temperature for each trial (Figure 4A). Data from each temperature bin were selected if the number of data points in each bin was greater than a cutoff percentage of the total number of data points (Figure 4B). Cutoff percentages were chosen for each trial by visual inspection of the histogram and based off of the lowest percentage for which the data would be separated into the five temperature treatments. (range from 0.45 to 0.9% of total data points). Selected temperature data (Figure 4C) were partitioned into the five temperature treatments using the kmeans function in R, with the MacQueen algorithm (Figure 4D). Mean for temperature and movement were calculated for each temperature treatment. Overall movement rate was compared for each temperature treatment using a one-way repeated measures ANOVA.

Respirometry experiments data was selected and partitioned by temperature as in movement rate experiments. This was done to eliminate the temperatures of transition between the treatments and to capture the range of temperatures for each temperature treatment. Q10 of octopuses was calculated using the mean temperature and respiration rate from the first and fourth temperature treatments.

Temperature selection of octopuses was compared in thermal preference trials. Octopuses preferred corners of the tank, and true temperature preference could not be determined because octopuses showed a strong preference for the corners of the aquarium. The temperature gradient was split into two categories, warm and cold, based on the median temperature for each trial. The median temperature was used to divide the holding tank into two equal spatial regions with two corners each. A binomial test was used to determine if there was a significant difference in what side of the holding tank the octopus selected.

Results

Movement Rate

I measured movement of 15 *Muusoctopus leioderma* in temperature controlled enclosures over the course of 10 hours at five different temperature treatments (targeted temperatures: 13, 11, 9, 7, and 4°C). The mean temperatures for the five treatments were 12.9, 10.7, 8.5, 5.7 and 3.6°C. Movement increased with increasing temperature, with the greatest movement at 12.9°C and the lowest at 5.7°C (Figure 5). Movement at 12.9°C was significantly greater than all other temperature treatments other than 10.7°C (One-way repeated measures ANOVA, Tukey post-hoc test, $df = 14$, $p\text{-value} = 0.0002$).

Metabolic Rate

I measured oxygen consumption of 11 *M. leioderma* in respirometers over the course of 13 h at four different temperature treatments (12.6, 10.7, 8.8, and 6.9°C). Five octopuses were excluded from analysis; three of them were excluded because of unrealistic routine metabolic rates (mean metabolic rate at any one temperature was less than 0 or greater than $20 \mu\text{molO}_2 \text{ g}^{-1} \text{ hr}^{-1}$), one was excluded because the octopus died in the respirometer, and one due to poor temperature control in the respirometer (temperature range from 12-18°C). The metabolic rate of the remaining 6 octopuses generally increased with increasing temperature (Figure 6). Metabolic rates changed as a function of temperature less than movement rates as a function of temperature (Figure 7). The mean metabolic rates for the remaining 6 octopuses ranged from $0.5 \mu\text{molO}_2 \text{ g}^{-1} \text{ hr}^{-1}$ and $3.2 \mu\text{molO}_2 \text{ g}^{-1} \text{ hr}^{-1}$ and the mean temperatures ranged from 5.6 to 13.5°C. These rates are comparable to those found in a review of metabolic rates for nine species of

benthic octopuses, which ranged from 0.42-3.38 $\mu\text{molO}_2 \text{ g}^{-1} \text{ hr}^{-1}$ (Seibel & Childress, 2000). Metabolic rates observed for the final resting at 9°C period were not significantly different than those during the initial 9°C period, so order of temperatures exposed is unlikely to have influenced the results (paired t-test, p-value = 0.1). The Q_{10} of individual octopuses ranged between 1.61 and 5.86 (Table 1).

Thermal Preference

Thermal preference of 15 *M. leioderma* was measured in a temperature gradient over the course of 3 h (Figure 8). Position of octopuses throughout the holding tank varied between trials, with some showing a preference for corners while others constantly moved throughout the tank. There did not seem to be a relationship between amount of movement of an octopus in the thermal gradient and its preference for the cold or warm side. Significantly more octopuses spent the majority of their time on the side colder than the median than on the side warmer than the median ($9.3 \pm 1.4^\circ\text{C}$, Figure 3) of the thermal gradient (Binomial test, n=15, cold side =12, warm side=3, p-value = 0.035).

Discussion

Thermal Preference

In the laboratory thermal preference experiments *Muusoctopus leioderma* showed a preference for cooler temperatures than temperatures recorded by dive computers at their Burrows Bay collection location (Figure 8). These results match the prediction that the octopuses would be best acclimated to, and thus prefer cooler temperatures than those measured in Burrows Bay based on the hypothesis that these octopuses are recent arrivals

to shallow water. If the octopuses had been long term inhabitants of Burrows Bay, then a temperature preference not approximating the collection location would be surprising.

Movement Rate and Metabolic Rate

Muusoctopus leioderma had significantly greater movement at 12.9°C than at all temperatures colder than 10.7°C (Figure 5) and movement rate changed as a function of temperature more than metabolic rate as a function of temperature (Figure 7), which suggests either elevated movement at high temperatures or reduced movement at low temperatures. However, these data alone do not clarify whether octopuses have low movement at cooler temperatures or elevated movement at warmer temperatures. When combined with the results demonstrating preference for temperatures lower than 9.3°C, this suggests elevated movement at 12.9°C, which perhaps could be a thermoregulatory response.

It could be that all temps are equally suboptimal, or that the temperature increments were not fine enough to fully detect the octopuses optimal temperature, however if even the optimal temperature was not found there should still be a hierarchy of more suitable temperatures. Previous studies have been able to quantify activity and movement of *Enteroctopus dofleini* by using sonic transmitters to track octopuses in the wild (Hartwick et al., 1984; Mather et al., 1985; Scheel & Bisson, 2012), but it is difficult to compare movement rates in this study to these previous studies as *M. leioderma* is much smaller than *E. dofleini*.

If the Burrows Bay population of *M. leioderma* had been long-term inhabitants of Burrows Bay and been previously unnoticed, then I would expect them to prefer

temperatures consistent with the collection location. However, the preference for temperatures cooler than those measured at the collection location is consistent with the hypothesis that the *M. leioderma* found in Burrows Bay have recently expanded into shallow water.

Range Expansion

Several studies have suggested recent global cephalopod population expansions as evidenced by reports of increased cephalopod landings from cephalopod fisheries, and geographic range expansion (Caddy & Rodhouse, 1998; Doubleday et al., 2016; Ramos et al., 2014; Zeidberg & Robison, 2007). The Burrows Bay population of *M. leioderma* may illustrate a depth range expansion for this octopus species instead of a geographic range expansion or a population expansion. This phenomenon is not limited to cephalopods, as range expansions have occurred in other taxa, including bivalves, echinoderms, and algae (Krug, Jablonski, & Valentine, 2008; Ling, 2008; Lyons & Scheibling, 2009).

A range shift in marine organisms may occur as a result of biotic factors such as prey abundance, reduction of predators, and reduced competition. Population and range expansion of cephalopods may be influenced by depletion of fish stocks resulting in reduced predation and competition, allowing them to expand to areas that were previously high risk (Caddy & Rodhouse, 1998). This depth range expansion may be in response to reduced predation and competition from groundfish, as groundfish landings have recently declined in the Pacific Northwest. (Caddy & Rodhouse, 1998; National Oceanographic and Atmospheric Administration, 2012)

Range shifts in cephalopods could also be attributed to abiotic factors such as temperature, salinity, pH, and oxygen, which are associated with anthropogenic climate change. Decreases in pH are more dramatic at shallow depths, as anthropogenic CO₂ concentrations increase inversely to depth (Sabine et al., 2004). Therefore, deep water species such as *M. leioderma* would be more likely to move deeper rather than shallower in response to anthropogenic pH change.

Changing temperatures can also impact cephalopod distributions by influencing reproductive success or fitness of paralarvae (Higgins et al., 2012; Juárez et al., 2015). Increasing ocean temperatures have been implicated in geographic range expansion in several cephalopod species including *Octopus tetricus*, *Loligo forbesii*, *Loligo vulgaris* and *Alloteuthis subulata* (Ramos et al., 2014; van der Kooij et al., 2016). The recent polewards range shift of *Octopus tetricus* to the coastal waters of southeastern Australia may be related to the southern extension of the warm East Australian Current (Ramos et al., 2014). Warming sea surface temperatures of 1.7°C in the North Sea over the past 35 years has been positively correlated with geographic range expansion of *L. forbesii*, *L. vulgaris* and *Alloteuthis subulata*, with *L. forbesii* and *L. vulgaris* shifting southward and *A. subulata* shifting northward (van der Kooij et al., 2016).

Although changes in temperature are largely associated with latitude shifts, there have been reports of fish moving deeper in response to warming ocean temperatures (Perry et al., 2005). While changing temperatures are associated with both latitudinal and depth range expansion in marine organisms, it is unlikely that the depth range expansion into shallow water of *M. leioderma* was influenced by warming temperatures. Shallow water is warming to a greater degree than deep water (Levitus et al., 2012), thus a deep

water species such as *M. leioderma* would be more likely to move deeper rather than shallower in response to warming ocean temperatures.

Another abiotic factor that may influence geographic ranges in marine organisms is dissolved oxygen. Low oxygen can prevent some organisms from using a certain area, and this may allow others to take advantage of those areas. One example of oxygen playing a role in changes of cephalopod geographic range is that of *Dosidicus gigas*. *Dosidicus gigas* will suppress its metabolic rate at low oxygen levels, allowing this species to inhabit areas of the water column that have low oxygen levels and are therefore limiting to the distribution of other marine organisms (Gilly et al., 2006; Rosa & Seibel, 2008). *Dosidicus gigas* has extended its range northward in the eastern North Pacific, likely in response to the expansion of hypoxic conditions, as they may prohibit competing top predators (Rosa & Seibel, 2010; Zeidberg & Robison, 2007). Between 2004 and 2014 low dissolved oxygen events in the Salish Sea have been increasingly common, which could possibly induce distribution changes of organisms in the area (Moore et al., 2015; Roberts et al., 2014).

Changes in water current may also influence range expansion by effecting the settling of larva. It seems unlikely that changes in currents could influence the depth distribution in *M. leioderma* because they most likely have benthic young as the most closely related species to *M. leioderma*, *Octopus californicus*, is known to have benthic young (Hochberg, 1998; Kore et al., in review).

Depth Shift in M. leioderma

The unique nature of this depth shift in *M. leioderma* makes it difficult to determine the exact cause(s) for the appearance of this octopus species in Burrows Bay. The previously known depth range for *M. leioderma* (90 to 1400 m, most abundant between 450 to 600 m) has made it difficult to study this octopus species, however the depth shift may allow for a closer look into the life history and physiology of this octopus. Physiological studies should concentrate on the influence of environmental factors such as temperature and pH on metabolic processes. The depth shift of *M. leioderma* may not be limited to Burrows Bay, thus further investigations should include cephalopod surveys of Puget Sound and other areas of the Pacific Northwest to look for other shallow water populations of *M. leioderma*.

The distribution of a marine organism is influenced by both biotic and abiotic factors. Changes in one or both of these major factors may result in a shift in distribution. In the case of the depth range expansion of *M. leioderma* it is most likely that biotic factors had a more prominent role than did abiotic factors. The response of a deep water species to changes in pH and temperature would be more likely to move deeper rather than shallower. However, the Burrows Bay population of *M. leioderma* may have shifted from deep water to shallow water, suggesting this depth change is not influenced by pH and temperature, but could instead be influenced by biotic factors. Biotic factors such as reduction in predation and competition may have allowed *M. leioderma* to expand its range to areas that were previously high risk.

Conclusions

Overall, these results suggest that *M. leioderma* has a preference for temperatures that are more similar to those found at depth (90 to 1400 m), and *M. leioderma* increases its movement at shallow water temperatures. In conclusion these results suggest that the Burrows Bay population of *M. leioderma* may be recent immigrants to shallow water.

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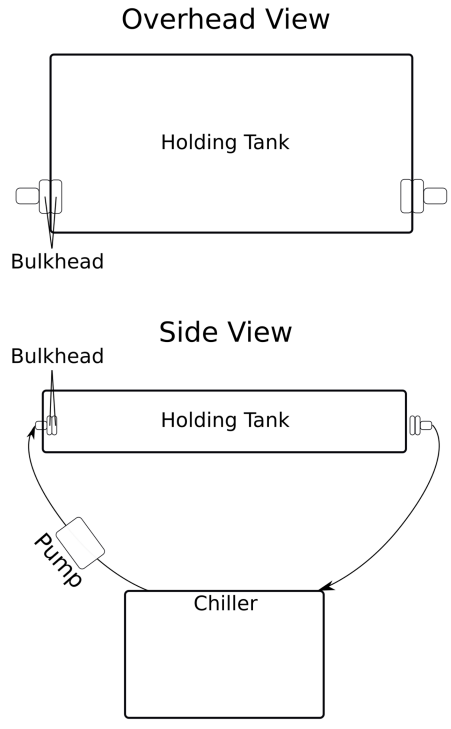


Figure 1: Design of holding tank used for movement rate trials.

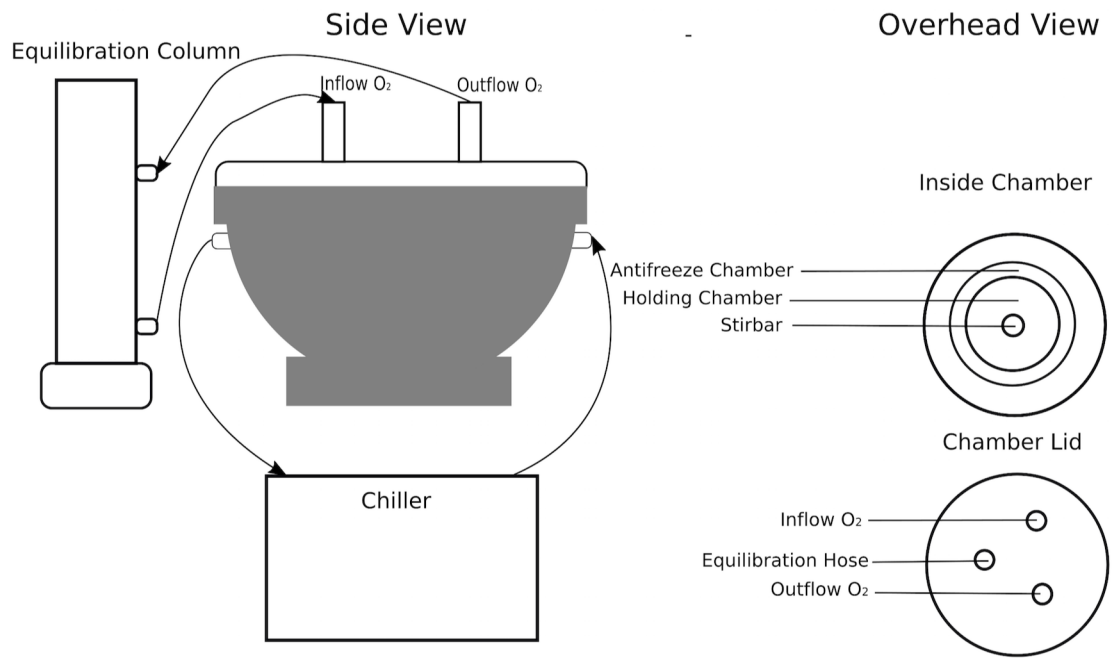


Figure 2: Design of open respirometry system used for metabolic rate trials.

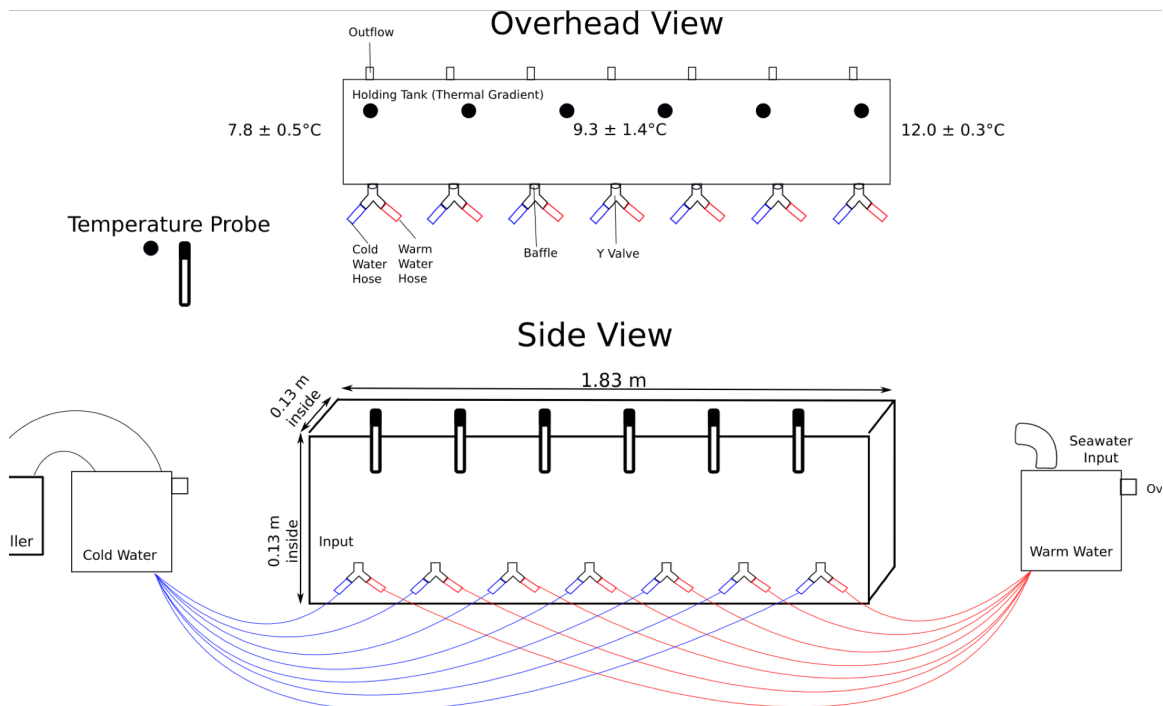


Figure 3: Thermal gradient tank design. The blue lines represent cold water hoses and the red lines represent warm water hoses. The average temperature on the cold side of the thermal gradient was $7.8 \pm 0.5^\circ\text{C}$, the median was $9.3 \pm 1.4^\circ\text{C}$ and the warm side was $12.0 \pm 0.3^\circ\text{C}$.

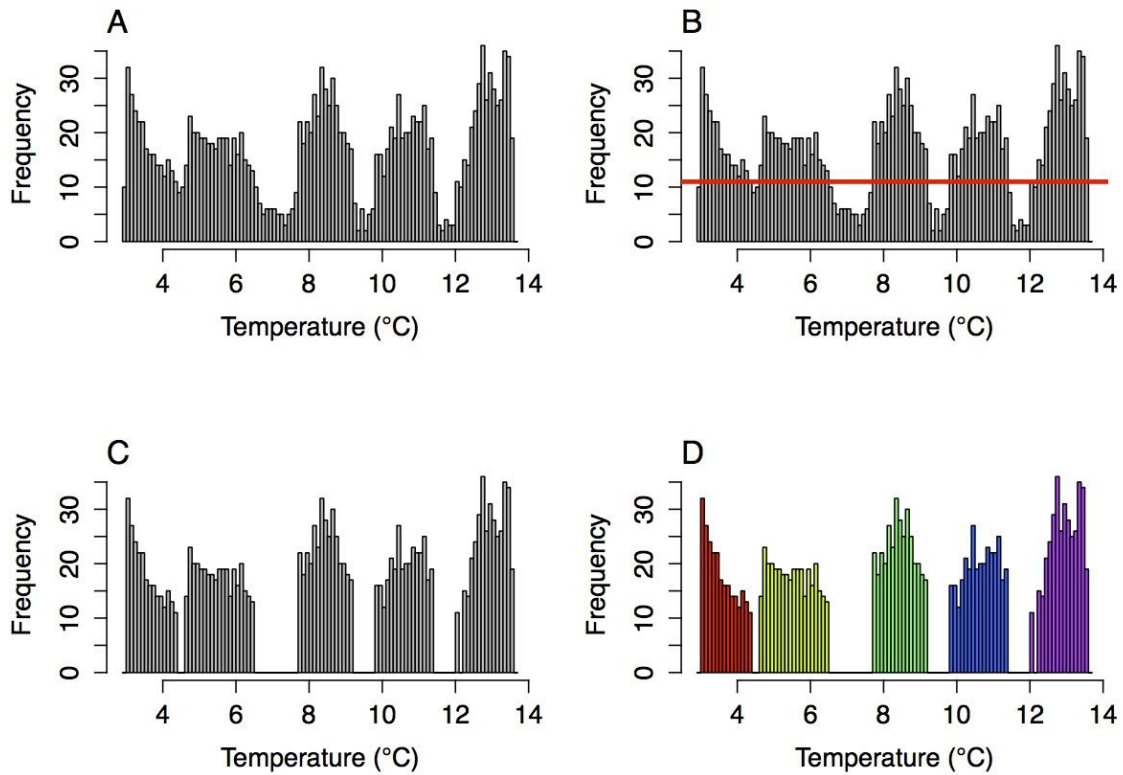


Figure 4: Stepwise illustration of how data were selected and partitioned by temperature for movement rate and respirometry experiments. Frequency, or the height of the bars represents the number of times a temperature occurred per frame. **A:** Temperature data was sorted into 0.1°C bins. **B:** Then all data in a bin were selected if the number of data points in each temperature bin was greater than a cutoff percentage (range from 0.45 to 0.9% of total data points) of the total number of data points (red line). **C:** Selected temperature data **D:** kmeans function in R, with the MacQueen algorithm was used to partition the selected temperature data into the five temperature treatments.

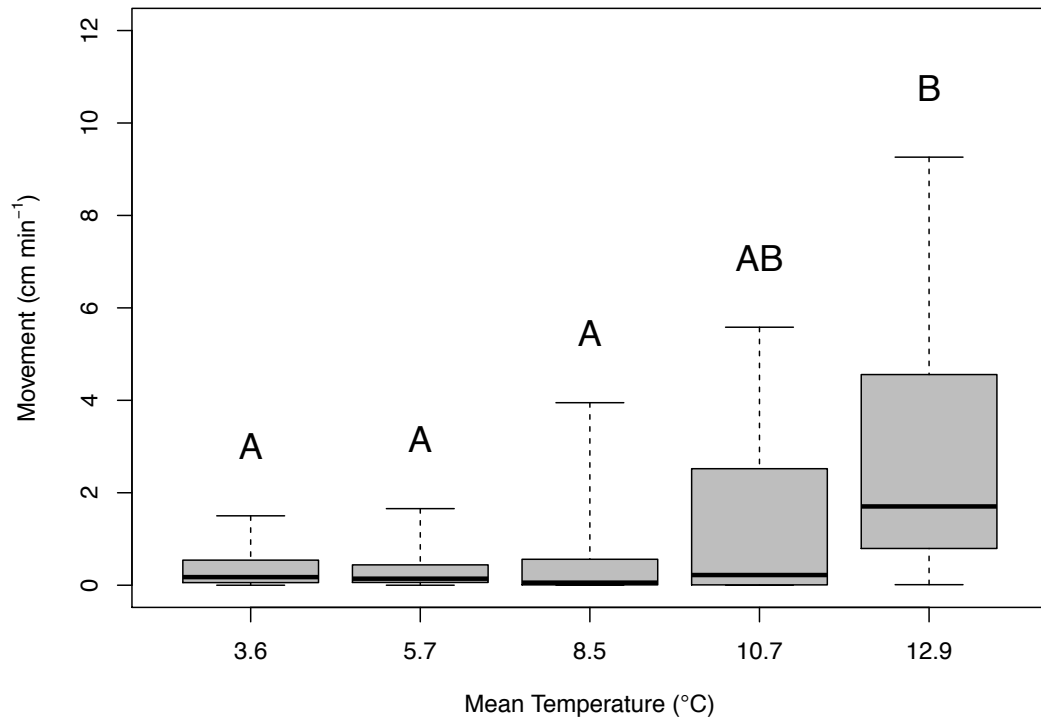


Figure 5: Effect of temperature on movement rates of *M. leioderma*. Movement at 12.9°C was significantly greater than at other temperature treatments except 10.7°C (One-way repeated measures ANOVA, Tukey post-hoc test, n = 15, df = 14, p-value = 0.0002). Means with the same letter are not statistically different. Dark black line represents the median, upper box indicated the first quartile, lower the third quartile, and whiskers indicate the most extreme data points within the normal range (1.5x the interquartile region)

Table 1: Q_{10} values of respiration rates for 6 *M. leioderma*. Q_{10} of octopuses was calculated using the mean temperature and respiration rate from the first and fourth temperature treatments (13-7°C).

| Octopus | Q_{10} |
|---------|----------|
| L003 | 5.86 |
| L010 | 4.68 |
| M003 | 2.52 |
| M004 | 1.61 |
| M007 | 1.69 |
| M008 | 1.61 |

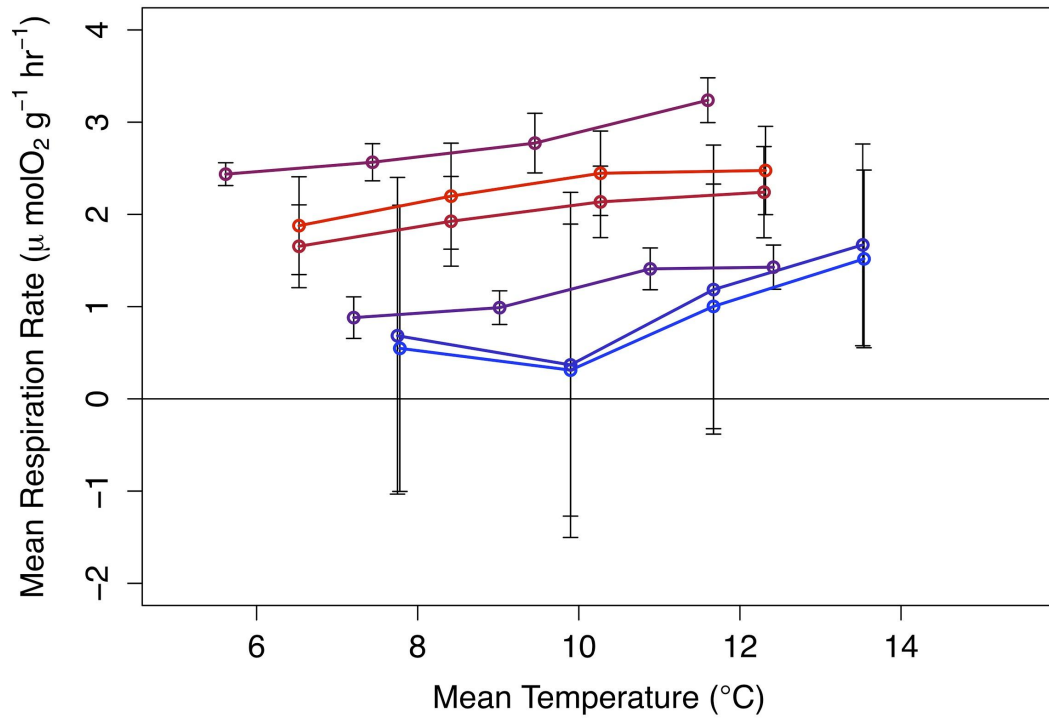


Figure 6: Effect of temperature on respiration rates of *M. leioderma* (n=6). Each colored line represents an individual octopus and each dot represents the mean respiration rate at each of the four temperature treatments. Error bars are shown as black vertical lines.

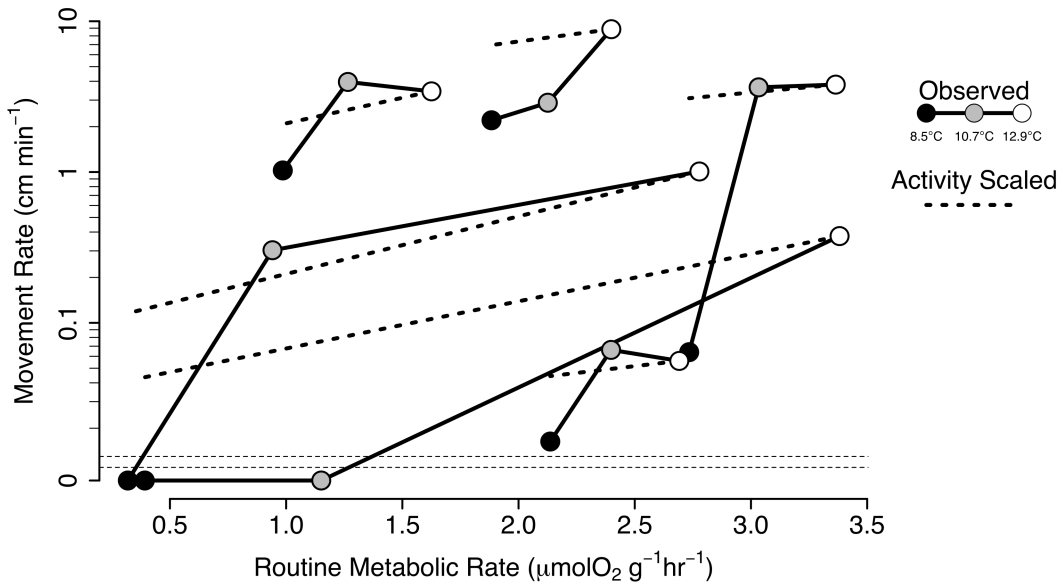


Figure 7: Effect of routine metabolic rate on movement rate of *M. leioderma* (n=6). Each solid line connecting three points represents one octopus. The shade of the points indicate different temperature treatments (black = 8.5°C, gray = 10.7°C, white = 12.9°C). Each solid line represents the observed trends in movement rate at different temperatures and the dashed line represents scaled trend in movement rate if it is proportional to routine metabolic rate. Metabolic rates were interpolated between measured points based on the Q₁₀ found in respirometry trials.

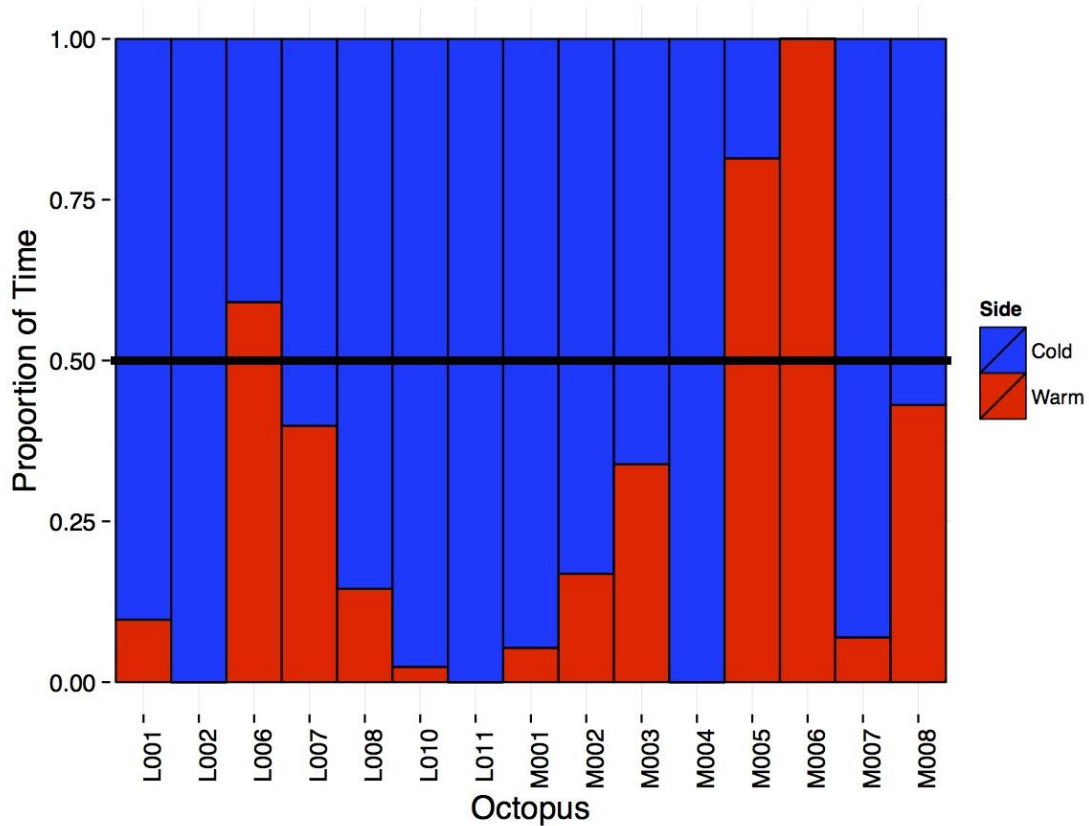


Figure 8: The proportion of time octopuses spent on either the warm (red) or cold (blue) side of the thermal gradient for each trial. The temperature gradient was split into two categories, warm and cold, based on the median temperature for each trial. Each octopus is represented with a column. The black line indicates half of the trial duration. Significantly more octopuses spent the majority of their time on the cold side (12) than the warm side (3) of the thermal gradient (Binomial test, $n=15$, $p\text{-value} = 0.035$).