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Thermosensitivity of the Lobster, *Homarus americanus*, as Determined by Cardiac Assay

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Abstract. It is generally accepted that crustaceans detect, and respond to, changes in water temperature, yet few studies have directly addressed their thermosensitivity. In this investigation a cardiac assay was used as an indicator that lobsters (*Homarus americanus*) sensed a change in temperature. The typical cardiac response of lobsters to a 1-min application of a thermal stimulus, either warmer (n = 19) or colder (n = 17) than the holding temperature of 15 °C, consisted of a short bradycardia (39.5 ± 8.0 s) followed by a prolonged tachycardia (188.2 ± 10.7 s). Lobsters exposed to a range of rates of temperature change (0.7, 1.4, 2.6, 5.0 °C/min) responded in a dose-dependent manner, with fewer lobsters responding at slower rates of temperature change. The location of temperature receptors could not be determined, but lesioning of the cardioregulatory nerves eliminated the cardiac response. Although the absolute detection threshold is not known, it is conservatively estimated that lobsters can detect temperature changes of greater than 1 °C, and probably as small as 0.15 °C. A comparison of winter and summer lobsters, both held at 15 °C for more than 4 weeks, revealed that although their responses to temperature changes were similar, winter lobsters (n = 18) had a significantly lower baseline heart rate (34.8 ± 4.4 bpm) and a shorter duration cardiac response (174 s) than summer lobsters (n = 18; 49.9 ± 5.0 bpm, and 320 s respectively). This suggests that some temperature-independent seasonal modulation of cardiac activity may be occurring.

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Introduction

Temperature is one of the most important and pervasive environmental influences on the American lobster, *Homarus americanus* (Cobb and Phillips, 1980; Aiken and Waddy, 1986; Factor, 1995). It is generally accepted that locomotory activity in this species is temperature dependent (McLeese and Wilder, 1958; Reynolds and Casterlin, 1979; Haakonsen and Anoruo, 1994) and that it carries out seasonal inshore to offshore migrations to gain the developmental benefits of warmer coastal temperatures in the spring and summer (Cooper and Uzmann, 1971; Pezzack and Duggan, 1986; Karnofsky *et al.*, 1989; Haakonsen and Anoruo, 1994; Factor, 1995; Watson *et al.*, 1999). Laboratory studies have demonstrated that *H. americanus* has a thermal preference of about 16 °C (Reynolds and Casterlin, 1979; Crossin *et al.*, 1998), and it has been proposed that behavioral thermoregulation may allow members of the species to occupy thermal niches which maximize their metabolic or behavioral efficiency. The behavioral responses of lobsters to thermal gradients suggest they have some mechanism to *sense* temperature so that they may effectively respond to the thermal properties of their environment.

Thermosensitivity in lobsters may be mediated by distinct thermoreceptors or thermosensitive neurons as in some other invertebrates (Prosser and Nelson, 1981; Mori and Ohshima, 1995). Although behavioral studies strongly suggest that *H. americanus* can sense temperature (Reynolds and Casterlin, 1979; Crossin *et al.*, 1998), to our knowledge only one study has addressed how neurons respond to changes in temperature in this species. In that study, firing of cells associated with thoracic ganglia connectives generally showed no spontaneous activity below 14 °C, but most became spontaneously active above this temperature. Interestingly, these cells "cycle reversibly from silent to continuously active to bursting and back as the temperature is

increased and decreased" (Konishi and Kravitz, 1978). Other than these cells, which may or may not play a role in thermally guided behaviors, we know little about the location of putative thermoreceptors, or the mechanisms used to detect temperature, in lobsters and most other crustaceans (Dorai Raj and Murray, 1962; Ache, 1982).

In situations where the precise receptors have not been identified, or are not readily accessible to electrophysiological investigation, cardiac assays are a valuable tool for preliminary investigations of sensory capabilities (Larimer, 1964; Offutt, 1970; Florey and Kriebel, 1974; Dufort, 1997). For example, many crustaceans exhibit a drop in heart rate in response to novel stimuli (Maynard, 1960; Larimer, 1964; McMahon and Wilkens, 1972; DeWachter and McMahon, 1996). This cardiac response has been used to measure the ability of *H. americanus* to detect sound (Offutt, 1970) and salinity (Dufort, 1997). Although a number of studies have addressed the effect of temperature on decapod heart rates at time scales ranging from hours to days (Ahsanullah and Newell, 1971; Florey and Kriebel, 1974; DeFur and Magnum, 1979; DeWachter and McMahon, 1996; DeWachter and Wilkens, 1996; Hokkanen and Demont, 1997), few have characterized the initial response (*i.e.*, <5 min.) to brief changes in water temperature. The present study used a cardiac assay to demonstrate that American lobsters are consistently capable of sensing increases or decreases in temperature that are greater than 1 °C. The typical response elicited by both cold and warm stimuli was a brief slowing of the heart rate, followed by prolonged cardioacceleration. Winter and summer lobsters responded somewhat differently to thermal stimuli, suggesting some type of seasonal temperature-independent modulation of their responsiveness to thermal stimuli.

Materials and Methods

Animals

Adult (82–92 mm carapace length), intermolt lobsters were held at 15 ± 1 °C (salinity 30 ± 1 ppt) for more than 4 weeks prior to use, and experiments were initiated at this temperature. All lobsters were captured from coastal New Hampshire waters, and experiments were conducted at the University of New Hampshire, Durham, New Hampshire. Experiments were carried out in both summer and winter under ambient light conditions. In the summer, the thermosensitivity of 18 lobsters was determined (cold stimuli, n = 9; warm stimuli, n = 9); in the winter, lobsters kept at the same temperature (15 °C) as summer lobsters were used in identical experiments (cold stimuli, n = 8; warm stimuli, n = 10).

Recording of temperature and heart activity

Small wire electrodes were inserted through the dorsal carapace above the heart and used with a UFI impedance converter (model #2991) to record heart rate (Dyer and Uglow, 1970). Because the impedance recording technique can be sensitive to temperature, the method was verified by using a second pair of electrodes and a Grass model 7D polygraph to simultaneously monitor the electrical activity associated with lobster heart contractions (see Watson and Wyse, 1978; Watson, 1980). External temperature was recorded using a small (3 mm × 1 mm) thermistor (C & B Sciences/iWorx, Inc., Dover, NH) placed on the dorsal carapace. The thermistor was calibrated weekly over the range of temperatures used in the experiments. The time constant of the thermistor was 2.0 s (time to achieve 67% of the final response). The absolute resolution of the thermistor was ±0.15 °C, but it could accurately detect *changes* in temperature as small as 0.01 °C. However, because of turbulent mixing within the recording chamber, the slight time delay due to the time constant of the thermistor (Fig. 1), and the unknown location of temperature-sensitive neural elements relative to the location of the thermistor, it was not possible to assess the thermal detection threshold with great accuracy. All temperatures presented are those recorded by the externally located thermistor above the dorsal carapace. These should be interpreted conservatively, in the context of the methods used and the unknown location of the sensory receptors.

Experimental chamber

After insertion of the electrodes, lobsters were placed in a recording chamber consisting of an 18-cm-diameter PVC pipe covered on the top and bottom by perforated plates through which seawater (temperature 15 ± 1 °C) continuously flowed (Fig. 1). This arrangement kept lobsters relatively immobile and ensured that changes in temperature within the recording chamber were rapid and relatively homogeneous. The chamber was placed in an acrylic plastic insert (30 × 30 × 30 cm) that was immersed in a temperature-controlled 120-l aquarium (the ambient bath). Ambient seawater was continuously pumped (2 l/min) from the aquarium through the recording chamber, into the insert, and back to the aquarium. Thermal stimuli were delivered by switching the source of seawater from the ambient bath to the stimulus bath. This switching was accomplished by turning a stopcock and was considered the initiation of the stimulus (see arrows in Fig. 2). The stimulus bath was filled from the ambient bath to minimize novel chamber chemosensory cues (Fig. 1) and brought to the appropriate experimental temperature using aquarium heaters or cooling coils. The recording chamber was covered with black plastic to minimize visual disturbance, and the lobster was left in the experimental apparatus overnight before an experiment. Lobsters are much more sensitive to stimuli if allowed to recover from electrode insertion and become accustomed to the recording chamber (Larimer, 1964; Dufort, 1997).

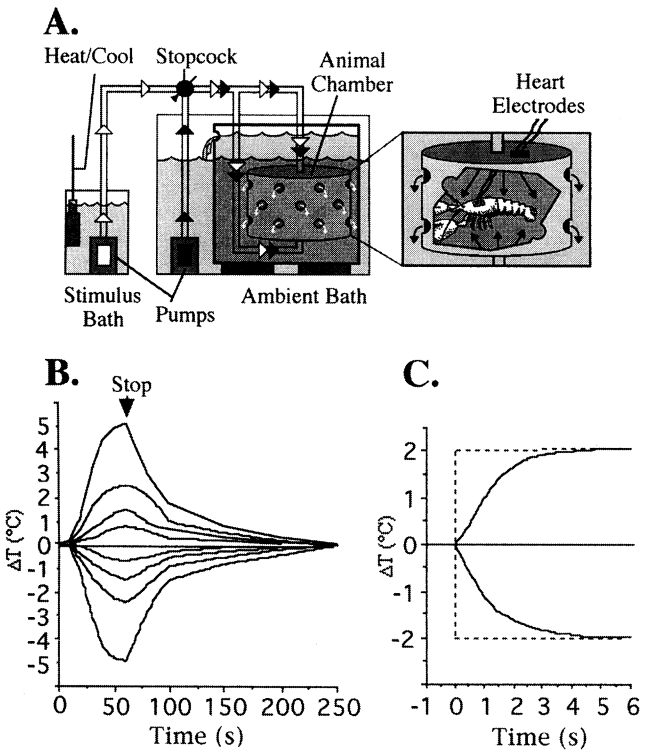

Figure 1. Experimental apparatus used to record lobster cardiac responses to changes in temperature. (A) Seawater (15 °C) flows continuously from the ambient bath into the animal chamber through perforated plates located above and below the lobster (direction of flow indicated by dark arrows). Heart rate is recorded before, during, and after exposure to a temperature stimulus. Switching the stopcock changes the source of seawater from the ambient bath to seawater from the stimulus bath (direction of flow indicated by white arrows). A thermistor on the dorsal carapace is used to monitor temperature during each trial. (B) Rates of temperature change in a typical experiment in response to 1 min stimuli (turned on at time = 0 and off at arrow) of ± 0.7, 1.4, 2.6, and 5.0 °C warmer or colder than the ambient temperature. (C) Time constant of the thermistor when exposed to a step change in temperature of ± 2 °C (dotted line). The estimated time to achieve 67% of final temperature is 2.0 s.

The following day, after basal heart rate was measured for at least 30 min, each animal was exposed for 1 min to a warm or cold stimulus that changed the temperature in the recording chamber at a rate of ±0.7 °C/min. This was followed by stimuli delivered at targeted rates of ±1.5 °C/min, ±2.5 °C/min, and ±5.0 °C/min for 1 min. Temperature was allowed to return to ambient (Fig. 2) between each treatment. Treatments were separated by at least 30 min. The temperature in the recording chamber was monitored with the dorsally located thermistor, and the actual mean rates achieved were 0.72 ± 0.04, 1.37 ± 0.06, 2.61 ± 0.10, and 4.95 ± 0.16 °C/min. Thus, the average maximum warm stimuli after 60 s were 15.7, 16.4, 17.6, and 20.0 °C, and the maximum cold stimuli were 14.3, 13.6, 12.4, and 10.0 °C.

Which stimulus (warm or cold) was tested on the first day was assigned randomly, and the other set of stimuli (warm or cold) were tested on the following day. A 25% change in heart rate—bradycardia (decrease) or tachycardia (increase)—was used as an indicator that lobsters sensed a change in water temperature (Offutt, 1970; Dufort, 1997). All records were digitized using a MacLab system (C & B Sciences/iWorx, Inc.) and were analyzed to determine the following: (1) delay to a response; (2) duration of bradycardia, tachycardia, or both; (3) heart rate (bpm) during bradycardia; and (4) heart rate (bpm) during tachycardia. In addition, thermosensitivity thresholds were estimated from the water temperature measured above the dorsal carapace at the time of the initial cardiac response. Controls were conducted before any thermal stimuli were applied; the same protocol described above was followed, but without changing the temperature in the stimulus bath.

Localization of putative temperature receptors

In an attempt to localize regions with putative temperature receptors, lobsters missing antennae (n = 4) or missing antennae and antennules (n = 4) were tested for a response to a temperature change of +2.5 °C/min. Antennae or antennules were removed bilaterally at their base, the wounds were sealed with wax to prevent blood loss, and the lobsters were allowed more than 24 h to recover.

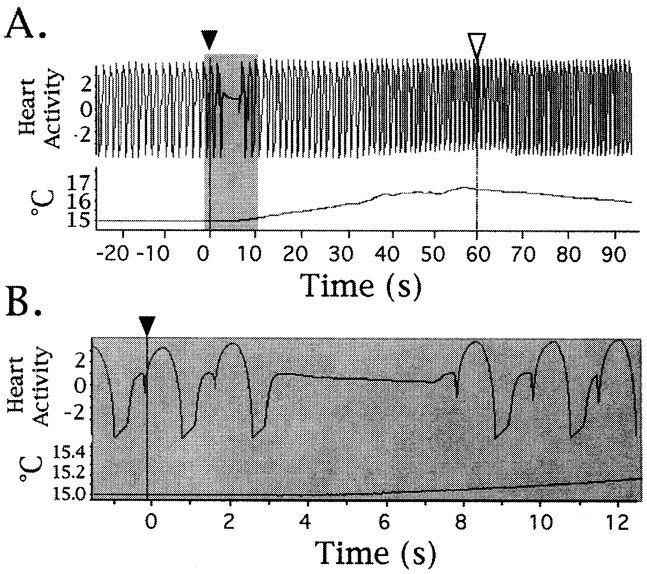

Figure 2. Typical cardiac response to a change in temperature. (A) The top trace shows the typical response to a +1.4 °C/min stimulus; the lower trace is a plot of the temperature change during the 60-s trial. The dark closed arrow shows when the stimulus flow was turned on, and the white open arrow shows when it was turned off. (B) An enlargement of the highlighted area from (A), showing the time course of the bradycardia and associated temperature change. Note that the rapid response may be a result of the combination of the location of the thermistor relative to the location of the unknown temperature sensitive receptors and the slight delay due to the time constant of the thermistor. There was no response to controls when the flow was switched but the temperature was not changed.

To determine whether changes in cardiac activity were mediated by the cardioregulatory nerves, responses to thermal stimuli were measured before and after nerve lesions (n = 5). Changes in heart rate were initially recorded in response to thermal stimuli of +1.5 °C/min and −1.5 °C/min. Then the cardioregulatory nerves were cut, and lobsters were allowed at least 2 days to recover. Finally their cardiac responses were measured again in response to the same stimuli that were applied before the lesions. Lesions were made as described in Guirguis and Wilkens (1995). A small (3-cm^2) rectangular piece of dorsal carapace just above the heart was removed, and superficial cuts were made with fine scissors through the connective tissue along the border of the opening. The shell was then replaced and fastened in place with tape. Sham-operated control animals (n = 4) were treated in the same manner except that no cuts were made in the connective tissue.

Statistical analysis

Throughout the text, variation is presented as standard error of the mean (*i.e.*, mean ± SEM). A P value of <0.05 was considered to be significant for all statistical tests.

Results

Typical response to a change in temperature

The typical cardiac response to both warm and cold stimuli consisted of a short bradycardia (39.5 ± 8.0 s), followed by a significantly (paired t test) longer tachycardia (188.2 ± 10.7 s; Fig. 2). In general, changes in heart activity were similar in response to both warm (n = 19) and cold (n = 17) stimuli. Although the intensity and duration of cardiac responses were similar for all temperatures tested (ANOVA, $P > 0.05$), some lobsters did not respond to slower rates of change (0.7 and 1.4 °C/min), whereas almost all lobsters responded to the maximum rate of change (5.0 °C/min; Fig. 3). There was no cardiac response in control trials (n = 36), where temperature was not changed but ambient water was pumped through the chamber from the stimulus bath (Fig. 4).

Sensitivity to warm and cold stimuli

Lobsters were extremely sensitive to both warm and cold stimuli (Fig. 3). For example, when subjected to a +2.6 °C/min stimulus, lobsters responded after just 3.8 ± 0.5 s, when the temperature in the chamber had changed by only 0.09 ± 0.04 °C. Lobsters exposed to the −2.6 °C/min stimulus responded after a drop of only 0.13 ± 0.09 °C, and the latency to respond (4.6 ± 1.8 s) was not significantly different (paired t test) than during a warm stimulus (Fig. 3).

Temperature change measured at the initiation of a cardiac response by individual lobsters ranged from 0.01 to 0.79 °C. There were no significant differences (unpaired t test) between the sexes in the temperature change at initial response; when lobsters responded, they exhibited comparable thresholds, at all measured rates of change (Fig. 3, Kruskal-Wallis test). The average temperature-detection threshold, for all trials in which animals responded, was 0.15 ± 0.03 °C. This is considered to be only an estimate because of the inherent time constant and resolution of the thermistor, the flow of water in the chamber, and the location of the thermistor relative to the still *unknown* location of the receptors mediating the response. Nonetheless, this assay demonstrates that lobsters are sensitive to very small changes in temperature.

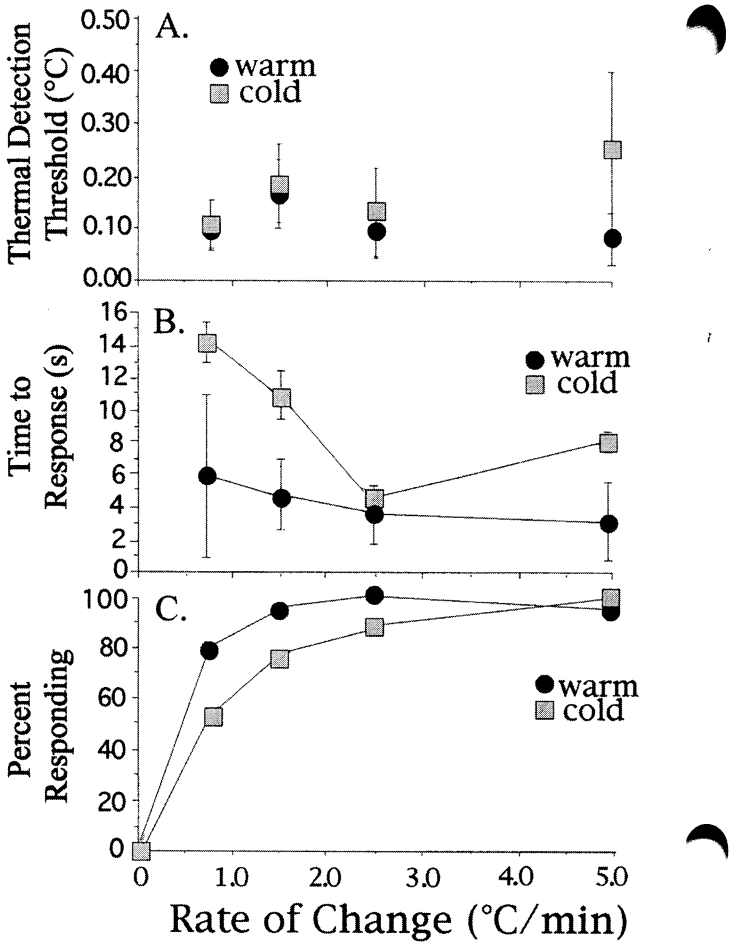

Figure 3. Responses to thermal stimuli at different rates of change. (A) The thermal detection threshold, or the amount of temperature change required to elicit a cardiac response, was similar even when hot and cold stimuli were applied at different rates. (B) When thermal stimuli were applied at slow rates of change, the delay to respond was longer, especially in the case of cold stimuli. (C) Although lobsters responded similarly to thermal stimuli applied at fast and slow rates of change, some animals did not respond at all to slow rates of change, while all animals responded to higher rates of change.