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Detection of Salinity by the Lobster, *Homarus americanus*

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Abstract. Changes in the heart rates of lobsters (*Homarus americanus*) were used as an indicator that the animals were capable of sensing a reduction in the salinity of the ambient seawater. The typical response to a gradual (1 to 2 ppt/min) reduction in salinity consisted of a rapid increase in heart rate at a mean threshold of 26.6 ± 0.7 ppt, followed by a reduction in heart rate when the salinity reached 22.1 ± 0.5 ppt. Animals with lesioned cardioregulatory nerves did not exhibit a cardiac response to changes in salinity. A cardiac response was elicited from lobsters exposed to isotonic chloride-free salines but not to isotonic sodium-, magnesium- or calcium-free salines. There was little change in the blood osmolarity of lobsters when bradycardia occurred, suggesting that the receptors involved are external. Furthermore, lobsters without antennae, antennules, or legs showed typical cardiac responses to low salinity, indicating the receptors are not located in these areas. Lobsters exposed to reductions in the salinity of the ambient seawater while both branchial chambers were perfused with full-strength seawater did not display a cardiac response until the external salinity reached 21.6 ± 1.8 ppt. In contrast, when their branchial chambers were exposed to reductions in salinity while the external salinity was maintained at normal levels, changes in heart rate were rapidly elicited in response to very small reductions in salinity (down to 29.5 ± 0.9 ppt in the branchial chamber and 31.5 ± 0.3 ppt externally). We conclude that the primary receptors responsible for detecting reductions in salinity in *H. americanus* are located within or near the branchial chambers and are primarily sensitive to chloride ions.

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Introduction

Several studies have provided evidence for osmolarity or salinity receptors in crustaceans, but the location of such receptors and the precise ionic stimuli that activate them are not fully understood. In a study designed to localize the salinity receptors of the green crab *Carcinus maenas,* Hume and Berlind (1976) selectively exposed different parts of crabs to seawater with a salinity of 15 ppt. They concluded that the salinity receptors were located in or near the excurrent opening of the branchial chambers. In the crayfish *Procambarus simulans,* the branchial chamber also appears to be the location of receptors that mediate cardiovascular responses to changes in salinity (Larimer, 1964). Although the antennae and antennules of lobsters are exquisitely sensitive to a wide range of chemicals (Tierney *et al.,* 1988; Johnson *et al.,* 1989; see review by Atema and Voigt, 1995), it is unclear whether they play a role in sensing salinity. During Hume and Berlind's (1976) investigation of salinity detection in *C. maenas,* removal of the antennae and antennules had no effect. In contrast, Lagerspetz and Mattila (1961), demonstrated that the antennules and antennae played an important role in the detection of low salinity in the isopod *Asellus* sp. and the amphipod *Gammarus oceanicus,* and Tazaki and Tanino (1973) concluded that the antennae of the spiny lobster *Panuliris japonicus* have mechanoreceptors that also function as osmoreceptors. There is also evidence that the legs of crustaceans have receptors that provide important information about salinity. The porcelain crab *Porcellana platycheles* is able to discriminate between water of different salinities by using its walking legs (Davenport and Wankowski, 1973), and Schmidt (1989) recorded electrophysiological responses to changes in salinity from sensilla on the legs of *C. maenas.* Thus, there is some limited evidence for receptors capable of sensing salinity changes in crustaceans, but the locations

of these receptors and the transduction mechanisms involved are poorly understood.

Little is known about how marine invertebrates detect changes in salinity. The bivalves *Mytilus edulis* and *Scrobicularia plana* close their shells in response to salinity reductions, and the receptors controlling these shell closures are primarily sensitive to Na^+, Mg^{++}, Ca^{++}, and possibly Cl^-, rather than to osmolarity (Davenport, 1981; Akberali and Davenport, 1982). However, there is also evidence for osmoreceptors in both marine molluscs and crustaceans (Davenport, 1972; Davenport and Wankowski, 1973; Tazaki, 1975; Schmidt, 1989). One goal of this study was to determine whether lobsters detect reductions in salinity by using a transduction mechanism that is sensitive to changes in the concentration of certain ions, or one that responds to alterations in ambient osmolarity.

Homarus americanus, the American lobster, is an excellent organism in which to investigate responses to changing salinity, both in terms of the sensory processes involved and how this behavior is adaptive in certain habitats. Although the American lobster is generally considered to be stenohaline and intolerant to salinities below 25 ppt (Dall, 1970), adult and juvenile lobsters are known to inhabit salt marshes, bays, and estuaries that are characterized by frequent fluctuations in salinity (Thomas and White, 1969; Munro and Therriault, 1983; Able *et al.,* 1988; Jury *et al.,* 1995; Howell *et al.,* 1999; Watson *et al.,* 1999; reviewed by Charmantier *et al.,* 2001). For example, lobsters are regularly found in the Great Bay Estuary, New Hampshire, where the salinity is normally between 22 and 28 ppt in the summer but routinely drops below 15 ppt each spring (Loder *et al.,* 1983; Short, 1992; Watson *et al.,* 1999). After heavy storms, the salinity can also fall to less than 5 ppt (Jury *et al.,* 1995), a value below the lower lethal limit for adult lobsters, which is from 8 to 14 ppt, depending on temperature, oxygen and acclimation conditions (McLeese, 1956). Moreover, even if lobsters are able to survive short-term exposure to low salinity, the resulting physiological stress may have deleterious long-term effects on growth or reproduction (Jury *et al.,* 1994a; Houchens, 1996).

Field studies have shown that lobster movements in estuaries tend to be correlated with seasonal changes in temperature and salinity (Munro and Therriault, 1983; Watson *et al.,* 1999), or with storms that cause substantial decreases in salinity (Jury *et al.,* 1995). Laboratory studies have also demonstrated that both adult lobsters (Jury *et al.,* 1994b) and larval lobsters (Scarratt and Raine, 1967) avoid low-salinity water. For example, when given a choice between two passageways, one containing water held at a low salinity (10–15 ppt) and one at a higher salinity (20–25 ppt), 93% of the lobsters tested moved through the high-salinity passageway. Lobsters also moved out of their shelters if the salinity in those shelters was lowered below 12.5 ppt (Jury *et al.,* 1994b). This avoidance response to low salinity strongly suggests that lobsters possess the ability to detect decreases in either osmolarity or the concentrations of specific ions.

It is well known that many crustaceans will exhibit a drop in heart rate (bradycardia) or ventilation rate (apnea) in response to novel stimuli (Maynard, 1960; McMahon, 1999). Therefore, as was pointed out by Florey and Kreibel (1974), heart rate "can serve as a most sensitive indicator of sensory perception and it could well be used in studies on perceptual physiology." For example, Larimer (1964) showed that crayfish exhibited changes in heart rate when exposed to (1) solutions low in oxygen; (2) different concentrations of NaCl and L-glutamic acid; and (3) sudden changes in temperature. A cardiac assay was also used by Offutt (1970) to measure the ability of *H. americanus* to detect sounds of different frequencies, and by Jury and Watson (2000) to measure the thermosensitivity of *H. americanus.*

In the present study, we employed a cardiac assay to demonstrate that lobsters are able to sense drops in salinity of greater than 4 ppt. Although removal of the legs, antennae, and antennules had little impact on their responsiveness, selective perfusion of the branchial chamber revealed that this is the most likely location of receptors sensitive to changes in salinity. Finally, by exposing lobsters to seawater deficient in certain ions, we determined that lobsters probably detect changes in salinity by monitoring the concentration of chloride rather than by sensing changes in osmolarity.

Materials and Methods

Adult (>82 mm carapace length), intermolt lobsters of both sexes were captured in research traps in the Gulf of Maine, near New Castle, New Hampshire. They were held in recirculating tanks at a salinity of 32 ppt and a temperature of 12 to 14 °C for up to 2 weeks prior to use. Throughout this paper, "normal" seawater refers to full-strength (along the NH coast), 32 ppt seawater.

Cardiac assay

Changes in heart and ventilation rates were used as indicators that lobsters sensed an alteration in their environment. Two pairs of wire electrodes, insulated except at the tips, were inserted through the carapace of each lobster and then fastened to the shell with tape and cyanoacrylate glue. Typically, one pair was implanted through the dorsal carapace on either side of the heart, and the second pair was inserted through the lateral carapace over the scaphognathites (gill bailers). The electrodes were connected to an impedance converter (UFI, Morro Bay, CA) that produced analog signals proportional to the magnitude of the movements of the heart or gill bailer. The impedance converter output was then digitized using a MacLab analog-to-digital interface (AD Instruments, Grand Junction, CO), and this digitized signal was recorded on a Macintosh computer. In

some cases, data were also recorded on an AstroMed Dash 4 polygraph (Grass Instruments, Quincy, MA).

Lobsters were placed individually in a 3-l light-tight acrylic plastic chamber that was continuously perfused with cooled (12 to 14 °C) normal seawater taken from a large holding aquarium. The experimental chamber was connected by tubing to two 1-l stimulus bottles, one containing experimental (0 ppt) water, and the other containing control seawater (32 ppt). Valves were used to control whether the lobster received an experimental or a control stimulus. During the ion-sensitivity experiments (see subsequent section of Materials and Methods), the experimental water consisted of solutions that were isotonic to the seawater in the recording chamber (950–1050 mOsm) but deficient in one or more specific ions. The temperature of the water in the stimulus bottles was held constant by placing them in a 25-l water bath that was maintained at the same temperature as the chamber holding the lobster.

Lobsters were secured in the chamber with elastic bands fastened loosely across their dorsal carapace and left overnight to adjust to this new environment. Previous studies have indicated that cardiac responses are more pronounced and are elicited with smaller stimulus intensities when lobsters are left in the chamber overnight instead of being tested shortly after electrode implantation (Offutt, 1970; Florey and Kriebel, 1974; Jury and Watson, 2000).

All lobsters were first tested to determine whether their heart rates altered in response to tactile stimulation, 10^{-4} betaine (Atema and Voigt, 1995), or shadows (Larimer, 1964). Only lobsters that exhibited a cardiac response to these stimuli were used in subsequent experiments. A Quicktime video showing a lobster cardiac response to a low-salinity stimulus can be viewed at the following website: http://zoology.unh.edu/faculty/win/winvideos.htm.

Salinity detection threshold

In this experiment, 30 lobsters (15 male, 15 female) were tested for their ability to sense changes in salinity. For each animal, the salinity in the recording chamber was gradually lowered from a starting value of 32 ppt to less than 20 ppt, at a rate of 1–2 ppt/min, while heart and scaphognathite rates were continuously recorded. To monitor salinity, a piece of tubing was placed in the experimental chamber near the inhalent opening to the lobster's branchial chamber. Throughout the experiment water from this area of the chamber continuously dripped out of this tubing. At 1-min intervals, the salinity of the water flowing from this tubing was determined, in parts per thousand, using a refractometer. It took 10 s for water to flow from the chamber to the end of the tubing, and data were adjusted for this time lag.

During these experiments, under these controlled conditions, the heart rates were very stable, deviating less than 4% from one minute to the next. Thus, a sudden, stimulus-induced increase or decrease in heart rate was very obvious

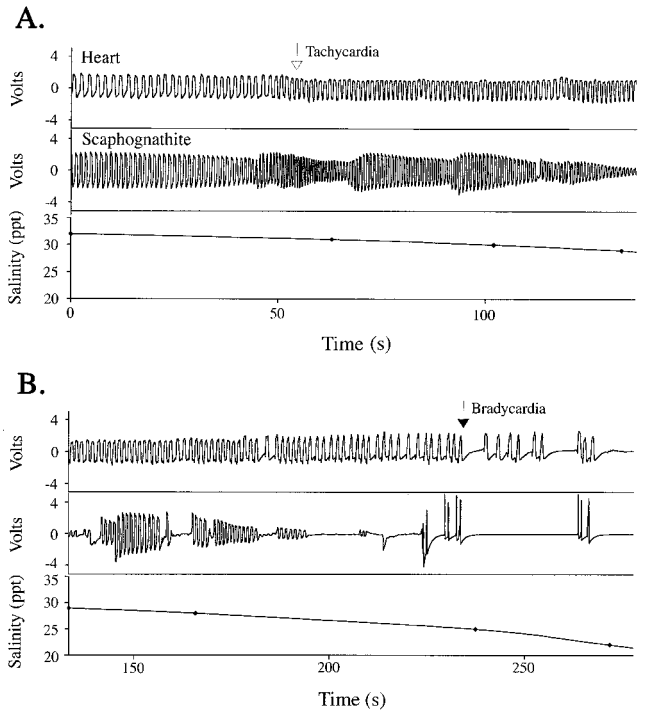

Figure 1. Impedance recordings (in volts) of heart and scaphognathite activity during reductions in salinity for a typical test animal. The salinity was decreased at a rate of 1–2 ppt/min. (A) The initial response was typically a rapid increase in heart rate, or tachycardia, which took place in this experiment at a salinity of 31 ppt and was accompanied by an increase in ventilation rate, as can be seen in the scaphognathite recording. Initiation of tachycardia is indicated by the open arrow. (B) As the salinity was decreased further, to 25 ppt, the lobster responded with a rapid decrease in heart rate, or bradycardia. Initiation of bradycardia is indicated by the closed arrow. Bradycardia was usually accompanied by a decrease in ventilation rate (*i.e.*, apnea).

(see Fig. 1 for example). However, even under the most stable conditions, occasionally lobsters will spontaneously skip a heartbeat, or ventilate their gill bailers in the reverse direction, which is often accompanied by a small change in heart rate. Therefore, to avoid counting these events as responses to salinity drops, we set a more obvious and conservative criterion for designating a change in rate as either a tachycardia or bradycardia response. This criterion was either an increase or a decrease of at least 25% from the baseline heart rate that lasted for more than 10 s. When a cardiac response occurred, the salinity measured at the beginning of the response (taking into account the time lag) was considered to be the salinity detection threshold (SDT) for that animal. All results are reported as a mean ± SEM.

Ion sensitivity assay

To determine which ions were used to detect differences in salinity, cardiac responses were measured while exposing lobsters ($n = 37$) to artificial saline solutions that were deficient in one or more specific ions. Most lobsters were

exposed to at least two different salines, yielding a total of 61 trials. All artificial saline solutions were isotonic with seawater, so the osmolarity did not change as they were introduced into the experimental chamber, but the concentrations of certain ions did change.

Each artificial saline solution was deficient in one, or a combination, of the following ions: sodium, chloride, magnesium, and calcium. The solutions tested were: 550 m*M* sodium bicarbonate, 530 m*M* sodium acetate, 530 m*M* sodium phosphate, 590 m*M* choline chloride, and 530 m*M* sodium chloride, as well as artificial seawater (423 m*M* NaCl, 9 m*M* KCl, 9.27 m*M* $CaCl_2$, 22.94 m*M* $MgCl_2$, 25.50 m*M* $MgSO_4$, 2.14 m*M* $NaHCO_3$), sodium-free seawater (9.40 m*M* KCl, 9.00 m*M* $CaCl_2$, 22.10 m*M* $MgCl_2$, 25.60 m*M* $MgSO_4$, 455 m*M* choline chloride, and 2.10 m*M* $KHCO_3$) and chloride-free seawater (25.50 m*M* $MgSO_4$, 2.14 m*M* $NaHCO_3$, 422.30 m*M* $NaNO_3$, 9.69 m*M* KNO_3, and 9.27 m*M* $Ca(NO_3)_2$). The pH of most solutions was adjusted to 7.6–7.7 with hydrogen chloride, acetic acid, sodium hydroxide, or potassium hydroxide, depending on the ions being tested. A few solutions, such as sodium acetate and choline chloride, were allowed a larger pH range (6.7–8.1), because adjusting the pH would alter the concentration of either Na^+, K^+, or Cl^- ions, as well as the overall osmolarity. In separate tests, lobsters did not exhibit cardiac responses when only the pH of natural seawater was changed over the range 6.2 to 8.1.

Blood osmolarity experiments

These experiments were carried out to determine whether significant changes in hemolymph osmolarity take place during the type of salinity reduction protocol used in the salinity detection studies. Individual lobsters ($n = 8$) were placed in the experimental chamber, and the salinity was decreased at a rate of 1.5 ppt/min. Before the salinity was decreased, and every 2 min during the study, the lobster was quickly removed from the chamber and 0.3 ml of hemolymph was removed from the base of one of the walking legs using a 1-ml tuberculin syringe and a 26-gauge needle. Because all SDTs in previous experiments occurred less than 16 min after exposure to low salinity, these experiments were conducted for 16 min. Hemolymph samples were placed in 1-ml eppendorf tubes on ice. Seawater samples were also taken from the experimental chamber every 2 min and placed in tubes on ice. Control lobsters ($n = 8$) were subjected to a similar protocol, except that the salinity of the seawater was kept constant. The osmolarities of all the hemolymph and water samples were measured using a Wescor vapor-pressure osmometer. The heart and ventilation rates were not measured from these lobsters because the repetitive blood sampling caused dramatic changes in heart rate that were not related to reductions in salinity.

Cardioregulatory nerve lesions

To determine whether changes in heart activity are mediated by the cardioregulatory nerves, three groups of lobsters were tested for cardiac responses to salinity changes. The first group of lobsters ($n = 5$) had their cardioregulatory nerves cut (lesion); the second group ($n = 5$) had the same operation as the first group except that their cardioregulatory nerves were left intact (sham); and the third group ($n = 5$) was handled, but did not undergo an operation (control). The baseline heart rates of all lobsters were recorded for more than 1 h before surgery and again at least 4 days after surgery, to determine if lesioning the cardioregulatory nerves had any effect on baseline heart rates. Once baseline heart rates were recorded, all lobsters were then tested for a cardiac response to reduced salinity. All recordings were carried out as described above, after lobsters had become accustomed to the recording chamber overnight.

Lesions were performed as described in Guirguis and Wilkens (1995). Briefly, 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 secured with tape and cyanoacrylate glue.

Ablations

First, the SDTs of the experimental lobsters ($n = 15$) were determined by the cardiac assay method. Then, after chilling the animals for 30 min, their antennae ($n = 5$), antennules ($n = 5$), or all walking legs ($n = 5$) were removed. After at least 4 weeks of recovery in a flow-through tank at the UNH Coastal Marine Laboratory, the lobsters were tested again to determine the salinity reduction necessary to elicit bradycardia.

Selective perfusion of the branchial chambers

Since the branchial chamber cannot be isolated by lesioning, a different technique was employed to determine whether this region is receptive to reductions in salinity. Both branchial chambers of six lobsters were cannulated with polyethylene (PE) tubing (1.57 mm I.D.). Four lengths of PE tubing were inserted into each branchial chamber through small holes drilled in the carapace near the dorsal edge of the branchial chambers and glued into place. These four lengths of tubing were connected to a flow divider, which in turn was connected to a valve that permitted the perfusion of each branchial chamber with either normal seawater or reduced-salinity water. A fifth length of PE tubing (1.19 mm I.D.) was inserted through the shell posterior to the exhalent area of each branchial chamber to monitor water salinity. As in all previously described experiments, lobsters were left in the experimental chamber,

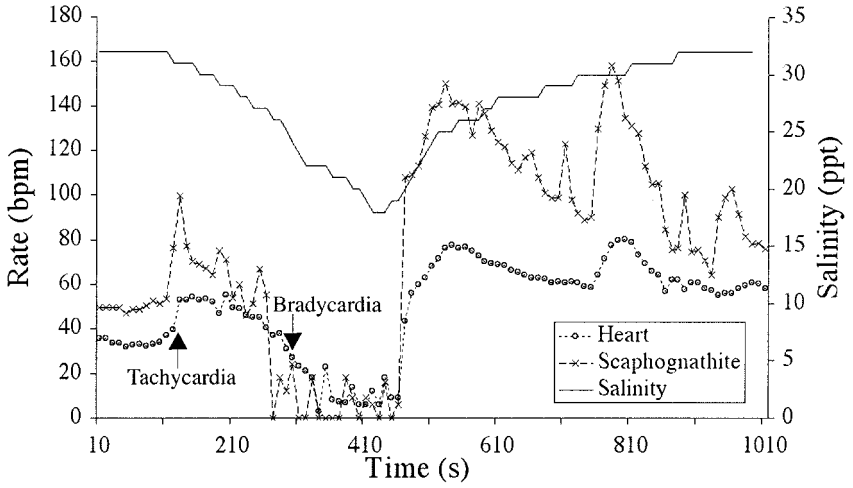

Figure 2. Changes in heart and scaphognathite rates in response to changes in salinity. As in Figure 1, salinity was decreased at a rate of 1–2 ppt/min. Each heart and scaphognathite data point is an average of 10 s of data from a digital ratemeter, while each salinity data point comes from a single refractometer measurement each min. Initial heart and scaphognathite rates were 35 and 50 bpm, respectively. Tachycardia first occurred at 31 ppt (upward arrow) and was accompanied by an increase in ventilation rate. When the salinity reached a value of 22 ppt, the lobster responded with bradycardia (downward arrow). The bradycardia response was accompanied by apnea. Shortly after the salinity began to increase, both heart and scaphognathite rates rebounded to levels well above baseline and then slowly recovered towards baseline over time.

with normal seawater flowing through both the branchial chambers and the experimental chamber (tank), overnight. The next day, lobsters were exposed to the following treatments. (1) Normal seawater (32 ppt) was perfused through the tank while the salinity in the branchial chambers was gradually reduced. (2) The salinity in the tank was reduced while the branchial chambers were perfused with normal seawater. (3) The salinity in the tank was reduced and no solutions were perfused through the branchial chamber, as in a typical salinity-reduction experiment. Treatment #1 was always carried out last; the other two treatments were randomized. Animals were given at least 1 h to recover between treatments. Water from both branchial chambers and the experimental chamber dripped into a reserve tank through PE tubing so that salinity could be sampled each minute using a refractometer.

Results

Control heart rates and cardiac response controls

After overnight acclimation in the experimental chamber, the lobsters tested before their salinity detection threshold (SDT) was measured ($n = 32$) had a mean heart rate of 52.2 ± 3.3 beats/min (bpm). The heart rates of lobsters under these control conditions were very consistent, and thus changes in heart rate in response to drops in salinity were quite evident and easy to identify. For example, in a separate experiment (cardioregulatory nerve lesion controls), when we averaged the heart rates for 5 consecutive min, in 10 different lobsters, the mean standard deviation was only 1.2 bpm, or a 4% deviation from the average heart rate (48.4 bpm).

In response to a variety of novel stimuli, 30 of the 32 lobsters tested exhibited a transient bradycardia that was usually, although not always, accompanied by a reduction in ventilation rate (apnea). Stimuli which were effective in eliciting bradycardia included 10^{-4} *M* betaine, shadows, and tactile stimulation of the carapace. The abrupt and transient reductions in heart rate typically lasted 30 to 120 s, although on one occasion the heart rate stayed below baseline for 10 min. None of the lobsters showed any response to control applications of full-strength ambient seawater, which was true in all subsequent experiments as well. Only lobsters that exhibited a cardiac response to novel stimuli were tested for their response to changes in salinity.

Responses to a reduction in salinity

All the lobsters tested for their response to a reduction in salinity exhibited a dramatic change in heart rate when the salinity detection threshold was reached. The typical response was an increase, followed by a decrease, in heart rate (Figs. 1, 2). The initial tachycardia lasted for 178.0 ± 13.0 s ($n = 22$ because not all lobsters tested responded with an increase in heart rate) and was often, but not always, accompanied by a significant increase in ventilation rate. On average, heart rate increased significantly from 45.0 ± 3.0 to 66.9 ± 2.9 bpm or 48% (paired *t* test, $P < 0.0001$, $n = 22$). The bradycardia that occurred next was usually accompanied by a transient decrease in scaphognathite pumping

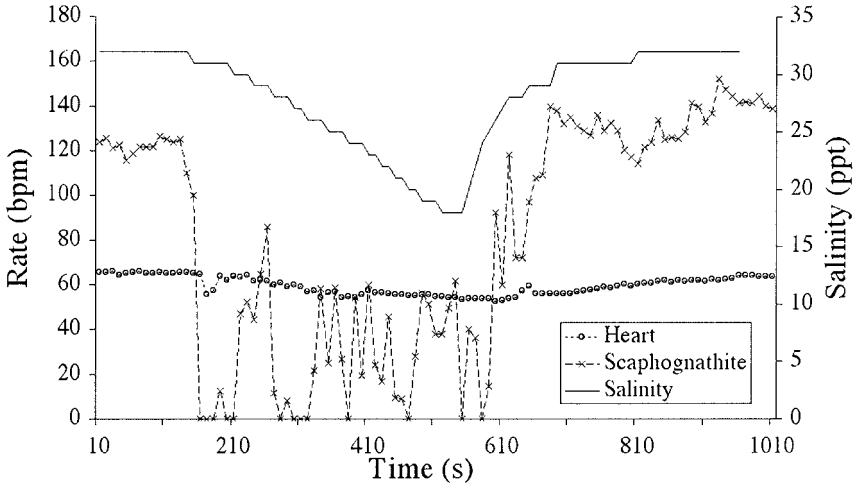

Figure 3. Heart and scaphognathite rates during a drop in salinity for a lobster with lesioned cardioregulatory nerves. The baseline heart and scaphognathite rates were 63 and 120 bpm, respectively. When the salinity reached 31 ppt, the scaphognathite rate dropped suddenly and continued a pattern of intermittent stops and starts until the salinity increased again. Over the course of the salinity drop, heart rate declined slowly to 53 bpm, a decrease of 16% from baseline, and then slowly increased back to baseline. Similar results with four additional lobsters indicate that cardiac responses to changes in salinity are mediated by the cardioregulatory nerves.

(Fig. 2). During bradycardia, the heart rate fell significantly from 50.3 ± 3.1 to 17.0 ± 1.33 bpm (paired t test, $P < 0.0001$, $n = 30$, a 66% decrease in rate) and remained below baseline for 123.0 ± 8.2 s. Following bradycardia, heart and ventilation rates usually increased above baseline for several minutes before full recovery (Fig. 2).

Seventy-three percent (22 of 30) of the lobsters exhibiting cardiac responses to drops in salinity expressed a biphasic change in heart rate; 27% expressed a bradycardia response with no tachycardia. Tachycardia, when present, always preceded the bradycardia and always occurred before the salinity reached 25 ppt. Although the bradycardia response was much more reliable, occurring in every lobster tested, it did not occur until the salinity had dropped to nearly 20 ppt. It is possible that the lobsters not exhibiting tachycardia may already have been in an excited state, because their average baseline heart rate was 64.6 ± 6.1 bpm and animals expressing tachycardia in response to reduced salinity increased their heart rate to 66.9 ± 2.9. In contrast, animals that did display a tachycardia response had a mean initial heart rate of 45.0 ± 3.0. Due to the more reliable nature of the bradycardia response, it was used in the ablation and ion-sensitivity experiments as an indicator that lobsters sensed changes in salinity.

Salinity detection threshold

Lobsters first expressed a tachycardia response when the salinity had fallen to 26.6 ± 0.7 ($n = 22$), representing a 5.4 ppt drop in salinity relative to ambient levels (32.0 ± 0.2 ppt). The salinity at which the tachycardia response occurred did not differ significantly (unpaired t test, $P > 0.5$) from females (26.3 ± 0.7 ppt) to males (26.9 ± 1.1 ppt). The bradycardia response in the 30 animals tested occurred at 22.1 ± 0.5 ppt, which represents an average drop of 9.9 ppt from the ambient salinity. For the lobsters that showed both bradycardia and tachycardia responses, the salinity at which bradycardia occurred was significantly lower than that at which tachycardia occurred (paired t test, $P < 0.001$). The SDT for bradycardia was also significantly higher (unpaired t test, $P < 0.05$) for females (23.1 ± 0.4 ppt) than for males (21.0 ± 1.0 ppt).

Involvement of cardioregulatory nerves

Under control conditions, prior to treatment, there was no significant difference ($P = 0.9775$, one-way ANOVA) between the baseline heart rates of control ($n = 5$, 43.7 ± 5.5 bpm), experimental ($n = 5$, 45.3 ± 4.0 bpm), and sham-lesioned lobsters ($n = 5$, 44.9 ± 6.9 bpm). After recovery from treatment (4–7 days), the heart rates of both the sham and lesioned groups were elevated in comparison to the control group, but this difference was not statistically significant ($P = 0.2994$, one-way ANOVA: control 40.8 ± 6.7 bpm; experimental 56.6 ± 7.8 bpm; sham-lesioned 51.3 ± 6.2 bpm). Lobsters in the control and sham groups ($n = 10$) all exhibited bradycardia in response to a 1 to 2 ppt/min reduction in salinity before the salinity in the experimental chamber reached 20 ppt. There was no significant difference ($P = 0.2362$, unpaired t test) in the mean SDTs of these two groups of lobsters (30.4 ± 1.7 ppt for controls and 25.8 ± 3.2 ppt for sham lesions). None of the lesioned animals ($n = 5$) exhibited bradycardia in response to salinity reductions down to 20 ppt (Fig. 3). Two of the 5

lesioned animals showed a slow decrease in heart rate during the course of the salinity reduction, but the magnitude of these rate decreases did not qualify them as a bradycardia under our criteria (Fig. 3). Interestingly, all of the lesioned lobsters exhibited reductions in ventilation rates during the course of the salinity reduction (Fig. 3). The salinity at which lesioned lobsters reduced their ventilation rates was not significantly different ($P = 0.8930$, one-way ANOVA), from that of control or sham-lesioned lobsters (control $n = 3$ [because scaphognathite records were poor in 2 of the 5 lobsters], 28.3 ± 2.6 ppt; lesion $n = 5$, 26.6 ± 2.2 ppt; sham-lesion $n = 5$, 26.4 ± 3.2 ppt), suggesting that the salinity response elements in the nervous system had been activated, but the lobsters were unable to modify their heart rates due to the lesions.

Ion sensitivity

Most (21 of 24) of the lobsters exposed to isotonic solutions lacking chloride showed a typical bradycardia response (Fig. 4). However, cardiac responses were seen in only 2 of 19 lobsters exposed to isotonic solutions lacking other ions, but containing chloride (Fig. 4). Statistically, the occurrence of a bradycardia was significantly dependent on the lack of chloride (Fig. 4). For example, lobsters did not exhibit bradycardia when exposed to an isotonic solution of choline chloride, but they did upon exposure to solutions of sodium phosphate and sodium acetate. Lobsters also expressed bradycardia in chloride-free but not in sodium-free artificial seawater. Thus, when only chloride was missing they detected a change, but when some combination of sodium, calcium, and magnesium was missing they responded as if the solution was normal seawater. The only exceptions were two lobsters that responded when exposed to NaCl solutions. It is not clear why they responded and seven other lobsters did not. These experiments indicate that (1) a change in osmolarity is not required for lobsters to sense a change in salinity; and (2) as long as chloride is present at normal concentrations, lobsters do not sense changes in the concentrations of other ions.

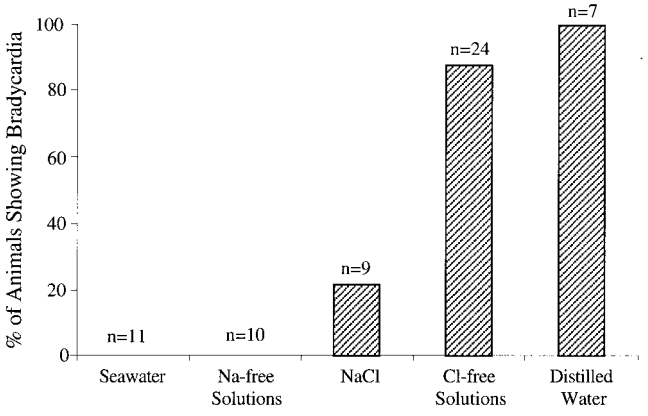

Figure 4. Percentage of trials ($n = 61$) in which lobsters exhibited bradycardia responses when exposed to natural and artificial solutions containing various amounts of certain ions. Lobsters did not usually respond when exposed to solutions containing chloride, such as seawater (natural [$n = 7$, 32 ppt] and artificial [$n = 4$]), sodium-free solutions (choline chloride [$n = 7$] and sodium-free seawater [$n = 3$]), and NaCl ($n = 9$). However, lobsters did usually exhibit bradycardia when exposed to solutions lacking chloride, such as distilled water ($n = 7$) and chloride-free solutions (sodium bicarbonate [$n = 8$], sodium acetate [$n = 7$], sodium phosphate [$n = 5$], and chloride-free seawater [$n = 4$]). Statistically, the occurrence of bradycardia was significantly dependent on the lack of chloride (Fisher's exact test, $P < 0.0001$). Each lobster ($n = 37$) was usually subjected to one to four different solutions, with sufficient time between solutions for the heart to recover to its baseline rate (>2 h). Distilled water, when used, was always the last solution tested.

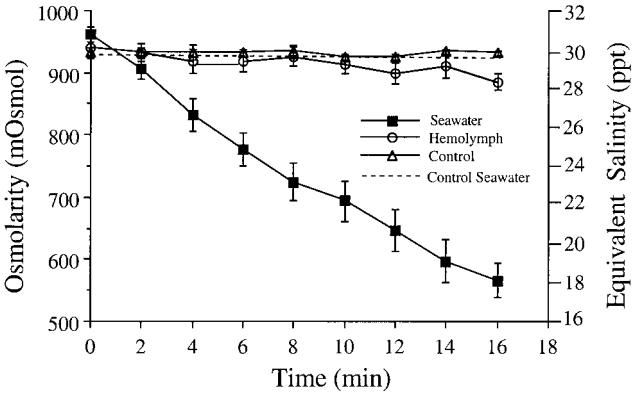

Figure 5. Comparison of the osmolarity of ambient seawater and lobster hemolymph during a typical salinity-reduction experiment. Blood samples and water samples were taken every 2 min, from eight lobsters, and averaged (± SEM). Control hemolymph values are also shown for eight lobsters held at a constant salinity for 16 min. There was no statistically significant difference between the hemolymph osmolarity of control and experimental animals after 10 min. However, after 16 min there was a slight, but statistically significant, difference. Equivalent salinity values, in parts per thousand, are shown on the right-hand vertical axis for comparison.

Changes in blood osmolarity as ambient salinity is reduced

In these experiments, the salinity of the seawater in the experimental chamber was reduced from 31 to 18 ppt over 16 min, and the osmolarity of lobster hemolymph and the seawater in the chamber were measured every 2 min. The control study was identical, except the salinity was not changed. After 10 min there was no statistically significant change (2-way ANOVA with replication $P > 0.10$) in the blood osmolarity of the test animals ($n = 8$) when compared to the blood osmolarity of control lobsters ($n = 8$) (Fig. 5). For comparison, in the salinity reduction experiments, the external salinity had dropped almost to 20 ppt after 10 min, which was usually sufficient to elicit a bradycardia response. After 16 min there was a small but statistically significant difference (2-way ANOVA with replication, $P < 0.01$) in experimental blood osmolarity

compared to controls (Fig. 5). Thus, although it is possible that sensitive internal receptors could detect this slight decrease in blood osmolarity, the time course and magnitude of the change—in comparison to the response of the lobsters—make it more likely that external salinity receptors detect the more robust declines that occur in the ambient seawater.

Ablation experiments

Lobsters with antennules ($n = 5$), antennae ($n = 5$), or walking legs ($n = 5$) ablated were responsive to declining salinity both before and after removal of these putative receptor sites (Fig. 6). There was no statistically significant difference between the mean SDTs obtained before and after removal of these structures (paired t test, $P > 0.5$ in all three groups; antennae $P = 0.94$, antennules $P = 0.30$, legs $P = 0.80$).

Branchial perfusion

Both branchial chambers in six lobsters were cannulated so that the salinity could be differentially controlled in both the branchial chambers and the experimental chamber. The day after cannulation, when the lobsters were exposed to different treatments, the mean heart rate of the lobsters was 51.8 ± 6.3 bpm. The SDTs were then determined in response to (1) a typical drop in external salinity; (2) a drop in external salinity with the branchial chambers perfused with normal salinity seawater; and (3) perfusion of the branchial chambers with low-salinity seawater while exposing the animal to normal seawater.

When these cannulated lobsters were exposed to a typical drop in external salinity, with no seawater perfusion of their branchial chambers, their SDT was 26.7 ± 1.4 ppt. During these experiments, the salinities in both branchial chambers and the experimental chamber were recorded each minute. These data showed that the salinity in the branchial chambers was always 1–2 ppt higher than the changes in salinity recorded in the ambient seawater. For example, in one lobster, the salinity in the branchial chambers was 28.0 ppt when the SDT was 26.7 ppt.

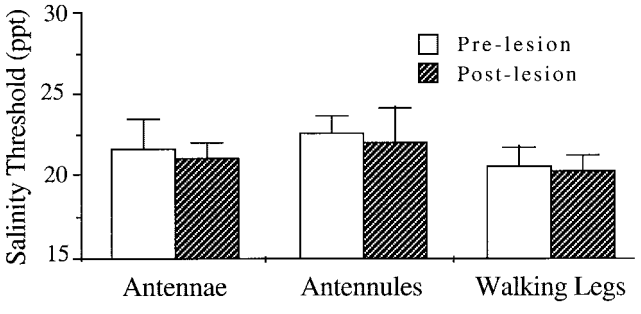

Figure 6. Mean salinity detection thresholds (SDTs) for pre- and post-lesion lobsters. The salinity level at which bradycardia occurred was measured prior to removal of antennules, antennae, and legs (pre-lesion SDT), and then compared to values obtained 4 weeks after ablations (post-lesion SDT). Five lobsters were tested after ablation of each putative receptor site. There were no statistically significant differences between any of the means (paired t test, $P > 0.5$).

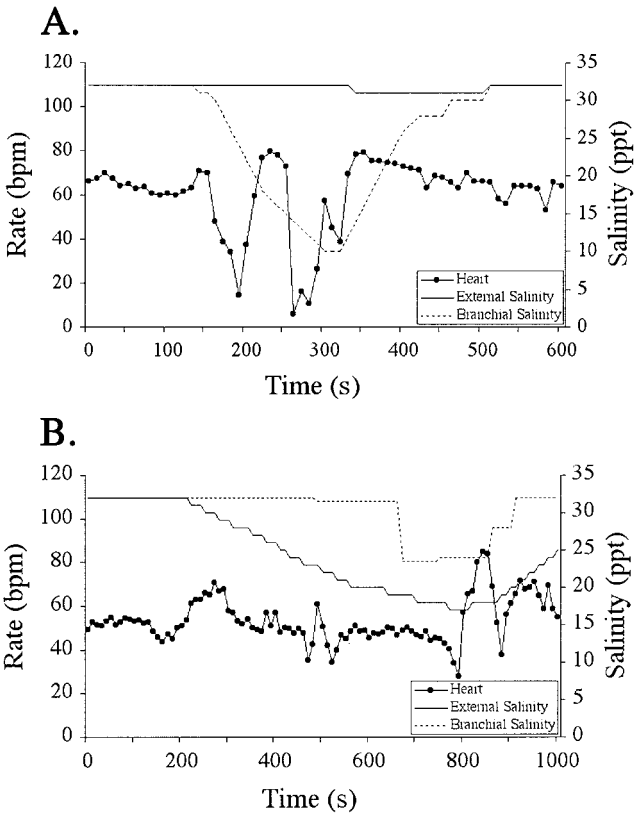

Figure 7. Cardiac responses of two lobsters to (A) perfusion of the branchial chambers with low-salinity water while providing the experimental chamber with normal seawater; and (B) perfusion of the branchial chambers with normal seawater while the salinity in the experimental tank was lowered. In both experiments, the salinity in the branchial chambers and the experimental tank was recorded every minute. The heart rates shown were averaged every 10 s. Compare how fast the lobster responded when low-salinity water was perfused directly into its branchial chambers with how long it took the other lobster to respond when the salinity in its branchial chambers was maintained close to 32 ppt, while the salinity in the experimental tank was lowered.

Perfusing the branchial chambers with normal seawater while dropping the external salinity caused the cardiac response to occur at an average external salinity of 21.6 ± 1.8 ppt (Fig. 7), which was lower than the SDT obtained from these same lobsters during the typical experiment described above. However, this difference in thresholds was not statistically significant (Mann-Whitney U test, $P = 0.06$). At the time the lobsters showed a cardiac response, the salinity in their branchial chambers was still significantly higher than the external salinity (Mann-Whitney U test, $P < 0.05$) due to the perfusion with normal seawater. However, it had decreased to a value of 29.0 ± 1.2 ppt due to dilution with the lower salinity water in the experimental tank that was being pumped through the branchial chambers

by the scaphognathites. In contrast, when the salinity in the branchial chambers was lowered, while the lobster was being perfused with normal seawater, a cardiac response was expressed almost immediately, when the branchial chamber salinity had only dropped to 29.5 ± 0.9 ppt and the external salinity had only been reduced to 31.5 ± 0.3 ppt. Interestingly, in all three experiments, there was no statistically significant difference between the salinity values in the branchial chambers when a cardiac response took place (repeated measures ANOVA, $P > 0.5$ ($P = .66$)). These data, taken together, suggest that some, if not all, of the salinity receptors are located in or near the branchial chambers.

Discussion

The ability of American lobsters to detect changes in salinity was examined by monitoring heart and ventilation rates while exposing the animals to a gradual reduction in salinity, either in the ambient seawater or in water directly flowing into the branchial chambers. The typical response to a reduction in salinity consisted of tachycardia followed by bradycardia. In the first set of experiments, tachycardia occurred when the salinity had decreased from 32 to 26.6 ± 0.7 ppt, whereas bradycardia was not expressed until the salinity dropped to 22.1 ± 0.5 ppt. During direct perfusion of the branchial chamber, bradycardia was elicited in response to very small drops in salinity (SDT = 29.5 ± 0.9, measured in the branchial chamber); when the branchial chambers were perfused with normal seawater while the external salinity was dropped, lobsters were less responsive than during control experiments. These data suggest that the primary salinity receptors mediating the cardiac responses investigated in this study are located in or very near the branchial chambers.

In the behavioral avoidance experiments conducted by Jury *et al.* (1994b), lobsters first became restless and started to move out of their shelters when the salinity in their shelters dropped below 18 ppt. These observations, along with observations of lobsters during cardiac assays and more recent electrocardiogram recordings obtained from freely moving lobsters (D. O'Grady, University of New Hampshire, unpubl. data), indicate that the bradycardia response, and not the more sensitive tachycardia response, is more often correlated with avoidance behaviors. Therefore, even though lobsters can detect relatively small reductions in salinity, which may cause them to become aroused and may increase their heart rate, they may not exhibit avoidance behaviors until the salinity drops to about 18 ppt, a level well below that necessary to elicit bradycardia. Thus, as suggested by McGaw and McMahon (1996) and Guirguis and Wilkens (1995), bradycardia is probably a shock or startle response, indicating that animals sense a potentially dangerous stimulus and are initiating an avoidance behavior.

We suggest that tachycardia is one of the earliest indicators that lobsters have sensed a change in salinity, and that this sensory input leads to arousal and a readiness for a change in behavior. Most of the lobsters in our study that did not exhibit tachycardia had elevated heart rates before the stimulus was applied, so they may already have been in a relatively excited state. In *Callinectes sapidus*, the blue crab, drops in salinity trigger a similar tachycardia, and the available data suggest this increase in heart rate facilitates certain behaviors associated with low osmolarity (McGaw and Reiber, 1998). Lobsters induced to walk on a treadmill exhibit a very rapid increase in heart rate at the onset of activity, which is comparable to the changes observed in our experiments (Guirguis and Wilkens, 1995; Rose *et al.*, 1998; O'Grady *et al.*, 2001). This increase is mediated by the cardioregulatory nerves, and as in our experiments, the tachycardia is probably an arousal response that helps prepare the lobster for activity.

Although the physiological role of brief changes in heart and ventilation rates is not obvious, the physiological role of long-term changes is clear. Increased oxygen uptake and enhanced circulation of the hemolymph are necessary to serve the metabolic demands associated with osmoregulation (Jury *et al.*, 1994a; Houchens, 1996), locomotion (Guirguis and Wilkens, 1995; Rose *et al.*, 1998), and higher temperatures (S. Schreiber, University of New Hampshire, unpubl. data). Under these circumstances, the initial and rapid changes in heart rate appear to be mediated by the cardioregulatory nerves, whereas circulating hormones appear to be involved in long-term modulation (Guirguis and Wilkens, 1995; McMahon, 1999; Jury and Watson, 2000; O'Grady *et al.*, 2001).

Marine animals may sense drops in ambient salinity by detecting a change in osmolarity (Davenport, 1972; Davenport and Wankowski, 1973; Tazaki, 1975; Schmidt, 1989), or they may utilize a sensory mechanism that is sensitive to the concentration of one or more of the ions present in seawater (Davenport, 1981; Akberali and Davenport, 1982). One further possibility is that a change in osmolarity could alter the responsiveness of another type of receptor. For example, in *Callinectes sapidus*, the sensitivity of olfactory sensilla decreases in low-salinity water because the osmotic stress causes the outer dendritic segments to change size (Gleeson *et al.*, 1996, 1997). However, in the two species of molluscs that have been studied in the most detail, *Mytilus edulis* and *Scrobicularia plana*, and in two crustaceans, *Carcinus maenus* (Hume and Berlind, 1976) and *Homarus americanus* (present study), the salinity detection systems involved are sensitive to the concentration of certain ions rather than to overall osmolarity. Both molluscs are primarily sensitive to sodium, magnesium, and calcium, and only slightly responsive to changes in chloride levels (Davenport, 1981; Akberali and Davenport, 1982). Hume and Berlind (1976) were unable to determine if any single ion was detected during salinity reductions in *Carcinus maenus*. In

contrast, the lobsters in the present study exhibited the typical low-salinity response when exposed to saline solutions lacking chloride, even though the osmolarity of the artificial saline was identical to that of seawater. Moreover, they did not exhibit that response when exposed to solutions that lacked other ions but contained appropriate concentrations of chloride ions. Thus, although marine crustaceans may employ any of several mechanisms to detect changes in salinity, the American lobster appears to detect drops in salinity by monitoring changes in the concentration of chloride ions.

This study provides evidence that at least some salinity receptors in lobsters are located in or near the branchial chamber. A similar conclusion was reached by Hume and Berlind (1976) for *Carcinus maenas* and by Larimer (1964) for crayfish. Previous studies of various crustaceans have suggested that osmoreceptors might be located on the antennules or antennae (Lagerspetz and Mattila, 1961; Van Weel and Christofferson, 1966; Tazaki and Tanino, 1973), and the dactyls (Case *et al.,* 1960; Davenport, 1972; Davenport and Wankowski, 1973; Schmidt, 1989). However, in this investigation we did not find any evidence that the antennules, antennae, or legs were necessary for the detection of salinity changes in *Homarus americanus.*

There may be internal receptors for salinity or osmolarity in lobsters, but three lines of evidence strongly implicate external receptors. First, there was no statistically significant change in blood osmolarity during the first 10 min of our experiments, even though changes in heart rate typically occur within the first 5 min, when the external salinity had been reduced by 6 to 8 ppt. Second, when the branchial chamber was perfused with low-salinity water while the rest of the animal was exposed to normal seawater, bradycardia occurred very rapidly in response to very small drops in salinity. Finally, when the branchial chamber was selectively perfused with normal seawater, animals became less responsive to changes in external salinity. Their eventual response was typically correlated with a slight decrease in the branchial chamber salinity, which was difficult to maintain at 32 ppt when the external salinity reached low levels. Thus, while the available evidence suggests that external salinity receptors probably exist, further studies are clearly needed to better localize and characterize these sensory structures.

Lobsters inhabit estuarine and coastal habitats where storms and spring runoff often produce large drops in salinity that may last for days or weeks (Charmantier *et al.,* 2001). This puts a tremendous demand upon the limited ability of the animals to osmoregulate, causing a marked increase in metabolism and, at salinities less than 10 ppt, extensive mortality (McLeese, 1956; Thomas and White, 1969; Jury *et al.,* 1994a; Houchens, 1996). The avoidance responses to drops in salinity that lobsters exhibit in the laboratory probably serve in their natural habitat to move them to an area that might have a higher salinity (Jury *et al.,* 1994b, 1995). Although we have used bradycardia as an assay for detection of salinity and possibly as an index of an impending avoidance response, the true adaptive significance of this response still needs to be resolved. In the field, bradycardia would probably be triggered when reductions in salinity are rapid, long-lasting, or of sufficient magnitude to cause osmoregulatory stress. During the spring runoff season in the Great Bay estuary, the salinity typically drops at a rate of 0.2 ppt/min; the rate of decrease is probably even greater during a storm with heavy rains (see the UNH/CICEET IDEMS website: www.ciceet.unh.edu). It is likely that lobsters would detect such a change, and their reaction would be twofold. First, they would avoid the low-salinity water and seek deeper water, closer to the coast, that would have a higher salinity (Jury *et al.,* 1994b, 1995). Second, they would increase their metabolism and heart and ventilation rates to help fuel the Na^+/K^+-ATPases necessary to keep their blood osmolarity higher than the ambient water (Jury *et al.,* 1994a; Charmantier *et al.,* 2001). The metabolic demands of these behavioral and physiological adaptations are likely to be too large to allow both to occur simultaneously. Results from recent studies, in which we measured locomotion, ventilation, and heart rates while exposing lobsters to gradual drops in salinity, indicate that when they are faced with this dilemma, lobsters will eventually stop walking and give priority to osmoregulation (D. O'Grady, University of New Hampshire, unpubl. data). Field studies are necessary to further test this hypothesis and clarify how lobsters regulate their heart and ventilation rates in response to naturally occurring changes in their environment.

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Literature Cited

Able, K. W., K. L. Heck, Jr., M. P. Fahay, and C. T. Roman. 1988. Use of salt-marsh peat reefs by small juvenile lobsters on Cape Cod, Massachusetts. *Estuaries* **11:** 83–86.

Akberali, H. B., and J. Davenport. 1982. The detection of salinity changes by the marine bivalve molluscs *Scrobicularia plana* (da Costa) and *Mytilus edulis* L. *J. Exp. Mar. Biol. Ecol.* **58:** 59–71.

Atema, J., and R. Voigt. 1995. Behavior and sensory biology. Pp. 313–348 in *Biology of the Lobster,* Homarus americanus J. R. Factor, ed. Academic Press, New York.

Case, J., G. F. Gwillian, and F. Hanson. 1960. Dactyl chemoreceptors of brachyurans. *Biol. Bull.* **119:** 308.

Charmantier, G., C. Haond, J.-H. Lignot, and M. Charmantier-Daures. 2001. Ecophysiological adaptation to salinity throughout a life cycle: a review in homarid lobsters. *J. Exp. Biol.* **204:** 967–977.

Dall, W. 1970. Osmoregulation in the lobster *Homarus americanus. J. Fish. Res. Board Can.* **27:** 1123–1130.

Davenport, J. 1972. Salinity tolerances and preferences in the porcelain crab, *Porcellana platycheles* and *P. longicornis. Mar. Behav. Physiol.* **1:** 123–138.

Davenport, J. 1981. The opening response of mussels (*Mytilus edulis*) exposed to rising seawater concentrations. *J. Mar. Biol. Assoc. UK* **61:** 667–678.

Davenport, J., and J. Wankowski. 1973. Pre-immersion salinity-choice behavior in *Porcellana platycheles. Mar. Biol.* **22:** 313–316.

Florey, E., and M. E. Kriebel. 1974. The effects of temperature, anoxia and sensory stimulation on the heart rate of unrestrained crabs. *Comp. Biochem. Physiol.* **48A:** 285–300.

Gleeson, R. A., L. M. McDowell, and H. C. Aldrich. 1996. Structure of the aesthetasc (olfactory) sensilla of the blue crab, *Callinectes sapidus:* transformations as a function of salinity. *Cell Tissue Res.* **284:** 279–288.

Gleeson, R. A., M. G. Wheatly, and C. L. Reiber. 1997. Perireceptor mechanisms sustaining olfaction at low salinities: insight from the euryhaline blue crab *Callinectes sapidus. J. Exp. Biol.* **200:** 445–456.

Guirguis, M. S., and J. L. Wilkens. 1995. The role of the cardioregulatory nerves in mediating heart rate responses to locomotion, reduced stroke volume, and neurohormones in *Homarus americanus. Biol. Bull.* **188:** 179–185.

Houchens, C. R. 1996. A comparison of the osmoregulatory capabilities of estuarine and coastal populations of the American lobster, *Homarus americanus.* Master's thesis, University of New Hampshire, 76 pp.

Howell, W. H., W. H. Watson III, and S. H. Jury. 1999. Skewed sex ratio in an estuarine lobster (*Homarus americanus*) population. *J. Shellfish Res.* **18:** 193–201.

Hume, R. I., and A. Berlind. 1976. Heart and scaphognathite rate changes in a euryhaline crab, *Carcinus maenas,* exposed to dilute environmental medium. *Biol. Bull.* **150:** 241–254.

Johnson, B. R., R. Voigt, and J. Atema. 1989. Response properties of lobster chemoreceptor cells: response modulation by stimulus mixtures. *Physiol. Zool.* **62:** 559–579.

Jury, S. H., and W. H. Watson III. 2000. Thermosensitivity of the lobster, *Homarus americanus,* as determined by cardiac assay. *Biol. Bull.* **199:** 257–264.

Jury, S. H., M. T. Kinnison, W. H. Howell, and W. H. Watson III. 1994a. The effects of reduced salinity on lobster (*Homarus americanus* Milne Edwards) metabolism: implications for estuarine populations. *J. Exp. Mar. Biol. Ecol.* **176:** 167–185.

Jury, S. H., M. T. Kinnison, W. H. Howell, and W. H. Watson III. 1994b. The behavior of lobsters in response to reduced salinity. *J. Exp. Mar. Biol. Ecol.* **180:** 23–37.

Jury, S. H., W. H. Howell, and W. H. Watson III. 1995. Lobster movements in response to a hurricane. *Mar. Ecol. Prog. Ser.* **119:** 305–310.

Lagerspetz, K., and M. Mattila. 1961. Salinity reactions of some fresh- and brackish-water crustaceans. *Biol. Bull.* **120:** 44–53.

Larimer, J. 1964. Sensory-induced modifications of ventilation and heart rate in crayfish. *Comp. Biochem. Physiol.* **12:** 25–36.

Loder, T. C., J. A. Love, C. E. Penniman, and C. D. Neefus. 1983. Long-term environmental trends in nutrient and hydrographic data from the Great Bay Estuarine System, New Hampshire-Maine. University of New Hampshire Marine Program, UNH-MP-D/TR-SG-**83–6.** 65 pp.

Maynard, D. M. 1960. Circulation and heart function. Pp. 161–226 in *The Physiology of Crustacea,* Vol. I., T. H. Waterman, ed. Academic Press, New York.

McGaw, I. J., and B. R. McMahon. 1996. Cardiovascular responses resulting from variation in external salinity in the dungeness crab, *Cancer magister. Physiol. Zool.* **69:** 1384–1401.

McGaw, I. J., and C. L. Reiber. 1998. Circulatory modification in the blue crab *Callinectes sapidus,* during exposure and acclimation to low salinity. *Comp. Biochem. Physiol.* **121A:** 67–76.

McLeese, D. W. 1956. Effects of temperature, salinity and oxygen on the survival of the American lobster. *J. Fish. Res. Board Can.* **13:** 247–272.

McMahon, B. R. 1999. Intrinsic and extrinsic influences on cardiac rhythms in crustaceans. *Comp. Biochem. Physiol.* **124A:** 539–547.

Munro, J., and J. Therriault. 1983. Migrations saisonnières du homard (*Homarus americanus*) entre la côte et les lagunes des Îles-de-la-Madeleine. *Can. J. Fish. Aquat. Sci.* **40:** 905–918.

Offutt, G. C. 1970. Acoustic stimulus perception by the American lobster, *Homarus americanus* (Decapoda). *Experientia* **26:** 1276–1278.

O'Grady, D. F., S. H. Jury, and W. H. Watson III. 2001. The use of a treadmill to study the relationship between locomotion, ventilation and heart rate in the lobster, *Homarus americanus. Mar. Freshw. Res.* (In press).

Rose, R. A., J. L. Wilkens, and R. L. Walker. 1998. The effects of walking on heart rate, ventilation rate and acid-base status in the lobster *Homarus americanus. J. Exp. Biol.* **201:** 2601–2608.

Scarratt, D. J., and G. E. Raine. 1967. Avoidance of low salinity by newly hatched lobster larvae. *J. Fish. Res. Board Can.* **24:** 1403–1406.

Schmidt, M. 1989. The hair-peg organs of the shore crab, *Carcinus maenas* (Crustacea, Decapoda): ultrastructure and functional properties of sensilla sensitive to changes in seawater concentration. *Cell Tissue Res.* **257:** 609–621.

Short, F. T., ed. 1992. *The Ecology of the Great Bay Estuary, New Hampshire and Maine: An Estuarine Profile and Bibliography*. NOAA Coastal Ocean Program Publ. 222 pages.

Tazaki, K. 1975. Sensory units responsive to osmotic stimuli in the antennae of the spiny lobster, *Panulirus japonicus. Comp. Biochem. Physiol.* **51A:** 647–653.

Tazaki, K., and T. Tanino. 1973. Responses of osmotic concentration changes in the lobster antenna. *Experientia* **29:** 1090–1091.

Thomas, M. L. H., and G. N. White. 1969. Mass mortality of estuarine fauna at Biddeford P. E. associated with abnormally low salinities. *J. Fish. Res. Board Can.* **26:** 701–704.

Tierney, A. J., R. Voigt, and J. Atema. 1988. Response properties of chemoreceptors from the medial antennular filament of the lobster *Homarus americanus. Biol. Bull.* **174:** 364–372.

Van Weel, P. B. and J. P. Christofferson. 1966. Electrophysiological studies on perception in the antennules of certain crabs. *Physiol. Zool.* **39:** 317–325.

Watson, W. H. III, A. Vetrovs, and W. H. Howell. 1999. Lobster movements in an estuary. *Mar. Biol.* **134:** 65–75.