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Temperature and acid–base balance in the American lobster *Homarus americanus*

Syed Aman Qadri[1], Joseph Camacho[1], Hongkun Wang[2], Josi R. Taylor[3], Martin Grosell[3] and Mary Kate Worden[1,*]

[1]*Department of Neuroscience and* [2]*Division of Biostatistics and Epidemiology, University of Virginia, Charlottesville, VA 22908, USA and* [3]*Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, FL 33149, USA*

*Author for correspondence (e-mail: mkw3k@virginia.edu)

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Summary

Lobsters (*Homarus americanus*) in the wild inhabit ocean waters where temperature can vary over a broad range (0–25°C). To examine how environmental thermal variability might affect lobster physiology, we examine the effects of temperature and thermal change on the acid–base status of the lobster hemolymph. Total CO_2, pH, P_{CO_2} and HCO_3^- were measured in hemolymph sampled from lobsters acclimated to temperature in the laboratory as well as from lobsters acclimated to seasonal temperatures in the wild. Our results demonstrate that the change in hemolymph pH as a function of temperature follows the rule of constant relative alkalinity in lobsters acclimated to temperature over a period of weeks. However, thermal change can alter lobster acid–base status over a time course of minutes. Acute increases in temperature trigger a respiratory compensated metabolic acidosis of the hemolymph. Both the strength and frequency of the lobster heartbeat *in vitro* are modulated by changes in pH within the physiological range measured *in vivo*. These observations suggest that changes in acid–base status triggered by thermal variations in the environment might modulate lobster cardiac performance *in vivo*.

Key words: acid–base balance, lobster, pH, temperature, hemolymph.

Introduction

The marine environment inhabited by the American lobster *Homarus americanus* varies in temperature from 0 to 25°C depending on the seasons, the winds and the tides (Lawton and Lavalli, 1995). The breadth of this temperature range poses an interesting physiological challenge for the lobster because as poikilotherms they are unable to regulate their own body temperature. The body temperature of the lobster closely matches the external temperature of the seawater (Worden et al., 2006), therefore changes in seawater temperature also warm and cool lobster physiological systems. As demonstrated for other marine arthropods (Florey and Hoyle, 1976; Stenseng et al., 2005; Stillman and Somero, 2000; Young et al., 2006), temperature can be a potent modulator of the excitability of muscles and nerves because it alters the activity of ion channels and pumps in excitable membranes. The heart rate, stroke volume and heartbeat kinetics in *H. americanus* are all temperature dependent within the thermal range the lobster encounters in the wild (Worden et al., 2006). Moreover, the water temperature to which lobsters are acclimated determines the upper and lower thermal limits of lobster cardiac performance (Camacho et al., 2006).

Understanding the effects of temperature change and thermal stress on lobster physiology is particularly important given that a recent period of unusually warm water temperatures in Long Island Sound correlated with lobster mortality rates sufficiently high to collapse the commercial *H. americanus* fishery in that region (Pearce and Balcom, 2005). Furthermore, understanding how the signaling properties of the *H. americanus* nervous system are affected by the environmental conditions in which this species lives is also important given the long history of this species as a model system for studying the excitability of neural circuits, the dynamics of synaptic plasticity and the neural control of behavior (e.g. Battelle and Kravitz, 1978; Bucher et al., 2005; Edwards and Kravitz, 1997; Harris-Warrick and Kravitz, 1984; Heinrich et al., 1999; Huber et al., 1997; Kravitz, 1988; Kravitz et al., 1983; Kravitz et al., 1963; Livingstone et al., 1980; Mahadevan et al., 2004; Prinz et al., 2005; Richards et al., 1999).

The overall goal of this study is to determine how thermal change and thermal stress might affect the properties of lobster hemolymph that are important for neural and muscle physiology *in vivo*. Hemolymph is the blood that bathes the internal organs, muscles and synapses of the lobster to supply

gasses, nutrients and metabolites to the neurons and muscles (Martin and Hose, 1995). The ionic composition of the hemolymph is critically important for physiological function because it determines the transmembrane ion gradients that provide the driving force for sodium, calcium and potassium flux across neural and muscle membranes. Moreover, in other decapod crustaceans extracellular pH varies with temperature (Truchot, 1978), and the acid–base status of the hemolymph might regulate neural and muscle excitability.

Although the effects of temperature on hemolymph properties have been investigated in several species of marine crabs (Truchot, 1978; Wood and Cameron, 1985), the effects of temperature on the hemolymph of the lobster *H. americanus* are relatively unexplored. Recent observations that hemolymph of *H. americanus* acidifies by 0.2 pH units when lobsters acclimated to 16°C seawater are exposed to a temperature of 23°C for 3 weeks have been interpreted to suggest that prolonged thermal stress disrupts acid–base homeostasis in lobsters (Dove et al., 2005). However, in other decapod crustaceans and ectothermic vertebrate species the pH of the blood passively follows temperature to maintain a constant ratio of $[OH^-]/[H^+]$ according to the rule of constant relative alkalinity (Reeves, 1977; Truchot, 1978). In these species variations in pH as a function of thermal change are not interpreted as physiological anomalies attributable to thermal stress. Furthermore, because Dove and colleagues measured pH only at the end of the 3-week study period it is not clear whether the acidification they observed had occurred over a timescale of minutes, as demonstrated in crabs (Truchot, 1978), rather than over days or weeks.

To examine how water temperatures in the physiological range of 2–25°C might affect the acid–base properties of lobster hemolymph that bathes the excitable membranes of muscle and nerve, we investigated the effects of temperature change on the hemolymph of lobsters in the laboratory and in the wild. Our results demonstrate that temperature change alters lobster acid–base status both on long-term (weeks) and short-term (min) timescales. An abrupt temperature increase triggers a metabolic acidosis that is compensated by a temperature-dependent increase in ventilation rate. In addition, thermal change alters hemolymph pH within a physiological range that modulates cardiac activity *in vitro*, suggesting that the temperature dependence of cardiac performance *in vivo* is mediated in part by thermal changes in acid–base status.

Materials and methods

Lobsters (*Homarus americanus* Milne-Edwards 1837) were obtained from commercial sources and maintained in artificial seawater in a cold room (4–5°C) aquarium, in aquaria at room temperature (20–21°C), and in a temperature-controlled aquarium at 12°C. Animals were fed every 3 to 4 days. To facilitate sampling of postbranchial hemolymph, a sampling port overlying the pericardial sinus was drilled into the dorsal carapace and sealed with a latex covering. All animals were allowed a 48 h recovery period before hemolymph sampling began. Hemolymph samples of volumes 0.4–1.0 ml were withdrawn using iced gastight Hamilton syringes and centrifuged for 3 min at 13,000 ***g*** in a refrigerated (4°C) centrifuge to prevent clotting. The supernatant was removed to plastic Eppendorf tubes and brought to the temperature to which the lobster was acclimated.

pH was immediately measured using a pH meter (Accumet BASIC, Fisher Scientific) with a combination pH electrode with silver/silver chloride references (Fisher Scientific). Measurements were made non-anaerobically with reference to certified buffers at the same temperature. Hemolymph samples were stored overnight on ice before assaying total CO_2 levels. Preliminary experiments (N=3) on hemolymph from lobsters acclimated to a temperature of 12°C demonstrated that storing samples overnight on ice did not affect total CO_2 measurements (11.40±2.59 compared with 11.43±1.79; P=0.94 in a paired t-test). CO_2 was measured using a Corning 965 carbon dioxide analyzer (Corning, NY, USA). Levels of bicarbonate, carbonate and partial pressure of CO_2 (P_{CO_2}) were calculated from total CO_2 levels and pH using the Henderson-Hasselbach equation, the CO_2Sys software (Lewis, 1996) and seawater pK_I and pK_{II} constants appropriate for the experimental temperature.

Hemolymph samples were drawn both from lobsters acclimated to artificial seawater in laboratory aquaria and from lobsters acclimated to the ambient seasonal temperatures of natural seawater in February 2006 (5°C) at the Marine Biological Laboratory in Woods Hole, MA, USA and in July 2006 (16°C) at the Mount Desert Island Biological Laboratory in Salisbury Cove, ME, USA. The majority of samples drawn from laboratory lobsters were postbranchial hemolymph sampled from the pericardial sinus. In lobsters in Maine and Woods Hole (and occasionally in laboratory lobsters) prebranchial hemolymph was sampled from the infrabranchial sinus at the base of the walking legs. To verify whether prebranchial and postbranchial hemolymph would be comparable we compared hemolymph properties of pre- and postbranchial hemolymph from single individuals. In agreement with previous reports (Booth et al., 1984; Cameron and Batterton, 1978; Rose et al., 1998; Truchot, 1978) there were no significant differences in pH or total CO_2 in prebranchial and postbranchial samples in quiescent animals [For pH: P=0.21 (N=8) at 12°C, P=0.83 (N=9) at 22°C. For total CO_2: P=0.11 (N=4) at 12°C, P=0.92 (N=3) at 22°C; all values were tested with a paired t-test]. As discussed by Truchot, the similarity between prebranchial and postbrancial hemolymph values of pH and CO_2 can be attributed both to the low oxygen-carrying capacity of crustacean hemolymph and the high buffering capacity of hemolymph proteins (Truchot, 1978).

Recordings of cardiac activity *in vitro* were performed as described previously (Worden et al., 2006). Briefly, hemolymph enters the single chamber of the lobster heart through paired ostia and is pumped out through seven arteries. Isolated hearts continue to beat because of rhythmic neural output from the cardiac ganglion, located on the dorsal inner

wall of the heart. Following isolation of the heart, the sternal artery was cannulated and perfused with temperature-controlled lobster saline at 3.5 ml min^{-1} to maintain stretch. The antennal arteries were tied off with 6.0 surgical silk and attached to a tension transducer (model FT-03; Grass Instruments, Quincy, MA, USA) and amplifier (CyberAmp model 320; Axon Instruments, Union City, CA, USA) to record contractions of the heart during each heartbeat. All physiological signals were recorded on VCR and digitized by an analog to digital converter using pClamp software (Digidata1200 A-D converter and pClamp software from Axon Instruments-Molecular Devices, Union City, CA, USA).

Chronic electrodes were implanted to record heart rate and ventilation rate by measuring changes in impedance resulting from movements of the heart and the gill balers (scaphnogathites), respectively. The procedure for implantation of chronic electrodes for recording lobster cardiac activity *in vivo* has been described previously (Worden et al., 2006). Briefly, two wires insulated to within 0.5 cm of their tips were inserted through small holes drilled 3 cm apart in the dorsal carapace overlying the heart and glued in place. To record ventilation a second set of electrodes was implanted on one side of the ventrolateral thorax in the region overlying the gills. All animals were allowed a 48 h recovery period before physiological experiments began. Control experiments verified that implantation of the electrodes did not alter the pH of the hemolymph.

To measure heart and ventilation rates individual lobsters were placed in a temperature-controlled chamber and acclimated to a temperature of 2°C for at least 30 min before the water was warmed at a rate of approximately 0.75°C min^{-1} to a maximal value of 30°C. Heart and ventilation rates were measured in the same experiments by alternating recording periods of 30 s for each parameter as a function of temperature. Signals from the electrodes were input to an impedance converter (UFI model 2991), digitized by pClamp software and stored on VCR tapes. In contrast to the heart, which beat rhythmically at all temperatures, the movements of the gill balers were interrupted occasionally by periods when ventilation spontaneously ceased. If ventilation halted during the recording period, the ventilation rate was calculated over a period of at least 15 s where ventilation occurred. Trials in which ventilation could not be recorded were not included in calculations of mean ventilation rates (<2% of observations). Lobsters were cold acclimated by housing them in aquaria at a temperature of 4°C for 3 weeks or more. Warm acclimated lobsters were housed at a temperature of 20°C for a period of at least 2 weeks before data were recorded.

Repeated-measures models (Crowder and Hand, 1990) were used for the analyses of experiments involving multiple measurements to investigate specific hypotheses concerning comparisons between groups or to specific temperatures. F-tests were used to compare the values for cardiac parameters at different temperatures. Linear spline models were fit to analyze the relationship between the temperature and ventilation rate as well as heart rate. Analyses were performed using SAS software (The SAS Institute, Cary, NC, USA) version 9.1 module 'PROC MIXED'.

Results

Resting acid–base status depends on acclimation temperature

Hemolymph pH in *Homarus americanus* is inversely related to temperature (T) throughout the temperature range from 4–22°C (filled symbols in Fig. 1A), with a $\Delta pH/\Delta T$ coefficient of –0.011 pH $°C^{-1}$. Values of hemolymph pH measured in lobsters acclimated to natural seawater at ambient seasonal temperatures were in good agreement (open symbols). Lobsters acclimated to warmer temperatures also had significantly higher heart rates (see Fig. 1, inset) and relatively high levels of locomotor and startle activity (data not shown) compared with cold acclimated lobsters.

To our surprise, these pH values are significantly more alkaline than those reported in previous studies in which lobster hemolymph was stored on ice or frozen before pH was measured. Dove et al., for example, reported *H. americanus* hemolymph pH values in the range 7.2–7.4 (Dove et al., 2005). To test whether storing hemolymph might affect pH values we compared pH in the same samples before and after overnight storage on ice or at –80°C. In 20 samples drawn over a range of acclimation temperatures, the value of the pH dropped significantly [from 7.83±0.16 (mean ± s.e.m.) to 7.77±0.129;

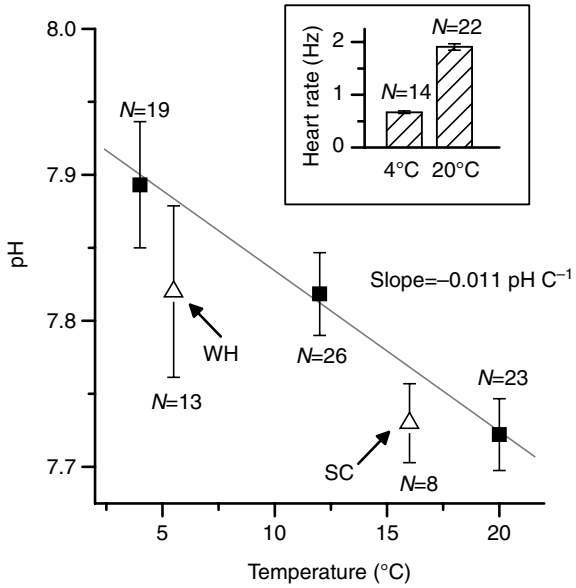

Fig. 1. Hemolymph pH varies as a function of acclimation temperature. Symbols represent mean (± s.e.m.) values for pH measured in lobsters acclimated to the indicated temperatures in artificial seawater in the laboratory (filled symbols) or to the ambient temperature of natural seawater in Woods Hole (MA, USA) in February (open symbols, WH) and in Salisbury Cove (ME, USA) in July (open symbols, SC). Numbers of samples measured under each condition are indicated. Inset: heart rates (mean ± s.e.m.) averaged over a period of 1 min in quiescent lobsters acclimated in the laboratory to water temperatures of 4 and 20°C.

two-sample paired *t*-test, P=0.02] after storage on ice overnight. In a similar experiment in which samples (N=11) were stored at –80°C for 12 days the pH value also dropped significantly (from 7.72±0.22 to 7.601±0.14; two-sample paired *t*-test, P=0.03). Slopes of linear fits to plots of the change in hemolymph pH relative to the original pH were –0.391 for samples stored on ice and –0.511 for samples stored at –80°C. Both values are significantly different from zero (P=0.0093 and P=0.0026, respectively), demonstrating that samples that were originally the most alkaline showed the greatest degree of acidification as a consequence of storage. Therefore, subsequent experiments were designed such that pH was always measured immediately after withdrawal of the hemolymph, to avoid potential artifacts associated with storage of the samples.

Other parameters of acid–base status in the lobster also differ as a function of the temperature to which the lobsters are acclimated. Fig. 2 shows the total CO_2 measured in the hemolymph of lobsters acclimated to different temperatures as well as the concentrations of bicarbonate, carbonate and P_{CO_2} in hemolymph calculated from the pH and total CO_2 values of the samples. Total CO_2 depends on water temperature, with the highest values measured at 12°C and significantly lower values at the extremes of 4°C and 20°C (Fig. 2A). Bicarbonate levels, like total CO_2 levels, were significantly higher at 12°C than they were at 4 or 20°C (Fig. 2B). Carbonate levels did not change between 4 and 12°C but were significantly lower at 20°C (Fig. 2C). Levels of P_{CO_2} doubled between 4 and 12°C, but did not change significantly at higher temperatures (Fig. 2D).

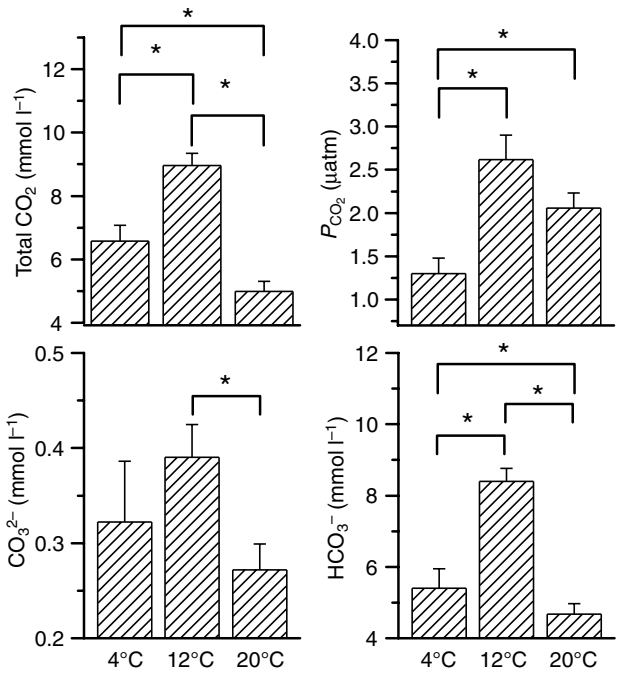

Fig. 2. Hemolymph levels (mean ± s.e.m.) of total CO_2, P_{CO_2}, CO_3^{2-} and HCO_3^- vary with acclimation temperature. *Data are significantly different at P<0.05 (two-sample independent *t*-test). Conversion factor: 1 Pa=9.86×10^{-6} atm.

The time course of acid–base changes in response to acute temperature change

A recent study reporting a significant drop in hemolymph pH in lobsters exposed to 3 weeks of warm (23°C) temperatures attributed the acidosis to 'prolonged thermal stress' (Dove et al., 2005). To test whether hemolymph pH acidifies over several weeks of acclimation over a range of temperatures, we repeatedly sampled hemolymph pH in populations of lobsters undergoing 3 weeks of acclimation to water temperatures of 4, 12 and 20°C. Hemolymph pH did not vary significantly over time at acclimation temperatures of 4 and 12°C (P values for linear fits were P=0.28 and P=0.09, respectively), suggesting that pH remains relatively stable at colder acclimation temperatures. However, pH values measured in lobsters housed at 20°C tended to become more acidic over the 3 weeks of acclimation, at a rate of 0.0075 pH units day^{-1} (P=0.004). At this rate, calculations of the change in hemolymph pH in lobsters over 3 weeks of exposure to 20°C would predict acidification by an average of approximately 0.16 pH units.

To test how quickly hemolymph pH might change during acute temperature shifts we measured pH repeatedly in a single lobster as the temperature of the surrounding seawater increased from 2 to 12°C over a 35-min time period. Fig. 3 illustrates the results from one of four experiments of this type. Hemolymph pH acidified rapidly as temperature warmed, dropping from 8.3 to 8.1 over a 3-min period before leveling off at 7.85 (Fig. 3A).

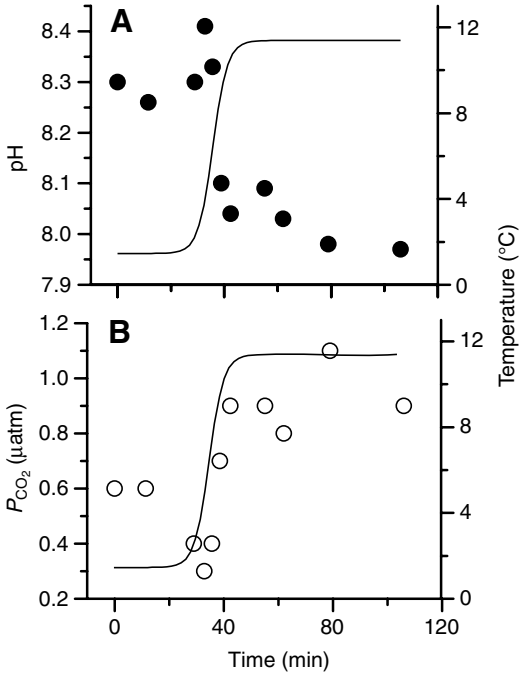

Fig. 3. The acid–base status of the hemolymph in a single lobster changes rapidly in response to a temperature increase. (A) Hemolymph pH (filled symbols) repeatedly sampled in a single lobster as the seawater temperature warmed from 2 to 12°C. (B) Hemolymph P_{CO_2} (open symbols) measured in the same samples. In both plots the line indicates the kinetics of the temperature change from 2 to 12°C. Conversion factor: 1 Pa=9.86×10^{-6} atm.

Over the same time course levels of hemolymph P_{CO_2} approximately doubled (Fig. 3B). These results qualitatively demonstrate that acute thermal change can strongly and rapidly alter the acid–base status of the hemolymph.

Quantitative measurements of the time course of changes in hemolymph acid–base status measured in a population of lobsters subjected to an abrupt temperature increase from 4 to 20°C are shown in Fig. 4. Hemolymph pH acidified by more than 0.3 pH units within the first 10 min and then remained stable for 6 h before recovering to control values at 24 h (Fig. 4A). The corresponding decreases in the concentrations of total CO_2 and HCO_3^- over 24 h were not statistically significant (Fig. 4B,C). However, values of P_{CO_2} (Fig. 4D) more than doubled over the first 10 min, remained stable at 2 h and recovered completely at 6 h. By 24 h, P_{CO_2} had decreased significantly compared with control values. The Davenport diagram illustrating these changes in acid–base status (Fig. 5) shows that the initial transient acidosis and subsequent recovery of hemolymph pH is not accompanied by significant changes in total CO_2.

Finally, to test whether changes in pH might alter lobster physiology we examined the effects of varying pH on the beating of the neurogenic lobster heart *in vitro*. Fig. 6 shows that shifting pH between the value of the traditional lobster saline (7.4) and a value close to the upper limit of the physiological range measured in the hemolymph *in vivo* (8.1) alters the frequency and strength of the lobster heartbeat. In this

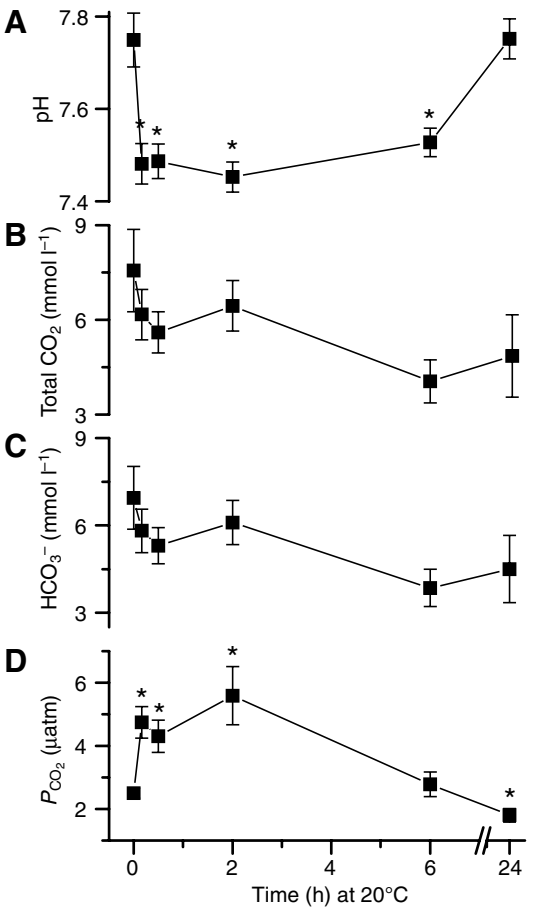

Fig. 4. The time course of the change in acid–base status of a population (N=9) of lobsters abruptly exposed to a temperature change from 4 to 22°C. Symbols represent means ± s.e.m. *Data are significantly different from those at time 0 at P<0.05. Conversion factor: 1 Pa=9.86×10^{-6} atm.

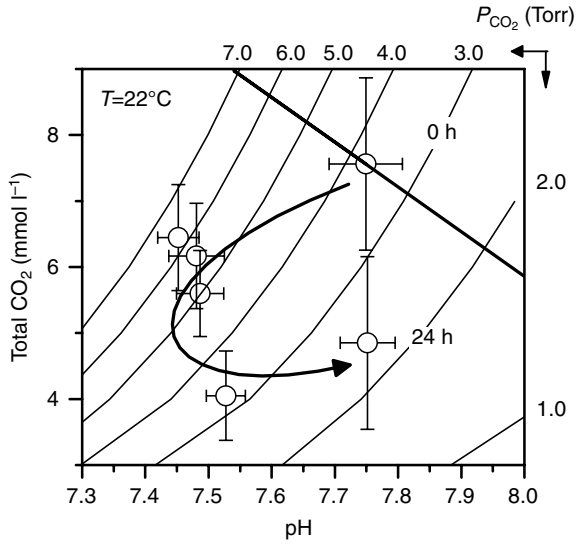

Fig. 5. Davenport diagram illustrating changes in acid–base status (mean ± s.e.m.) over 24 h following an abrupt temperature change from 4 to 22°C. Iso-P_{CO_2} (in µatm) lines are drawn. The buffer line at time 0 h demonstrates the hemolymph buffering capacity of *H. americanus* reported by Rose et al. (Rose et al., 1998) (–6.8 CO_2 l^{-1} pH^{-1} unit). Conversion factor: 1 Torr=133.3 Pa.

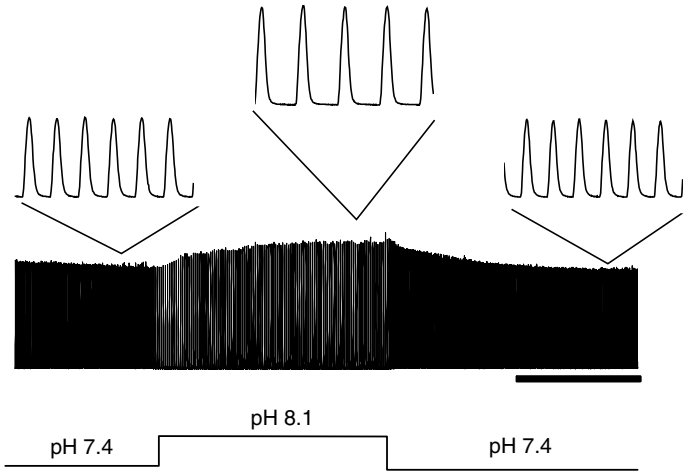

Fig. 6. Changes in pH modulate lobster cardiac activity. The middle trace shows a continuous 10 min tension record of the spontaneous beating of the neurogenic lobster heart *in vitro* at 16°C. The pH of the saline perfusing the heart is indicated below the trace. Upper traces show three 8 s samples of tension recordings selected from the indicated portions of the 10 min recording to illustrate changes in the amplitude and frequency of the heartbeat. All traces are shown with the same vertical amplification. Scale bar, 2 min.

experiment the change in pH decreased the frequency of the heartbeat by 17% and increased the amplitude of the heartbeat by 21%. Both effects were completely reversible. Similar results were obtained in two other heart preparations.

Respiratory rate increases as a function of temperature

Our observation that hemolymph pH, total CO_2 and P_{CO_2} levels are temperature dependent raises the possibility that the lobster's physiological response to thermal change might include changes in respiration. To test this directly we measured the temperature dependence of ventilation rates in individual lobsters and made simultaneous measurements of heart rates for comparison. In agreement with previous reports (Camacho et al., 2006; Worden et al., 2006), lobster heart rates increase over the temperature range between 2 and 20°C but decrease at higher temperatures (Fig. 7A). In addition, heart rates are significantly higher in warm acclimated lobsters exposed to temperatures of 22°C and above. In the same lobsters the rate of ventilation also increased as a function of temperature (Fig. 7B), with warm acclimated lobsters exhibiting significantly higher respiratory rates at temperatures >10°C in comparison with cold acclimated lobsters.

The rhythm of the ventilatory pattern also differed between cold acclimated and warm acclimated lobsters. In experiments on cold acclimated lobsters we frequently observed spontaneous cessations of gill baler movements lasting tens of seconds, particularly at temperatures of 14°C and warmer. Interruptions of the ventilatory rhythm were never observed in experiments on warm acclimated lobsters. It was not possible to verify whether these interruptions represent true cessation of respiration because electrodes were implanted only on one side of the body and crustaceans can independently regulate the movement of the gill baler on the left and right side (McMahon, 1999). However, it is important to note that because mean rates of ventilation were calculated by excluding periods when ventilation ceased (see Materials and methods), the plots in Fig. 7B overestimate the ventilation rates in cold acclimated lobsters and therefore underestimate the differences between ventilation rates in cold- and warm-acclimated lobsters.

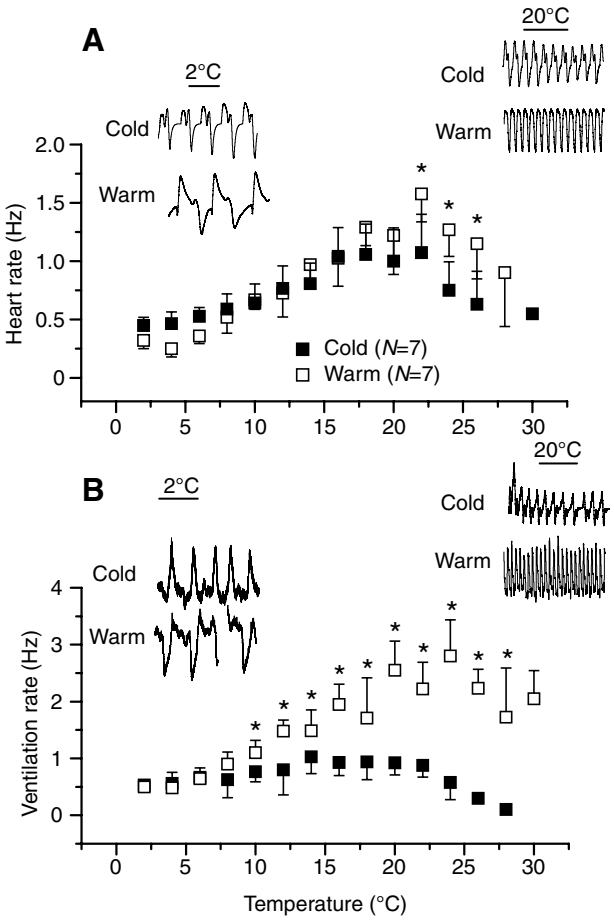

Fig. 7. Thermal acclimation alters the temperature dependence of heart rate and ventilation. Heart and ventilation rates (mean ± s.d.) were measured simultaneously in single lobsters acclimated to cold (4°C; N=7) and warm (20°C; N=7) temperatures. (A) Heart rates are temperature dependent. For warm-acclimated lobsters the temperature-dependent increase in heart rate (slope=0.0635) is statistically significant (P=0.0001), whereas for cold-acclimated lobsters it is not (slope=0.04; P=0.06). The temperature-dependent decrease in heart rate at temperatures of 20°C and above is significant for both warm-acclimated and cold-acclimated lobsters (slope=–0.056, P<0.0001 and slope=–0.036, P<0.0001, respectively). (B) Ventilation rate increases as a function of temperature up to 20°C. For both warm- and cold-acclimated lobsters the increase in ventilation at temperatures ⩽20°C is statistically significant (slope=0.1136; P<0.0001 for warm-acclimated lobsters; slope=0.0235, P=0.0001 for cold-acclimated lobsters). At temperatures >20°C ventilation decreases significantly in warm-acclimated lobsters (slope=–0.052, P=0.0001). In cold-acclimated lobsters, the temperature dependence of ventilation decreases at temperatures >20°C, although not to a significant extent (slope=–0.09, P=0.413). *Data from cold- and warm-acclimated lobsters are significantly different at P<0.05. Insets show samples of traces from ventilation and heart recordings in warm- and cold-acclimated lobsters at 2 and 20°C. Values of N are ⩾5, except as follows: heart rate in cold-acclimated animals at 30°C (N=1), heart rate in warm-acclimated animals at 26°C (N=4) and 30°C (N=2), ventilation in cold-acclimated animals at 6°C (N=3), at 22°C (N=4), at 24°C (N=4), at 26°C (N=1) and at 28°C (N=1), and ventilation in warm-acclimated animals at 2°C (N=4), at 28°C (N=4) and at 30°C (N=2).

Discussion

The temperature dependence of hemolymph pH

Temperature is an environmental factor of major importance to the lobster *Homarus americanus* in the wild because it regulates multiple biological processes, including synaptic signaling (Worden and Camacho, 2006), cardiac function (Camacho et al., 2006; Worden et al., 2006), growth and reproduction (Ennis, 1995; Waddy et al., 1995), and locomotion into lobster traps (Drinkwater et al., 2006). As reported for other crustacean species, we find the pH of the hemolymph of *H. americanus* varies inversely with temperature. The value for the coefficient $\Delta pH/\Delta T$ (–0.011 pH °C^{-1}) in *H. americanus* is in good agreement with that measured in other marine decapods (reviewed by Truchot, 1983), as well as with the coefficient describing the variations

of the neutral point of pure water with temperature, suggesting that lobster hemolymph follows the rule of relative alkalinity. Over the temperature range from 4 to 20°C hemolymph pH values in the lobster decrease from a mean value of 7.9 to nearly 7.7. These values are similar to those measured over the same thermal range in the crabs *Carcinus maenas* (Truchot, 1978) and over the thermal range 10–30°C in the blue crab *Callinectes* (Wood and Cameron, 1985) and at a temperature of 14–15°C in the lobster *Homarus vulgaris* (a species also known as *Homarus gammarus*) (McMahon et al., 1978).

However, previous studies of the properties of the hemolymph in *H. americanus* reported more acidic values for pH: 7.45–7.61 for lobsters in different seasons (Cole, 1940), 7.6 for lobsters at 15°C (Stewart et al., 1966), and values in the range 7.2–7.4 for lobsters maintained at 16 or 23°C (Dove et al., 2005). We suspect that some of these pH values might be artificially depressed because we have observed that hemolymph acidifies with storage (see Results), and authors of previous studies (Dove et al., 2005; Stewart et al., 1966) stored hemolymph before measuring pH. Moreover, earlier authors did not comment on whether their lobsters struggled during the sampling procedure. We noted that pH values were more acidic in lobsters that struggled during sampling (data not shown), in agreement with previous reports that pH of the hemolymph in *H. vulgaris* acidifies in response to air exposure and struggling during handling (McMahon et al., 1978) and that the acid–base chemistry of *H. americanus* hemolymph varies as a function of exercise, handling and disturbance (McMahon, 1995; Rose et al., 1998). Overall, these results suggest the importance of sampling from quiescent animals, minimizing handling procedures and assaying pH immediately after hemolymph withdrawal in order to obtain the most accurate measures.

Interestingly, many previous neurophysiological studies have employed a saline developed specifically for *H. americanus* in which pH is buffered to 7.4 (e.g. Bykhovskaia et al., 1999; Bykhovskaia et al., 2004; Golan et al., 1994; Golan et al., 1996; Goy and Kravitz, 1989; Grossman and Kendig, 1990; Kravitz et al., 1980; Vorob'eva et al., 1999; Worden et al., 2006; Worden et al., 1997; Worden and Camacho, 2006; Worden et al., 1995). The composition of this saline is based on a 'perfusing solution' that Cole developed more than 60 years ago by comparing a series of solutions with varying concentrations of sodium, potassium, calcium, magnesium and sulfate to determine which was the optimal mixture for maintaining the strength and frequency of the lobster heartbeat *in vitro* at 17°C (Cole, 1941). The pH of all the solutions was buffered to 7.3–7.5, slightly lower than the author's previous measures of hemolymph pH values of 7.45–7.61 in intact lobsters (Cole, 1940). A value of 7.4 is more acidic than any we measured in this study, even in samples from lobsters housed at the warmest acclimation temperature. Whether the pH of the traditional lobster saline is appropriate physiologically should be of particular concern in the design of experiments performed at cold temperatures, in which hemolymph in the intact animal is most alkaline and the discrepancy between hemolymph pH and the traditional *Homarus* saline pH is the greatest. Cold (≤5°C) temperature protocols have been used in studies of lobster neuromuscular transmission as a method of improving quantal resolution by decreasing the synchrony of quantal neurotransmitter release (Bykhovskaia et al., 1999; Worden et al., 1997) and enhancing the strength of inhibitory synaptic potentials (Worden and Camacho, 2006).

Our demonstration that shifting pH within the physiological range 7.4–8.1 alters both the frequency and the strength of the lobster heartbeat (Fig. 6) suggests that thermally triggered changes in hemolymph pH could modulate cardiac physiology *in vivo*. Previous studies of the temperature dependence of lobster cardiac performance have demonstrated that temperature change directly modulates the heartbeat strength, frequency and kinetics of isolated hearts bathed in saline (at pH 7.4) *in vitro*, whereas heart rates measured *in vivo* are similarly temperature dependent but faster (Worden et al., 2006). The higher heart rates observed *in vivo* have been ascribed to the presence of neural and hormonal inputs in the intact animal that are absent in the isolated heart. However, the results of the present study suggest another possibility: intact lobsters exposed to increases in seawater temperature will experience a fall in hemolymph pH in addition to warming of the hemolymph and internal tissues, and both effects tend to increase heart rate. Additional experiments will be required to determine whether pH might affect the neurophysiological properties of neurocardiac synapses on the heart or the process of excitation–contraction of lobster cardiac muscle. Precedence for the idea that extracellular pH modulates muscle membrane currents has been described for crayfish skeletal muscle (Pasternack et al., 1992).

Finally, Dove et al. observed a depression in hemolymph pH by –0.2 pH units in lobsters moved from 16°C to 23°C for a period of 3 weeks and attributed the acidosis to the 'prolonged thermal stress' (Dove et al., 2005). Although our results confirm that the pH of lobster hemolymph decreases slowly by approximately –0.16 units over 3 weeks of acclimation to warm (20°C) water, we also observed that hemolymph pH changed by 0.2 to 0.3 pH units within minutes of a temperature increase from 2 to 12°C or from 4 to 20°C (see Figs 3 and 4, respectively). These results demonstrate that thermal stimuli that are neither prolonged nor sufficiently warm to be physiologically stressful can trigger significant changes in hemolymph pH. A temperature change from 4 to 12°C (see Fig. 3), for example, is within the cooler half of the entire temperature range lobsters inhabit in their natural habitat and yet it depresses hemolymph pH within minutes. We interpret the temperature-dependent changes in lobster hemolymph pH as a passive response to temperature that approximates to that of water (the rule of relative constant alkalinity), rather than as a true acidosis.

Temperature and acid–base balance in crustaceans

Although the temperature dependence of hemolymph pH in *H. americanus* is similar to that previously reported for the crab *C. meanus*, a species that shares its geographical and thermal

habitat, our data also suggest that these two species differ in terms of the temperature dependence of total CO_2. Crabs acclimated to different water temperatures show a monotonic decrease in total CO_2 as temperature increases from 5 to 25°C (Truchot, 1978). By contrast, in temperature-acclimated lobsters total CO_2 peaks at 12°C and is lower at cold (4°C) and warm (20°C) temperature extremes (see Fig. 2), an effect than can be attributed primarily to the temperature dependence of the lobster hemolymph bicarbonate concentration. In addition, P_{CO_2} in *C. maenas* increases linearly as a function of temperature, doubling between 5 and 25°C. By contrast, in lobster P_{CO_2} doubles between 4 and 12°C and then decreases by approximately 20% at 20°C. Overall, the primary differences between temperature-acclimated lobster and crabs in terms of the temperature dependence of total CO_2, HCO_3^- and P_{CO_2} appear at the coldest temperatures. Compared with the mid-range temperature of 12°C, levels of CO_2 and HCO_3^- at 4°C are depressed in lobster but elevated in the crab.

Another difference between these two species is that their acid–base status undergoes different changes in response to abrupt temperature increases. Both *C. maenas* and *H. americanus* initially respond to a temperature increase with hemolymph acidosis and an increase in blood P_{CO_2} that occurs within the first 10 min and is sustained over the next 6 h. By 24 h both parameters recover to near control values in the lobster (see Fig. 4). By contrast, neither parameter returns to control values in the crab; pH remains relatively stable and acidotic whereas P_{CO_2} remains stable and elevated by more than 50% [see fig. 2 of Truchot (Truchot, 1978)]. Truchot concluded that crab hemolymph maintains a constant acid–base state as defined by the relative alkalinity. Whereas lobsters acclimated to temperature over days to weeks also follow the rule of relative alkalinity (see Fig. 1), an abrupt temperature increase from 4 to 20°C produces changes in lobster hemolymph acid–base status over 24 h that are consistent with a respiratory compensated metabolic acidosis (see Fig. 5). Respiratory compensated metabolic acidosis has previously been observed in the crab *C. magister* following strenuous exercise (McDonald et al., 1979), but not in the lobster *H. americanus*, where exercise triggers a predominantly respiratory acidosis (Rose et al., 1998). The results of the present study suggest that the acute and transient decrease in hemolymph pH that occurs when lobsters are subjected to an abrupt temperature elevation will be followed by a gradual decrease in pH over the subsequent days and weeks of acclimation to warm temperature.

It is unclear as to what accounts for the differences between our observations on *H. americanus* and earlier reports describing acid–base regulation in the crab. Although it is possible that there are true species-specific differences in respiratory physiology between these species, it may also be the case that differences in experimental approaches can explain the apparent discrepancies in the results. For example, Truchot reported that gill ventilation does not increase as a function of temperature in *C. maenas*, based on an indirect method in which he calculated water convection requirements from oxygen consumption records recorded intermittently over a period of days and only after (not during) a temperature change (Truchot, 1983). By contrast, we directly and continually observed ventilation rates in *H. americanus* both during and after a temperature change. Further experiments will be required to resolve the interesting issue of whether there are true differences between the respiratory physiology of lobsters and crabs, and how these differences might relate to the temperature dependence of the behavioral ecology of each species.

Our observation that ventilation rates increase significantly in laboratory lobsters as temperature warms (Fig. 7B) are in agreement with previous observations that lobster ventilation rates increase in the wild as the ocean waters seasonally warm (Mercaldoallen and Thurberg, 1987). In the present study, lobsters acclimated to warm (20°C) temperatures exhibited especially strong temperature-dependent increases in ventilation: at temperatures of 20–26°C ventilation rates in warm-acclimated lobsters were 2.5- to 7-fold higher than those measured in cold (4°C)-acclimated lobsters. Warm acclimation also increases heart rates at warm (20–26°C) temperatures, although the magnitude of this increase is much smaller, in the order of 50–60% (see Fig. 7A), in agreement with previous results (Camacho et al., 2006). Overall, these observations suggest (1) that thermal acclimation alters the neural output of the circuits that drive the beating of the heart and the movement of the gill balers, and (2) that the circuitry driving respiration is more sensitive to warm acclimation than the circuitry driving the heart. The factors that determine the upper limit of thermal tolerance in lobster are unknown but are likely to be related to the inability of ventilatory and circulatory systems to supply sufficient oxygen (or other metabolites) to the tissues, as shown for the spider crab *Maja squinado* (Frederich and Portner, 2000).

Overall, the results of this study suggest that several factors play a role in determining the temperature dependence of lobster hemolymph pH. From 12 to 20°C $\Delta pH/\Delta T$ can be attributed to the decrease in $[HCO_3^-]$ and increase in metabolism at high temperatures, as shown by the increase in heart rate (see Fig. 1, inset). However, from 4 to 12°C $\Delta pH/\Delta T$ can be attributed to the increase in P_{CO_2}, with this change explained by the classic closed system of the hemolymph (Reeves, 1977). Wood and Cameron observed a similar phenomenon in *Callinectes sapidus* and noted that it is not clear why this should occur in a water breather, because CO_2 is generally excreted easily across the gills (Wood and Cameron, 1985). However, because P_{CO_2} is relatively low in crustacean blood, even relatively small changes in P_{CO_2} can have a large effect on pH, as demonstrated for fish blood in which measured P_{CO_2} levels are in the same range (Perry and Wood, 1989).

In summary, thermal acclimation and thermal change alter not only the acid–base properties of lobster hemolymph but the properties of the neural circuits driving lobster ventilation and cardiac activity. In response to abrupt temperature increases lobsters undergo a respiratory compensated metabolic acidosis of sufficient magnitude that the fall in hemolymph pH can

modulate the strength and frequency of the heartbeat. These observations suggest that thermally triggered changes in hemolymph acid–base status can act to modulate lobster cardiac function, and perhaps other physiological systems as well. In their native habitat, lobsters experience thermal change as water currents flowing along the ocean floor transiently engulf them, as storms churn the ocean waters, as tides ebb and flow and as the seasons progress. Given that the environmental stress of high seawater temperatures has been linked both to the prevalence and spatial distribution of lobster shell disease (Glenn and Pugh, 2005) and to unusually high levels of lobster mortality and low levels of abundance of *H. americanus* in Southern New England and Long Island Sound waters in recent years (Howell et al., 2005), understanding the thermosensitivity and thermal tolerance of this commercially valuable species becomes especially important. Interestingly, lobsters acclimated to warm (20–22°C) temperatures appear especially physiologically efficient at delivering oxygen to the tissues and clearing P_{CO_2} from the hemolymph, suggesting that they can partially compensate for thermal stress that might otherwise endanger the health and survival of this species.

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