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Effects of temperature and thermal history on neuromuscular properties of two crustacean species

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Summary. The effect of temperature on the ability of neuromuscular junctions and muscle fibers to contract, release neurotransmitter, and maintain postsynaptic membrane properties, was measured in vivo and in vitro in claw closer muscles in stone crabs *Menippe mercenaria* (Say) and blue crabs *Callinectes sapidus* (Rathbun).

In vivo muscle stress (defined as force generated per unit of muscle cross-sectional area) was measured in crabs of both species collected from northern populations (annual temperature range ~2–30 °C) and southern populations (annual temperature range ~15–30 °C). Muscle stress was compared between (1) crabs of both species maintained in the laboratory at 30 °C (laboratory warmed); (2) crabs given a brief acclimation period (4 weeks for blue crabs; 7 weeks for stone crabs) at 8 °C in the laboratory (laboratory cooled), and (3) stone crabs that had been naturally acclimated from summer (30 °C) to winter (8 °C) temperatures over a 6 month period in the field (naturally cooled). No differences were found in the abilities of the northern and southern populations of either species to generate muscle stress when tested at summer temperatures (30 °C) common to both populations. Northern and southern blue crabs produced similar levels of muscle stress whether laboratory warmed (30 °C) or laboratory cooled (8 °C). Conversely, northern and southern stone crabs showed significantly reduced muscle stress in laboratory cooled crabs compared with laboratory warmed crabs. Stone crabs from both populations generated the same amount of muscle stress after having been naturally cooled to 8 °C as they had generated the previous summer (30 °C).

In vitro neuromuscular properties (i.e. (1) muscle stress as a measure of contractile ability; (2) excitatory junction potential (EJP) amplitude as a measure of neurotransmitter release; (3) specific membrane resistance (R_m) as a measure of postsynaptic membrane properties) were compared at 8, 20, and 30 °C between northern cold acclimated (naturally cooled stone crabs and laboratory cooled blue crabs) and non-cold acclimated (laboratory cooled stone crabs and laboratory warmed blue crabs) crabs. Muscle fibers in claws of stone crabs and blue crabs showing cold acclimation had higher R_m at 8 °C than non-cold acclimated crabs. This higher R_m resulted in a broadening of EJP's which enhanced EJP summation, muscle fiber depolarization, and muscle stress.

Introduction

The stone crab *Menippe mercenaria* is found from North Carolina to the tropics and the blue crab *Callinectes sapidus* from New England to Florida (Williams 1984). Northern populations of both species experience summer maximum temperatures ranging from 27 to 30 °C and winter minimum temperatures from 2 to 8 °C. Southern populations experience similar summer temperatures (28–31 °C) but are rarely exposed to winter temperatures below 15–20 °C. Blue crabs live in estuarine environments where large short term temperature fluctuations are much more common than in the marine subtidal environment of the stone crab.

This paper investigates the hypotheses that populations within a particular species that experience different annual temperatures ranges will have

Abbreviations: *EJP* excitatory junction potential; E_r resting membrane potential; F_e facilitation; R_m specific membrane resistance

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different neuromuscular responses to temperature changes, and that animals that frequently experience large temperature fluctuations (estuarine blue crabs) will have a greater ability to cope with such fluctuations than animals living in a more constant thermal environment (marine stone crabs). To test these hypotheses, claw contractile ability was measured in vivo in stone crabs and blue crabs collected from northern and southern populations and subjected to various temperature treatments. In vitro neuromuscular properties were recorded at 8, 20, and 30 °C saline in claws taken from cold acclimated and non-cold acclimated stone crabs and blue crabs of the northern populations. These in vitro experiments compare differences in neuromuscular performance between cold acclimated and non-cold acclimated animals that experience temperature fluctuations of different magnitudes.

Materials and methods

Maintenance of animals. Stone crabs were collected from Beaufort, NC ('northern' population) and Marathon, FL ('southern' population) during July 1984 and July 1985. Blue crabs were collected from Cambridge, MD (Chesapeake Bay, 'northern' population) and Cape Coral, FL (Caloosahatchee River, 'southern' population) during July and August of 1984. Water temperatures at the time of collection were 28–30 °C.

Crabs were held individually in 38 liter aquaria filled with artificial sea water (34 ppt salinity for stone crabs and 20 ppt for blue crabs). Water filtration and temperature control took place in a 208 liter aquarium; water was pumped from this aquarium to individual 38 liter aquaria. A light:dark cycle of 12:12 h was maintained in the aquarium room. Crabs were fed whole oysters twice weekly.

Temperature treatments. After collection, all crabs were placed in aquaria at 30 °C in the laboratory for 2 weeks (termed 'laboratory warmed' stone crabs and blue crabs in Table 1) at which time in vivo measurements of muscle stress were taken (controls). Some blue crabs (7 northern, 15 southern) were then transferred directly from the 30 °C aquaria into 8 °C aquaria for 4 weeks ('laboratory cooled' blue crabs in Table 1). All blue crabs survived this treatment. However, no northern or southern stone crab subjected to a similar 22 °C decrease in temperature survived ($n=12$). Therefore, a more gradual cold acclimation procedure was adopted for stone crabs. Aquarium water of laboratory cooled stone crabs (5 northern, 6 southern; Table 1) was gradually lowered from 30 °C to 8 °C over a 3 week period. All stone crabs survived when held at 8 °C for an additional 4 weeks. Northern ($n=7$) and southern ($n=4$) stone crabs were also held at 30 °C for 7 weeks as a control with no mortalities. Some northern and southern stone crabs were placed in outdoor sea water tanks (in July 1985, water temperature 30 °C) located at Duke University Marine Laboratory in Beaufort, NC (northern location). These crabs, referred to as 'naturally cooled' stone crabs (Table 1), were tested again 6 months later in January 1986 when water temperatures had decreased to 8 °C.

Muscle stress measured in vivo. The term 'muscle stress' refers to the force generated by the whole muscle per unit of muscle cross-sectional area; 'stress' does not imply any cost or load experienced by the animals as a result of changing environmental temperatures. Unlike muscle tension, muscle stress is less dependent upon claw size, muscle volume, or mechanical advantage and thus can be more readily compared between crabs with claws of different sizes and mechanical advantages.

Table 1. Acclimation protocols used to measure muscle stress (in vivo, 8° and 30 °C) and neuromuscular properties (in vitro, 8°, 20°, and 30 °C) of stone crabs and blue crabs collected from northern (*N*) and southern (*S*) populations

		Laboratory warmed	Laboratory cooled	Naturally cooled
Muscle stress				
Stone crabs	N	X	X	X
	S	X	X	X
Blue crabs	N	X	X	–
	S	X	X	–
Neuromuscular properties				
Stone crabs	N	–	X	X
Blue crabs	N	X	X	–

Muscle stress was compared between northern and southern populations of stone crabs exposed to three temperature treatments (laboratory warmed, laboratory cooled and naturally cooled) and blue crabs exposed to two temperature treatments (laboratory warmed and laboratory cooled) (Table 1). Stress was recorded initially at 30 °C before the temperature treatment and then at the end of the temperature treatment. Stress was also recorded in some northern stone crabs immediately upon field collection ($n=27$ in summer at 30 °C, $n=19$ in winter at 8 °C).

Muscle stress, expressed in newtons (N) per cm^2, was calculated with measurements of dactyl force (measured in claws of live crabs by placing a force transducer directly into the claw), closer muscle volume and the angle of muscle fiber attachment onto the apodeme of the closer muscle (see Govind and Blundon 1985 for details of specific measurements). A paired *t*-test (Sokal and Rohlf 1969) was used to compare muscle stress measurements in crabs of both species before and after they were subjected to temperature changes. Geometric mean regression analysis (ordinate = claw size, abscissa = muscle stress) was used to compare muscle stress between summer and winter stone crabs collected and immediately tested in the field (Ricker 1973). Because some crustacean muscles show a decrease in muscle stress with increasing muscle volume (Elner and Campbell 1981; Blundon 1988), a *t*-test or one-way analysis of variance would be confounded by differences in claw length between stone crabs tested in summer and in winter ($P<0.02$, unpaired *t*-test; mean claw length in summer stone crabs = 69.1 ± 14.6 mm (mean $\pm$SD), $n=27$; mean claw length in winter stone crabs = 80.0 ± 16.1 mm, $n=19$).

Neuromuscular properties measured in vitro. Neuromuscular properties from northern crabs collected during the summer at 30 °C were recorded at three saline temperatures (8, 20, and 30 °C) in isolated claws removed from: (a) stone crabs cooled from 30 °C to 8 °C over 7 weeks (laboratory cooled, $n=5$), (b) stone crabs cooled from 30 °C to 8 °C over 6 months (naturally cooled, $n=9$), (c) blue crabs maintained at 30 °C (laboratory warmed, $n=7$) and (d) blue crabs placed from 30 °C into 8 °C water and held for 4 weeks (laboratory cooled, $n=9$).

These experiments (Table 1) compared differences in neuromuscular performance between cold acclimated (b and d) and non-cold acclimated (a and c) animals of species that experience temperature fluctuations of different magnitudes.

Crabs were forced to autotomize their claws by having a dissecting pin inserted distal to the plane of autotomy between the basis and the ischium. The closer muscle was exposed by removing the dorsal surface of the propus, the opener muscle, and the tissue overlying the closer muscle. The claw was clamped in a plexiglas chamber with brass screws placed in the side of the chamber and submersed in a physiological saline of 470 m*M* NaCl, 8 m*M* KCl, 7 m*M* $MgCl_2$, 15 m*M* $CaCl_2$, 11 m*M* glucose, buffered with 5 m*M* HEPES to a pH of 7.4. The recording chamber was suspended in a larger plexiglas chamber which contained either an ice bath or a warm water bath to change and maintain saline temperature. These in vitro preparations were tested at three saline temperatures (8, 20 and 30 °C). Claws from laboratory warmed crabs were first tested at 30 °C and then cooled, while claws from all cold treatment crabs were tested at 8 °C and then warmed. The temperature of the preparation was changed by slowly perfusing either 5 °C or 30 °C saline into the recording chamber and cooling or warming the bath temperature surrounding the recording chamber. Claw temperature was monitored with a thermocouple placed next to the closer muscle (cooling was approximately −0.4 °C per min; heating was approximately +0.8 °C per min).

Axons innervating the muscle were exposed by breaking the joints between the merus and propus, cutting away all apodemes that attached to the propus, and gently sliding away limb segments proximal to the propus. The motor axon bundle was then placed across a pair of platinum hook electrodes. To confirm that excitatory axons were stimulated, action potentials in the motor axon bundle were monitored with a suction electrode placed just proximal to where the bundle entered the closer muscle.

Resting membrane potentials (E_r) and excitatory junction potentials (EJP's) were monitored in the dorsal fibers of the muscle with glass microelectrodes (10–30 MΩ, filled with 3 *M* KCl) with conventional amplification and recording techniques. A Grass SD9 square wave stimulator was used to stimulate the axon bundle (pulse duration 1.0 ms). EJP amplitudes were averaged over at least 10 EJP's using either a storage screen oscilloscope or a chart recorder (Brush 220, Gould Inc.). A depolarization ratio (which reflects EJP summation and facilitation) was calculated by dividing the level of depolarization above E_r evoked by 20 Hz EJP's (after this level had reached a plateau) by the amplitude of EJP's at 1 Hz.

Facilitation (F_e) of EJP's was measured as the amplitude of EJP's produced at 10 Hz (after the muscle fiber had reached a plateau of depolarization) divided by the amplitude of a single EJP (Bittner and Segundo, submitted for publication). If necessary, this value was adjusted to compensate for non-linear summation of EJP's (Martin 1955).

Specific membrane resistance (R_m) was determined by measuring the change in membrane potential in response to 200 ms pulses of hyperpolarizing current at various distances from the site of intracellular current injection. Current was injected with a broken glass microelectrode (5–10 MΩ) filled with 2 *M* K citrate. The distances between current and recording electrodes and muscle fiber diameters were measured with a calibrated ocular micrometer. Membrane input resistance and R_m were calculated as described by Junge (1981). Mean differences in R_m between populations and temperature treatments were tested with a one-way analysis of variance. If the ANOVA was significant, a Student-Neuman-Keuls multiple comparison procedure was used to determine which means were significantly different (Sokal and Rohlf 1969).

Muscle stress was determined in these in vitro claws by connecting a wire loop from the dactyl to a Grass FTOC3 force transducer. Contractions were evoked by stimulating the motor axon bundle at 80 Hz (the frequency which evoked maximum force). Muscle stress was calculated by use of the dactyl force measurement as previously defined.

Results

No northern-southern population differences of temperature effects on muscle stress were found for either stone crabs or blue crabs. Blue crabs cooled to 8 °C generated muscle stress levels similar to levels at 30 °C much sooner than did stone crabs. When given adequate time to acclimate to 8 °C, crabs of both species showed significant differences in several neuromuscular properties compared to non-cold acclimated crabs.

Muscle stress measured in vivo

Confinement of northern and southern stone crabs within aquaria for 7 weeks at 30 °C had no effect on muscle stress (laboratory warmed, Table 2). Northern and southern stone crabs showed significant decreases in muscle stress after 3 weeks of cooling from 30 to 8 °C followed by 4 weeks of exposure to 8 °C (laboratory cooled, Table 2). Results from field measurements suggested that the

Table 2. Laboratory measurements of claw closer muscle stress ($N \cdot cm^{-2} \pm SE$ (n)) over time in northern (N) and southern (S) stone crabs and blue crabs

	Stone crabs		
	0 weeks (30 °C)	7 weeks (30 °C)	*P*
N laboratory warmed	102± 9 (7)	105±10 (7)	ns
S laboratory warmed	159±30 (4)	159±44 (4)	ns
	0 weeks (30 °C)	7 weeks (8 °C)	*P*
N laboratory cooled	122±26 (5)	46±12 (5)	*
S laboratory cooled	103±18 (6)	41±10 (6)	**
	0 weeks (30 °C)	6 months (8 °C)	*P*
N naturally cooled	72± 5 (4)	51± 6 (4)	ns
S naturally cooled	49± 9 (4)	32± 4 (4)	ns
	Blue crabs		
	0 weeks (30 °C)	4 weeks (8 °C)	*P*
N laboratory cooled	93±23 (7)	83± 8 (7)	ns
S laboratory cooled	107± 6 (15)	82±13 (15)	ns

* $P<0.05$; ** $P<0.01$; *ns* not significant (t-test)

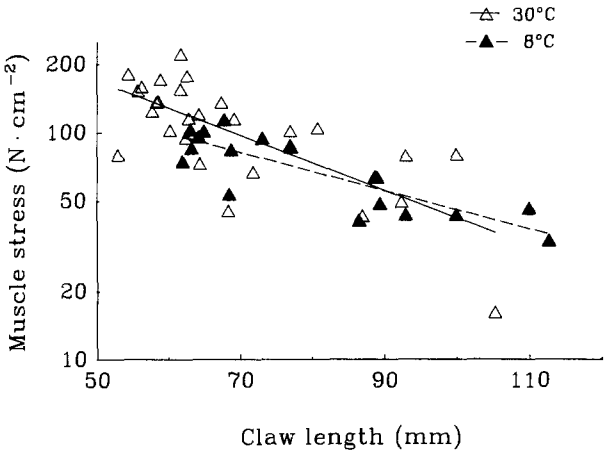

Fig. 1. Claw closer muscle stress as a function of claw length in summer (30 °C) and winter (8 °C) northern stone crabs. 30 °C: $\ln Y = -0.028X + 6.532$, $r^2 = 0.528$, $n = 27$. 8 °C: $\ln Y = -0.020X + 5.767$, $r^2 = 0.698$, $n = 19$. No significant differences in either slopes or elevations ($P > 0.05$)

significant reduction in muscle stress found in both northern and southern populations of laboratory cooled stone crabs may be an artifact of the rapidity of water temperature change or the duration of the exposure period. For example, measurements of muscle stress taken from northern stone crabs collected in the summer (30 °C, $n = 27$) and winter (8 °C, $n = 19$) were not significantly different (Fig. 1). Furthermore, no significant change in muscle stress was seen in naturally cooled northern and southern stone crabs collected and tested in July at 30 °C, and held in outdoor sea water tanks at the northern location and retested when the water temperature reached 8 °C in January (Table 2).

Muscle stress in northern and southern blue crabs was similar when measured at 30 °C and again after 4 weeks of exposure to 8 °C (laboratory cooled, Table 2).

Table 3. Comparisons of neuromuscular properties (mean ±SE (n)) in northern stone crabs and blue crabs of various temperature treatments at three saline temperatures

Saline temperature	8 °C	20 °C	30 °C
Stone crabs			
Resting potential (mV)			
Laboratory cooled	$-60.8^{a} \pm 3.2$ (5)	$-68.8^{ab} \pm 3.3$ (5)	$-77.6^{b} \pm 3.2$ (5)
Naturally cooled	$-62.6^{a} \pm 2.2$ (9)	$-71.7^{ab} \pm 4.2$ (9)	$-77.6^{b} \pm 2.6$ (9)
EJP amplitude (mV at 1 Hz)			
Laboratory cooled	$5.4^{a} \pm 1.4$ (5)	$8.8^{a} \pm 2.8$ (5)	$2.3^{b} \pm 0.9$ (5)
Naturally cooled	$1.9^{b} \pm 0.4$ (9)	$2.5^{b} \pm 0.6$ (9)	$1.1^{b} \pm 0.2$ (9)
Facilitation (F_c)			
Laboratory cooled	$1.2^{a} \pm 0.2$ (5)	$1.6^{ab} \pm 0.3$ (5)	$2.5^{bc} \pm 0.4$ (5)
Naturally cooled	$1.4^{a} \pm 0.1$ (9)	$1.6^{a} \pm 0.1$ (9)	$2.7^{c} \pm 0.4$ (9)
Muscle stress ($N \cdot cm^{-2}$ evoked by 80 Hz axonal stimulation)			
Laboratory cooled	$3.1^{a} \pm 0.2$ (5)	$2.6^{ab} \pm 0.7$ (5)	$0.6^{c} \pm 0.6$ (5)
Naturally cooled	$5.1^{b} \pm 0.6$ (6)	$2.8^{ab} \pm 1.0$ (6)	$0.1^{c} \pm 0.1$ (6)
Blue crabs			
Resting potential (mV)			
Warm	$-62.0^{a} \pm 2.7$ (7)	$-74.3^{b} \pm 2.2$ (7)	$-68.9^{ab} \pm 4.0$ (7)
Laboratory cooled	$-70.6^{ab} \pm 3.2$ (9)	$-76.0^{b} \pm 2.1$ (9)	$-76.6^{b} \pm 2.8$ (8)
EJP amplitude (mV at 1 Hz)			
Warm	$0.5^{a} \pm 0.1$ (6)	$1.8^{bc} \pm 0.4$ (6)	$2.0^{bc} \pm 0.5$ (6)
Laboratory cooled	$0.8^{ac} \pm 0.2$ (9)	$3.5^{b} \pm 1.0$ (9)	$1.5^{bc} \pm 0.4$ (9)
Facilitation (F_c)			
Warm	$1.8^{a} \pm 0.2$ (4)	$1.5^{a} \pm 0.1$ (5)	$1.7^{a} \pm 0.1$ (6)
Laboratory cooled	$1.7^{a} \pm 0.1$ (9)	$1.4^{a} \pm 0.1$ (9)	$1.8^{a} \pm 0.1$ (9)
Muscle stress ($N \cdot cm^{-2}$ evoked by 80 Hz axonal stimulation)			
Warm	$9.2^{a} \pm 1.9$ (7)	$14.9^{a} \pm 3.3$ (7)	$12.3^{a} \pm 3.8$ (7)
Laboratory cooled	$18.8^{b} \pm 5.5$ (5)	$14.4^{ab} \pm 6.5$ (5)	$9.3^{a} \pm 4.9$ (5)

$^{a-c}$ Means with at least one superscript in common are not significantly different ($P > 0.05$)

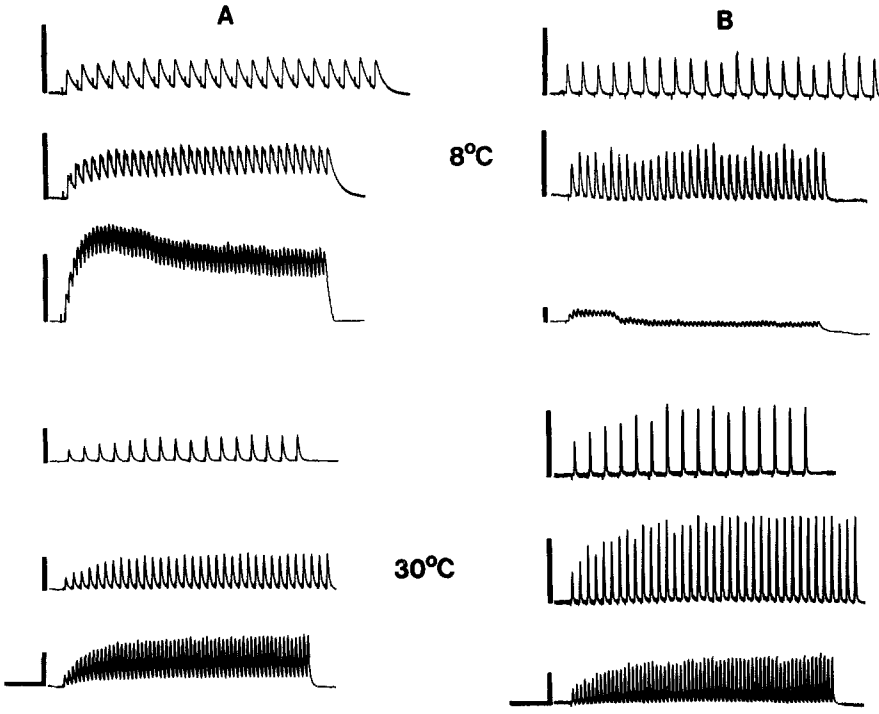

Fig. 2. Excitatory junction potentials evoked by axonal stimulation at 5, 10, and 20 Hz (for each saline temperature of 8 and 30 °C) from closer muscles of naturally cooled (**A**) and laboratory cooled (**B**) northern stone crabs. Horizontal scale = 500 ms; vertical scale = 5 mV

Neuromuscular properties measured in vitro

Resting membrane potential (E_r) measured in muscle fibers from both laboratory cooled and naturally cooled stone crabs showed significant changes with saline temperature; E_r was most hyperpolarized at 30 °C (Table 3). The E_r in muscle fibers from laboratory warmed blue crabs was most hyperpolarized at 20 °C, but did not change significantly with temperature in laboratory cooled blue crabs (Table 3).

The EJP amplitudes in fibers of laboratory cooled stone crabs were significantly larger at 8 °C and 20 °C than at 30 °C (Table 3), but showed no significant change with temperature in naturally cooled stone crabs. Furthermore, EJP amplitudes from laboratory cooled stone crabs were significantly greater at 8 °C and 20 °C than EJP amplitudes from naturally cooled stone crabs at the same temperature (Table 3). There was no significant difference in EJP amplitude between laboratory warmed and laboratory cooled blue crabs at each saline temperature. The EJP amplitude in all blue crabs was least at 8 °C (Table 3).

The EJP's showed increased duration at 8 °C in naturally cooled stone crabs compared to laboratory cooled stone crabs, as well as in laboratory cooled blue crabs compared to laboratory warmed blue crabs (Figs. 2 and 3). At 30 °C, there was no apparent difference in EJP duration between the temperature treatments of either crab species. Increased EJP duration resulted in greater muscle fiber depolarization at >20 Hz axonal stimulation. At 8 °C saline temperature, depolarization ratios (20 Hz EJP's/1 Hz EJP's: see methods) were significantly greater in muscle fibers of naturally cooled stone crabs than laboratory cooled stone crabs (4.7 ± 0.8 (mean $\pm$SE), $n=9$ for naturally cooled stone crabs vs. 2.0 ± 0.3, $n=5$ for laboratory cooled stone crabs; $P<0.05$). At 30 °C, there were no significant differences in depolarization ratios of fibers between laboratory cooled (3.8 ± 0.7) and naturally cooled (4.3 ± 0.6) stone crabs.

There were no significant differences in EJP facilitation (F_e) in muscle fibers between laboratory cooled and naturally cooled stone crabs or between laboratory warmed and laboratory cooled blue crabs. The amount of facilitation was nearly double at 30 °C relative to 8 °C for stone crabs (Table 3), but was unaffected by temperature in blue crabs (Table 3).

Differences in the rate of R_m change with cold exposure between stone crabs and blue crabs are reflected by in vivo and in vitro muscle stress measurements. Northern and southern stone crabs showed a significant increase in R_m at 8 °C after a seasonal 6 month decrease in temperature (Table 4). Muscle stress at 8 °C in these naturally cooled stone crabs was not significantly different from muscle stress measured at 30 °C in laboratory

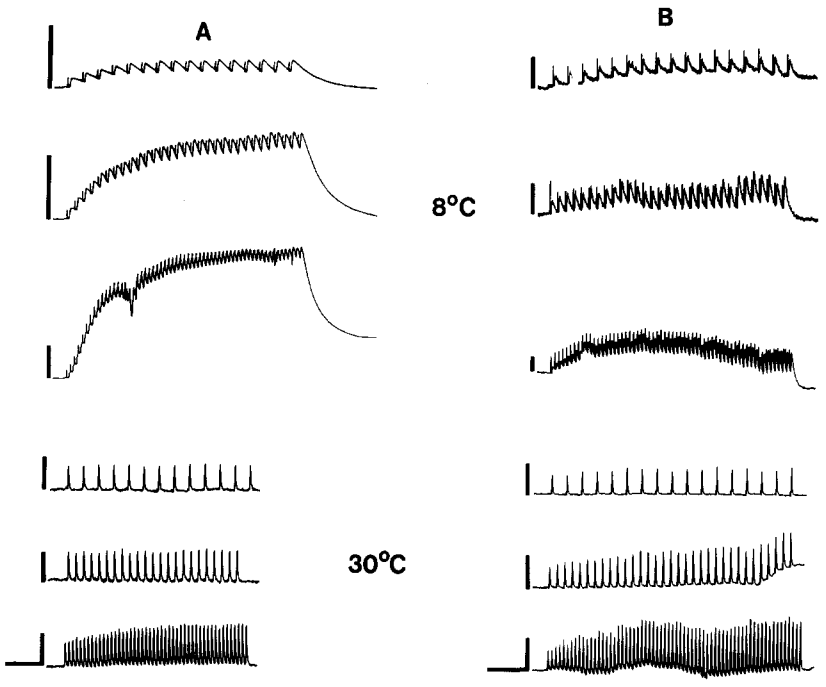

Fig. 3. Excitatory junction potentials evoked by axonal stimulation at 5, 10, and 20 Hz (for each saline temperature of 8 °C and 30 °C) from closer muscles of laboratory cooled (**A**) and laboratory warmed (**B**) northern blue crabs. Horizontal scale = 500 ms; vertical scale = 5 mV in **A** and lower **B**, 1 mV in upper **B**

Table 4. Measurements of specific membrane resistance (mean $\Omega \cdot cm^2 \pm SE$ (*n*)) at two saline temperatures in northern (*N*) and southern (*S*) stone crabs and blue crabs

Saline temperature	8 °C	30 °C	*P*
Stone crabs			
N laboratory cooled	1005±208 (7)	635± 95 (7)	ns
N naturally cooled	2571±363 (8)	877±155 (8)	*
S naturally cooled	2781±724 (4)	366± 29 (4)	*
Blue crabs			
N laboratory warmed	613± 79 (2)	421± 10 (2)	ns
S laboratory warmed	817±276 (4)	422± 32 (4)	ns
N laboratory cooled	1834±190 (4)	740±300 (4)	*
S laboratory cooled	1949±604 (3)	605±167 (3)	*

* $P < 0.05$; *ns* not significant (*t*-test)

warmed stone crabs (Table 2). Laboratory cooled stone crabs exposed to a more rapid decrease in temperature (30 °C to 8 °C in 3 weeks followed by 4 weeks at 8 °C) did not show a significant change in R_m with temperature (Table 4) and generated significantly less muscle stress at 8 °C compared to laboratory warmed stone crabs at 30 °C (Table 2). In vitro muscle stress was greater at 8 °C saline in claws of naturally cooled stone crabs than claws of laboratory cooled crabs (Table 3). Northern and southern blue crabs were able to acclimate to a 22 °C decrease in temperature much more rapidly than stone crabs. Laboratory cooled blue crabs placed directly from 30 °C water into 8 °C water and maintained at 8 °C for 4 weeks showed a significant increase in R_m at 8 °C saline relative to 30 °C (Table 4), and generated the same amount of muscle stress compared to 30 °C measurements (Table 2). In vitro muscle stress was greater at 8 °C saline in claws of laboratory cooled blue crabs than claws of laboratory warmed blue crabs (Table 3).

Discussion

The objective of this study was to investigate the effects of short and long term temperature changes on neuromuscular properties within species that have populations which experience different annual ranges of temperatures, and between species that are subjected to different degrees of temperature fluctuation. Northern and southern populations of stone crabs and blue crabs appear to be equally capable of muscular stress production given the appropriate acclimation time, although the southern populations do not experience the cold winter temperatures of their northern conspecifics. Blue crabs, estuarine animals that are exposed to greater short term temperature fluctuations than marine stone crabs, are able to acclimate to low temperatures much more rapidly than stone crabs.

Stone crabs naturally acclimated to 8 °C over 6 months and blue crabs cooled in the laboratory to 8 °C for 4 weeks (the two temperature treatments that produced successful cold acclimation

in the shortest time) showed two important differences in neuromuscular properties from non-cold acclimated crabs (stone crabs cooled in the laboratory to 8 °C for only 7 weeks and blue crabs held at 30 °C). At 8 °C, these cold acclimated crabs showed enhanced specific muscle fiber membrane resistance (Table 4). The EJP duration and EJP depolarization ratios (20 Hz EJP depolarization/1 Hz EJP depolarization) were also greater at 8 °C relative to 30 °C in fibers of cold acclimated crabs compared to non-cold acclimated crabs. Fischer and Florey (1981) proposed that the increase in R_m at cold temperatures in crayfish (*Astacus leptodactylus*) muscle fibers compensated for associated decreases in EJP amplitude by increasing EJP duration and summation. In cold acclimated stone crabs and blue crabs, as in the crayfish, high frequency trains of EJP's showed increased summation at 8 °C. Increased EJP summation at 8 °C in cold acclimated crabs resulted in levels of muscle fiber depolarization and stress comparable to levels in warm acclimated crabs at 30 °C.

Temperature independence of neuromuscular performance has been demonstrated in other crustaceans as well (the shore crab *Pachygrapsus crassipes*, Stephens and Atwood 1982; crayfish *Procambarus clarkii*, White 1983). In these crustaceans as in stone crabs and blue crabs, the decrease in EJP amplitude at low temperatures was offset by an increase in EJP duration and summation, whereas the decrease in EJP amplitude at high temperatures was offset by an increase in EJP facilitation.

Changes in EJP duration and summation may be due to changes in neurotransmitter reactivity with postsynaptic receptors, as well as changes in membrane cable properties such as capacitance and resistance. Fischer and Florey (1981) showed that changes in the decay time of EJP's in crayfish muscle fibers paralleled changes in the decay time of membrane potential responses to injected current, and that changes in neurotransmitter action were not necessary to explain changes in EJP duration. However, other aspects of neuromuscular performance not measured in this study may be modified by temperature-related changes in protein concentration or structure. Jacobs and Atwood (1981) speculate that the cold acclimation induced changes in long term facilitation and depression observed in crayfish neuromuscular junctions may be due to changes in sodium pump activity of the nerve terminals.

The time course of cold exposure in the laboratory was too rapid to permit the usual seasonal changes in stone crab muscle fiber membranes. Stone crabs cooled to 8 °C and held at that temperature for 4 weeks showed temperature associated changes in R_m that were similar to changes seen in laboratory warmed blue crabs (Figs. 2 and 3) and warm acclimated crayfish (calculated from White 1983). Temperature associated changes in R_m in stone crabs naturally cooled for 6 months were similar to those in blue crabs laboratory cooled to 8 °C for 4 weeks, and in crayfish cold acclimated for 6 weeks (White 1983). It is not known if the three week transition period from 30 °C to 8 °C or the four weeks at 8 °C was too short a period of cold exposure in the stone crabs to allow for otherwise seasonal physiological changes.

The ability to acclimate rapidly could be due to greater capacity to incorporate unsaturated fatty acids into lipid bilayers at low temperatures, perhaps a temperature related performance of a desaturase enzyme. Desaturase enzymes are responsible for maintaining membrane lipid fluidity at low temperatures in the bacterium *Bacillus megaterium* (Fujii and Fulco 1977) and the protist *Tetrahymena pyriformis* (Kasai et al. 1976). Alternatively, acclimation capacity may be associated with temperature related changes in protein performance or structure. Eurythermic organisms that experience substantial short term temperature changes may rely on cold and warm temperature variants of proteins that enable them to exist over a broad temperature range (Hochachka and Somero 1973).

Animals that are likely to experience more drastic short term fluctuations in temperature may have enhanced ability to acclimate rapidly. A cold water stenothermic species of rockfish (*Sebastes*) showed poorer acclimation capacity (as measured in cytochrome oxidase activity) than a eurythermic species of the same genus (Wilson et al. 1974). In this present study, both northern and southern blue crabs were capable of completely acclimating from 30 °C to 8 °C within 4 weeks. Harri and Florey (1979) reported cold acclimation in crayfish (from 24 °C to 12 °C) within two weeks. Crayfish are often found in fresh water ponds and streams where water temperature may fluctuate with rapidly changing air and land temperatures.

Eurytherms infrequently exposed to large temperature reductions and perhaps even warm water stenotherms may be able to cope with cold temperatures, but acclimation time may be much longer in these animals compared to that of an animal that is frequently exposed to significant short term temperature changes. Florey and Hoyle (1976) reported that ghost crabs *Ocypode ceratophthalma*

had lost the ability to adapt outside their normal temperature range. This crab lives within the very narrow temperature range of 26 °C to 28 °C. Florey and Hoyle (1976) found that the usual flight response of the crabs was abolished at ambient temperatures less than 23 °C. Movements of the animals were very sluggish below 20 °C. Tension and EJP amplitude in autotomized walking leg muscles declined outside the temperature range of 22 °C to 27 °C. Northern and southern stone crabs in this study which were acclimated to 8 °C for only 7 weeks appeared almost immobile, as did the cooled ghost crabs described by Florey and Hoyle (1976), but after 6 months of natural cooling, muscle stress production in stone crabs was unchanged (Table 2). Perhaps, given a longer period of cold exposure, normal neuromuscular function in ghost crabs might also be possible at colder temperatures.

The ability of both stone crabs and blue crabs to generate muscle stress irrespective of the temperature regime of their local environment no doubt plays an important role in the successful dispersal of the species across temperature gradients due to latitude or local geography. It is clear, however, that the ability of both northern and southern blue crabs to cope with sizable short term temperature changes is superior to that of stone crabs. This ability is undoubtedly essential in maintaining normal body function in the more thermally variable environment where blue crabs are found.

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