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Behavioral Influences on the Physiological Responses of *Cancer gracilis,* the Graceful Crab, During Hyposaline Exposure

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Abstract. The relationship between the behavioral and physiological responses to hyposaline exposure was investigated in *Cancer gracilis,* the graceful crab. The status of *C. gracilis* as an osmoconformer was confirmed. Survival decreased with salinity: the LT_{50} in 50% seawater (a practical salinity of 16, or 16‰) was 31.5 ± 22.7 h and in 25% seawater (a salinity of 8) was 8.0 ± 0.7 h. When exposed to a salinity gradient, most crabs moved towards the highest salinity. However, in the salinity range of 55% to 65% seawater, they became quiescent. This "closure response" was also evident at low salinities: the mouthparts were tightly closed and animals remained motionless for 2 to 2.5 h. During closure, crabs were able to maintain the salinity of water within the branchial chambers at a level that was about 30% higher than that of the surrounding medium. The closure response was closely linked to a short-term decrease in oxygen uptake. During closure, oxygen within the branchial chamber was rapidly depleted, with oxygen uptake returning to pretreatment levels upon the resumption of activity. In addition to the short-term decrease in oxygen uptake, there was a longer-term bradycardia, which may serve to further reduce diffusive ion loss across the gills. By exhibiting a closure response during acute hyposaline exposure and an avoidance reaction during prolonged or severe hyposaline exposure, *C. gracilis* is able to use behavior to exploit areas prone to frequent episodes of low salinity.

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Introduction

The responses of decapod crustaceans to hyposaline exposure have been extensively studied (for reviews, see Mantel and Farmer, 1983; Pequeux, 1995; McGaw *et al.,* 1999; Wolcott and Wolcott, 2001). Most studies have focused on decapods that demonstrate some osmoregulatory ability. However, much less is known about the behavioral and physiological responses of osmoconforming decapod crustaceans to hyposaline exposure.

Alterations in behavior are usually the first response to low-salinity exposure and help animals avoid unfavorable conditions. The primary behavioral response to decreased salinity is an increase in locomotor activity with dilution of the medium (Thomas *et al.,* 1981). In laboratory conditions, decapods have displayed a broad range of salinity preferences. These studies are most often carried out using choice-chamber experiments wherein animals are allowed to choose between two or more salinities until a range of preference is determined (Davenport, 1972; Davenport and Wankowski, 1973; Thomas *et al.,* 1981; Ameyaw-Akumfi and Naylor, 1987; McGaw, 2001). Stronger osmoregulators such as *Carcinus maenas* and *Hemigrapsus nudus* show avoidance of salinities below a certain threshold but do not discriminate between salinities above this level (McGaw and Naylor, 1992; McGaw, 2001). Sugarman *et al.* (1983) reported that *Cancer magister,* a weak osmoregulator, displayed a behavior in which it appeared that the branchial chambers were isolated when crabs were exposed to low salinity. However, to date there has been no quantitative evidence to support this finding.

Behavioral responses to salinity stress are not always possible, and most crustaceans inhabiting areas subject to

high levels of freshwater runoff show some physiological response to cope with acute hyposaline exposure. The primary means of dealing with osmotic challenges are directly through active ion transport or indirectly through decreased permeability, and these responses are reflected in changes in oxygen uptake and heart rate (Pequeux, 1995). Changes in oxygen uptake may result from increases in active transport of ions (Pequeux, 1995) or in locomotion associated with hyposaline exposure (Johansen *et al.,* 1970; McGaw *et al.,* 1999). Changes in heart rate and other cardiac parameters may aid in the efficient delivery or offloading of oxygen (Hume and Berlind, 1976; McGaw and McMahon, 1996).

During hyposaline exposure, oxygen uptake may increase, decrease, or remain constant (Kinne, 1966). It has been suggested that the way in which decreased salinity affects oxygen uptake in invertebrates is linked to habitat (Wheatly, 1988), but this claim has been difficult to substantiate. Efficient osmoregulators such as *Callinectes sapidus* and *Carcinus maenas* show an increase in oxygen uptake (Taylor, 1977; Guerin and Stickle, 1997; Piller *et al.,* 1995) that is likely the result of both increased ion transport and locomotion (Pequeux, 1995; McGaw *et al.,* 1999). Decapods with weaker osmoregulatory ability show intermediate responses. The weak osmoregulator *Cancer magister* (Hunter and Rudy, 1975) shows no change in oxygen uptake with dilution of the medium (Brown and Terwilliger, 1999). On the basis of a pattern in which oxygen uptake decreases with osmoregulatory ability in decapod crustaceans, we predict that osmoconformers will show a decrease in oxygen uptake with dilution of the medium.

Changes in cardiac parameters during hyposaline exposure may aid in redistributing hemolymph and delivering oxygen (McGaw and McMahon, 1996). Efficient osmoregulators such as *Callinectes sapidus* and *Carcinus maenas* react to exposure to low salinity with an increase in heart rate and cardiac output that is thought to be related to increases in locomotor activity (Hume and Berlind, 1976; McGaw and Reiber, 1998). Conversely, the weak osmoregulator *Cancer magister* shows an increase in heart rate but a decrease in cardiac output (McGaw and McMahon, 1996). The osmoconformer *Libinia emarginata* exhibits a decrease in heart rate that has been presumed to be associated with a decrease in cardiac output (Cornell, 1974). Ultimately, a reduction in cardiac output leads to a reduction in hemolymph flow through the gills, which may reduce the gradient for diffusive ion loss and osmotic water loading (Cornell, 1973, 1974; McGaw and McMahon, 1996). It has been speculated that in the absence of physiological adaptation, namely decreased permeability, behavior is used and animals select habitats with a higher salinity (Spaargaren, 1973).

Most investigations have focused on crustaceans that are classed as having some osmoregulatory ability, with much less attention being given to the effects of decreased salinity on crabs that are classed as osmoconformers. Although osmoregulatory ability may to some extent dictate a crustacean's ability to exploit euryhaline environments (Barnes, 1967; Spaargaren, 1973), those areas are not strictly the domain of efficient osmoregulators. Decapods that have been classified as very weak osmoregulators or even osmoconformers are regularly found within estuaries and bays that are subject to bouts of low salinity (*Cancer gracilis,* Orensanz and Gallucci, 1988; *Cancer productus,* Carroll and Winn, 1989; *Libinia emarginata,* O'Brien *et al.,* 1999; *Homarus americanus,* Watson *et al.,* 1999; *Cancer irroratus,* Stehlik *et al.,* 2004).

Cancer gracilis, the graceful crab, inhabits shallow isolated bays that may be subject to such bouts of low salinity (Orensanz and Gallucci, 1988; Jensen, 1995). This species has been reported to hyperregulate for brief periods (4 h), but to become iso-osmotic with the medium over longer time scales (Nelson *et al.,* 1998). Given that hemolymph osmolality typically takes more than 24 h to reach stable levels (Siebers *et al.,* 1972), the results of that report suggest that *C. gracilis* is indeed an osmoconformer, but that it may be employing short-term mechanisms to mitigate declines in hemolymph osmolality. In the present study, we confirm the status of *C. gracilis* as an osmoconformer and investigate the behavioral and physiological responses to dilution of the medium, providing insight into how low-salinity exposure affects an osmoconforming decapod.

Materials and Methods

From June to August 2005, adult male intermolt individuals of *Cancer gracilis* (Dana, 1852) weighing 150 to 220 g were collected in traps in Bamfield Inlet, British Columbia, Canada. Crabs were transferred to the Bamfield Marine Sciences Centre and held in running seawater (SW) at a practical salinity of 32 ± 1 (*i.e.,* 32‰ ± 1‰) and a temperature of 12 ± 1 °C. Animals were acclimated to laboratory conditions for one week prior to experimentation and fed fish twice weekly. Crabs used in experiments were isolated from the general population and starved for 2 d. Different concentrations of SW were prepared by mixing freshwater (FW) with SW and the salinity was checked with an YSI 30 conductivity meter.

Behavior

To determine the behavioral responses of *C. gracilis* to hyposaline exposure, crabs were exposed to 100%, 75%, 50%, or 25% SW (salinities 32, 24, 16, or 8, respectively). Individual crabs ($n = 16$) were housed in 20-l aquaria containing aerated SW. Crabs were allowed to settle in the tanks for 1 h, then salinity was changed to one of the experimental salinities by draining a portion of the tank and replacing it with aerated FW of similar temperature. The temperature was maintained at 12 ± 1 °C. The percentage

of time in a 30-min interval that the crabs ($n = 16$) exhibited a closure response was recorded during an observation period of 3 h. A closure response was defined as quiescence, with the mouthparts tightly closed, antennules retracted, antennae pointed downward, and chelae drawn in against the body. Differences in the percentage of time spent closed were compared across time and salinity, using two-way repeated measures ANOVA, and significant effects were further analyzed using a Tukey multiple comparison test.

In another experiment, the actual salinity at which crabs ($n = 15$) exhibited a closure response was determined. Individual crabs were placed in 20-l aquaria containing aerated 100% SW and allowed to settle for 15 min. Aerated FW was then added in increments that lowered the salinity at a rate of about 0.15 min^{-1}, and the salinity at which crabs adopted the closed posture described above was recorded.

Salinity preference was then determined using either a high-salinity gradient (a range of about 60%–100% SW) or a low-salinity gradient (about 30%–70% SW). The tank used for the salinity gradient measured 3.0 × 0.4 × 0.3 m and was divided into five chambers (Fig. 1). The gradient was maintained by altering the ratio of FW to SW input into each chamber. Mixing between chambers was minimized by having FW and SW inlets at the bottom of one side of the chamber and an outlet at the top on the opposite side of the chamber. For each trial, an individual crab was placed into one of the five chambers (6 per chamber; $n = 30$). Crabs were able to move unobstructed between chambers through a passageway in each divider measuring 15 × 15 cm. The tank was surrounded by black plastic to prevent outside disturbance, and constant dim light was maintained. Crabs were allowed to settle in the tank for 15 min after handling, after which movements were recorded for 3 h, using a time-lapse video system (Panasonic AG-RT650). Three hours has been shown to be long enough for crabs to exhibit a salinity preference (Thomas *et al.,* 1981; McGaw, 2001). The total amount of time spent in each chamber was compared using ANCOVA, with starting chamber as a covariate. Control trials ($n = 5$) were carried out with 100% SW in each of the chambers, and the total amount of time spent in each chamber during a 3-h period was compared using ANOVA.

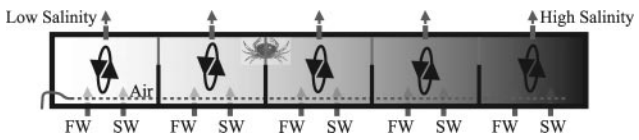

Figure 1. Diagram of the salinity-gradient tank. Arrows indicate the flow of seawater (SW) and freshwater (FW) in at the bottom edge of the tank. Water circulated within each chamber before flowing out of the tank at the top of each chamber, opposite the inlets. Passageways between each chamber (dashed line) allowed the crabs to freely move between salinity regimes. An air curtain was placed along the entire length of the chamber and maintained oxygen levels between 17 and 19 kPa.

Physiology

To determine the osmoregulatory ability of *C. gracilis,* crabs ($n = 20$) were progressively exposed to 100%, 75%, 50%, and 25% SW (salinities of 32, 24, 16, and 8, respectively) for 48 h. Crabs could only be acclimated to 25% SW for 9 h due to a high mortality rate in this salinity. At the end of each acclimation period, 200-μl samples of hemolymph were withdrawn from the arthrodial membrane of the walking legs. Samples were immediately frozen for later analysis. The osmolality of a 10-μl sample was determined using a vapor pressure osmometer (Wescor Inc. 5100B).

The percent survival over time was determined by placing crabs ($n = 15$) individually into tanks filled with 100%, 75%, 50%, or 25% aerated SW. At regular intervals, crabs were inspected, mortalities were recorded, and dead crabs were removed. The water in the tanks was replaced daily. Differences in mortality with salinity level were compared using a Kaplan-Meier survival test.

To determine the effects of hyposaline exposure on heart rate, a model 545-C pulsed-Doppler flowmeter (University of Iowa Bioengineering) was used to record changes in heart rate in eight crabs during a 3-h period in 100%, 75%, 50%, and 25% SW. A 3-h exposure period was used to emulate the short-term salinity exposures that *C. gracilis* encounters in the field (Curtis and McGaw, unpubl. obs.). Catheter-mounted peizo-electric crystal probes were guided to lie adjacent to the sternal artery. The phasic output of the flowmeter was recorded at 30-min intervals on an ADInstruments data acquisition system; the methodology is described in detail elsewhere (Airriess *et al.,* 1994). Differences in heart rate were compared across time and salinity using a two-way repeated measure ANOVA. Data showing a significant difference were further analyzed using a Tukey multiple comparison test.

An intermittent flow respirometer (Qubit Systems) was used to determine changes in oxygen uptake in eight crabs exposed to 100%, 75%, 50%, and 25% SW over a 3-h period. Water within the chamber was recirculated for 10 min at 30-min intervals, and the change in oxygen content within the chamber was determined. Data were recorded using a Loligo data acquisition system. Oxygen uptake expressed as milligrams of oxygen per kilogram of body mass per hour (mg O_2 kg body $mass^{-1}$ h^{-1}) was compared across salinity and time using a two-way repeated measure ANOVA. Data showing significant effects were further analyzed using a Tukey multiple comparison test.

Changes in the oxygen tension of the branchial chamber were measured during a closure response. A 3-mm hole was drilled in the carapace above the branchial chamber and sealed with dental dam. An oxygen microelectrode (Lazar Research Laboratories) was inserted through the dental dam to lie just inside the chamber, and output was recorded continually on an ADInstruments data acquisition system.

The crab was housed in a 20-l aquarium and allowed to settle for 30 min after handling. The salinity was then reduced to 50% SW by addition of a known volume of FW at ambient oxygen and temperature levels. The time when the crab exhibited a closure response was noted, and changes in oxygen tension were followed until the animal became active again.

To determine whether crabs are isolating water within the branchial chamber during a closure response, water within the brachial chamber of 15 crabs was sampled after 30 min of closure and compared with the external medium. To allow sample withdrawal, a 2-mm hole was drilled in the carapace above the branchial chamber and sealed with dental dam and cyanoacrylate adhesive. Crabs were then allowed to settle for 3 h before experimentation. To initiate a closure response, salinity within the tank was changed from 100% to 50% SW over 5 min by the addition of aerated FW of the same temperature. Thirty minutes after crabs initiated the closure response, a needle and syringe were used to remove 0.5 ml of water from the branchial chamber. Crabs showed minimal disturbance when samples were taken. Samples of the external medium (0.5 ml) were also taken at the same time. The osmolality of a 10-μl sample was determined using a vapor pressure osmometer (Wescor Inc. 5100B). Differences between water from the branchial chamber and the external medium were determined using a Mann-Whitney *U*-test.

Results

Behavior

At decreased salinities, *Cancer gracilis* individuals became quiescent, drawing legs and chelae tightly under the body. During this response the mouthparts were tightly closed, the antennules ceased flicking and were withdrawn into grooves in the carapace, and the antennae were lowered. In 100% SW, crabs did not exhibit closure behavior during any of the 3-h trials (Fig. 2). In all salinities below 100% SW there was a significant initial increase in the mean percentage of time spent closed (ANOVA, $F = 24.76$, df = 3, $P < 0.001$). There was also a significant salinity $\times$ time interaction (ANOVA, $F = 8.92$, df = 15, $P < 0.001$). In 75% SW, the mean percentage of time spent closed (26.3% $\pm$ 8.4%) was significantly elevated only during the initial exposure to low salinity. The initial increase in the percentage of time spent closed was most pronounced in 50% and 25% SW (mean percentages of 78.5% $\pm$ 7.0% and 67.7% $\pm$ 8.2%, respectively). In 50% and 25% SW, all crabs immediately adopted a closed posture. Once the closed posture was adopted, the crabs remained quiescent and only occasional mouthpart movements were observed. Over the duration of the 3-h trial, individual crabs opened up and resumed activity. Once activity resumed, crabs did not exhibit closure behavior again during the trial. Crabs in 50% SW resumed activity after 2 h. In 25% SW, 10 crabs resumed activity after 1 h, and all but one individual had resumed activity within 1.5 h (ANOVA, $F = 53.80$, df = 5, $P < 0.001$). When salinity was gradually decreased, the mean salinity at which crabs adopted the closed posture was 60.3% $\pm$ 1.9% SW.

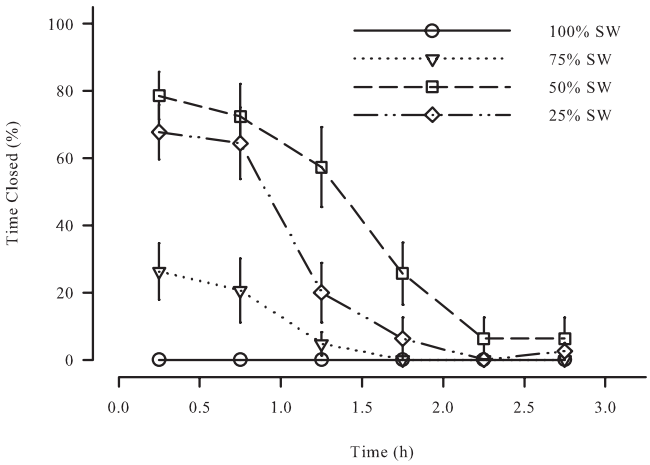

Figure 2. The percentage of time (mean $\pm$ SE) that *Cancer gracilis* ($n = 15$) exhibited closure behavior during 0.5-h intervals. Trials were carried out over a 3-h period with crabs being exposed to 25%, 50%, 75%, or 100% seawater (SW).

When exposed to a high-salinity gradient (65.0% $\pm$ 0.4% SW to 92.7% $\pm$ 1.4% SW), crabs showed an initial exploratory behavior, moving about the tank and remaining in each chamber only briefly (Fig. 3a). After this, the crabs typically moved toward the highest salinity offered and remained there for the duration of the experiment (ANCOVA, $F = 11.58$, df = 4, $P < 0.001$). The salinity of the chamber that the crabs were initially placed in was not a significant covariate (ANCOVA, $F = 0$, df = 1, $P > 0.05$). However, when crabs were initially placed in the low-salinity end of the tank (65% $\pm$ 0.4% SW), they became quiescent, exhibiting the closure response described above, and often remained in this chamber for the duration of the trial.

When exposed to a low-salinity gradient (32.7% $\pm$ 0.3% SW to 71.9% $\pm$ 0.3% SW), crabs again showed a short bout of exploratory behavior before settling and remaining in the chamber with the highest salinity (Fig. 3b; ANCOVA, $F = 20.34$, df = 4, $P < 0.001$). A few crabs in the low gradient became quiescent and displayed a closure response in chambers other than the highest salinity; this did not appear to be chamber-specific as it was in the high gradient. Again, the initial chamber that crabs were placed in was not a significant covariate (ANCOVA, $F = 0$, df = 1, $P > 0.05$). In control experiments where the entire tank was filled with 100% SW, crabs did not exhibit a preference for any chamber of the tank (ANOVA, $F = 0.50$, df = 4, $P > 0.05$).

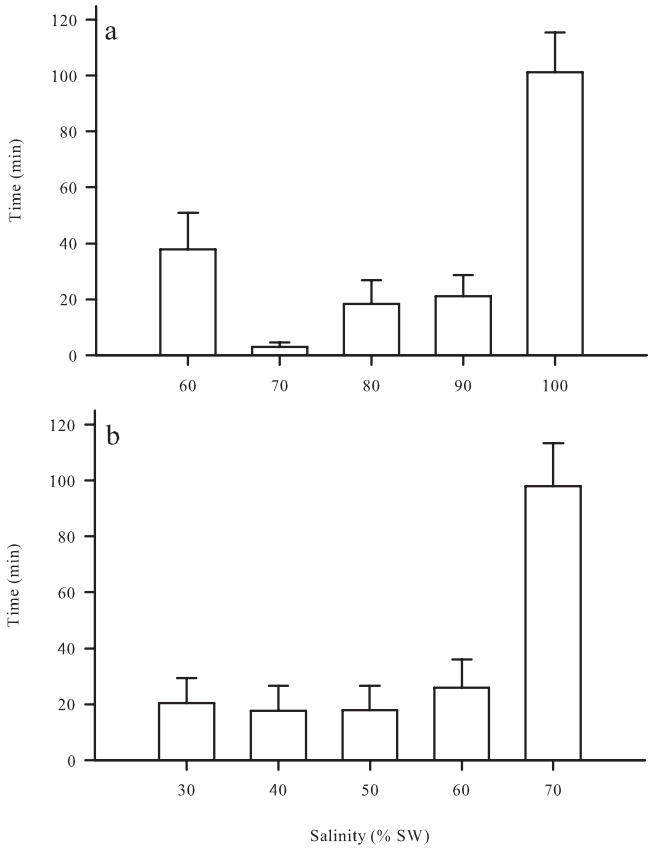

Figure 3. The amount of time (mean ± SE) *Cancer gracilis* ($n = 30$) spent in each chamber of either (a) a high-salinity gradient with a salinity range of about 60%–100% seawater (SW; salinity 19–32) or (b) a low-salinity gradient with a range of about 30%–70% SW (salinity 10–22) during a 3-h period.

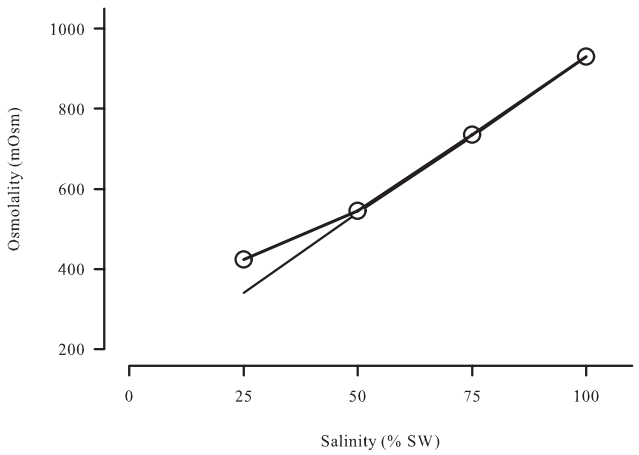

Figure 4. Hemolymph osmolality (mean ± SE) of 20 crabs exposed to 25%, 50%, 75%, or 100% seawater (SW), in relation to osmolality of the medium. In some cases, standard errors were small and do not show clearly on the figure.

Physiology

When crabs were exposed to salinities of 100% SW (927 mOsm) and 75% SW (735 mOsm), their hemolymph was iso-osmotic with the external medium at 930 ± 5 mOsm and 735 ± 1 mOsm, respectively (Fig. 4). In crabs exposed to 50% SW (521 mOsm) the hemolymph was slightly hyperosmotic to the medium at 545 ± 1 mOsm. After 9 h in 25% SW (275 mOsm), hemolymph was also hyperosmotic to the medium at 423 ± 6 mOsm.

The percent survival of *C. gracilis* declined with decreasing salinity (Fig. 5). In 100% SW, all 15 animals survived for 135 h, and 13 (87%) survived for the 500-h experimental period. In 75% SW, survival was similar to that in 100% SW: all 15 animals survived for 95 h, and 13 (87%) survived for the 500-h experimental period. In 50% SW, there was an initial decrease in percent survival: crabs reached 50% mortality (LT_{50}) at 31.5 ± 22.7 h (95% confidence level [CL]), and only two animals survived for the 500-h experimental period. For crabs exposed to 25% SW, mortality was significantly more rapid than in the other treatments: the LT_{50} was reached at 8.0 ± 0.7 h (95% CL), and all animals were dead within 15 h (Kaplan-Meier survival test).

Heart rate varied with both salinity and time (Fig. 6a). During exposure to salinities below 100% SW, there was a significant initial decrease in heart rate (ANOVA, $F = 86.64$, df = 3, $P < 0.001$). There was also a significant salinity × time interaction (ANOVA, $F = 8.73$, df = 18, $P < 0.001$). In 75% SW, heart rate decreased from a mean value of 72.4 ± 1.4 beats min^{-1} to 43.4 ± 6.5 beats min^{-1} during the first 30 min and remained stable for the remainder of the trial. The decrease in heart rate was most pronounced in 50% and 25% SW, dropping from mean values of 71 ± 4 and 65 ± 2 beats min^{-1}, respectively, to 15 ± 3 and 21 ± 3 beats min^{-1}, respectively, after 0.5 h. Thereafter

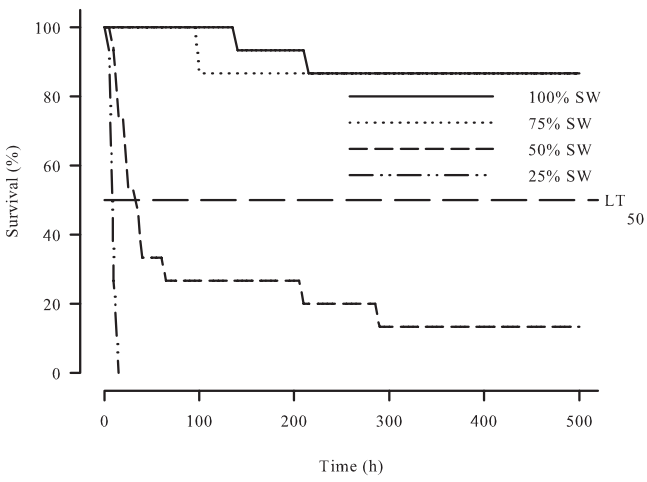

Figure 5. Percentage survival over time of 15 specimens of *Cancer gracilis* exposed to 25%, 50%, 75%, or 100% seawater (SW).

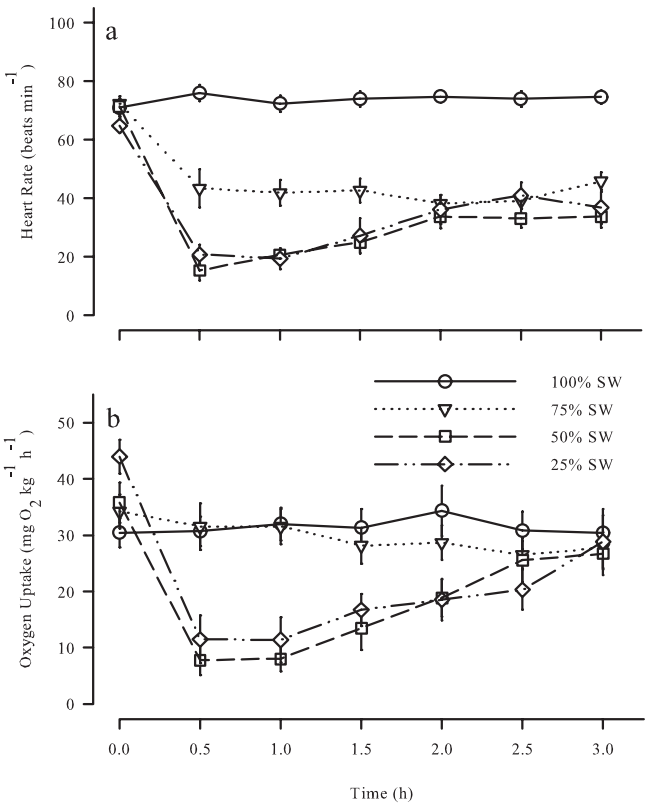

Figure 6. Changes in (a) heart rate (beats min^{-1}) and (b) oxygen uptake (mg O_2 kg^{-1} h^{-1}) of 8 crabs (mean ± SE) during 3-h exposure to 25%, 50%, 75%, or 100% seawater (SW).

heart rate gradually increased to 34 ± 4 and 36 ± 5 beats min^{-1}, respectively, by 2 h (ANOVA, $F = 45.24$, df = 6, $P < 0.001$). These levels were not significantly different from those in 75% SW, but were significantly lower than those observed for 100% SW.

Oxygen uptake also varied with both salinity and time (Fig. 6b), and there was a significant salinity × time interaction (ANOVA, $F = 8.38$, df = 18, $P < 0.001$). Oxygen uptake rates in 75% SW were not significantly different from those measured during exposure to 100% SW (Tukey test, $P > 0.05$). For crabs exposed to 50% SW, oxygen uptake decreased from 35.8 ± 3.6 mg O_2 kg^{-1} h^{-1} at 0 h to 7.8 ± 2.6 mg O_2 kg^{-1} h^{-1} at 0.5 h (ANOVA, $F = 4.13$, df = 3, $P < 0.05$). After an initial decrease, oxygen uptake increased to 25.6 ± 3.9 mg O_2 kg^{-1} h^{-1}, and pretreatment levels of oxygen uptake were reestablished at 2.5 h (ANOVA, $F = 8.38$, df = 6, $P < 0.001$). When crabs were exposed to 25% SW, there was an initial decrease in oxygen uptake, which rapidly dropped from 44.0 ± 3.0 mg O_2 kg^{-1} h^{-1} at 0 h to 11.5 ± 4.2 mg O_2 kg^{-1} h^{-1} at 0.5 h. After the initial decrease, oxygen uptake increased to 20.3 ± 3.6 mg O_2 kg^{-1} h^{-1} at 2.5 h, a level similar to that observed in 100% SW. The return of oxygen uptake to pretreatment levels corresponded to the cessation of closure behavior.

Changes in oxygen partial pressure within the branchial chamber during a closure response in a single crab are shown in Figure 7. During normal irrigation in 100% SW, oxygen levels in the branchial chamber varied between 17.5 and 18.5 kPa. The crabs adopted a closed posture within 1 min of exposure to 50% SW. Thereafter, oxygen levels in the branchial chamber declined rapidly, reaching 2 kPa 6 min after the initial closure response. A brief increase in oxygen levels occurred during a ventilatory reversal, after which oxygen levels decreased again, reaching 0.5 kPa. Once the crab became active, the mouthparts were opened and pretreatment oxygen partial pressures within the branchial chambers were regained within 1 min. When the osmolality of the medium was slowly decreased to about 50% SW, a closure response was initiated. The osmolality of water within the branchial chambers after 30 min of closure was 630 ± 28 mOsm (Fig. 8), significantly higher than the osmolality (442 ± 8 mOsm) of the external medium (Mann-Whitney, $P < 0.001$).

Discussion

In this study, the status of *Cancer gracilis* as an osmoconformer was confirmed. This observation contradicts a previous report that this species is a weak hyperosmoregulator with osmoregulatory ability similar to that of *Cancer magister* (Nelson *et al.,* 1998). However, that previous assumption was based on a 4-h acclimation period in low salinity, which is not sufficient time for hemolymph osmolality to reach new stable levels (Siebers *et al.,* 1972). Mantel and Farmer (1983) characterize an osmoconformer

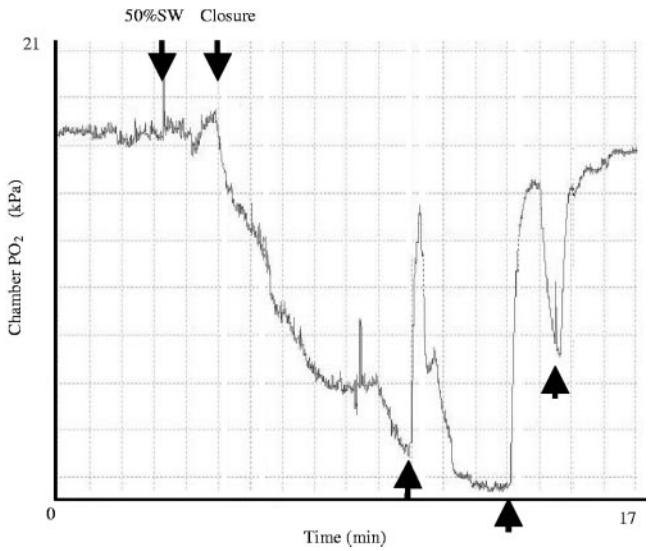

Figure 7. Changes in oxygen tension in the branchial chamber of a single specimen of *Cancer gracilis* during a closure response. Unlabeled arrows indicate when mouthpart movements occurred and the chamber was opened for a brief period of time.

by the inability to maintain the osmolality of the hemolymph at a level greater than 40 mOsm above the external medium. In 50% SW and above, the hemolymph osmolality of *C. gracilis* was less than 40 mOsm above the medium after 48 h of exposure. In 25% SW, hemolymph osmolality was greater than 40 mOsm above the medium (423 ± 6 mOsm), but crabs could only be maintained in those conditions for 9 h due to high mortality. Since 9 h is not long enough for the hemolymph osmolality to reach new stable levels (Siebers *et al.,* 1972), the short exposure time would be responsible for hemolymph being hyperosmotic to the external medium. Discriminating between an extremely weak osmoregulator and an osmoconformer on the basis of hemolymph osmolality alone can be difficult, and it may be necessary to examine gill ultrastructure to draw definite conclusions (Pequeux, 1995).

If *C. gracilis* cannot maintain hemolymph osmolality above the external medium, it follows that survival should decrease with salinity. In 50% SW the LT_{50} for *C. gracilis* was 31.5 ± 22.7 h (95% confidence limits), and in 25% SW no animals survived beyond 15 h. In areas subject to prolonged bouts of low salinity, death may be imminent if crabs are unable to avoid those conditions. Indeed, mass mortality of *C. gracilis* has been observed at Lucky Creek, in Barkley Sound, British Columbia (49°01.94′N, 125°18.34′W) after periods of heavy precipitation (McGaw, unpubl. obs.). Gradual acclimation to decreased salinity enhances the ability of *Libinia emarginata* to survive dilution of the medium, altering tolerance from about 80% to 40% SW (Gilles, 1970; Cornell, 1980). The rapid salinity changes used in this study may therefore overestimate the mortality incurred by *C. gracilis* in the field.

In its natural habitat, *C. gracilis* is usually exposed to only brief periods of low salinity. As the tide recedes, freshwater input from runoff is greater relative to the volume of seawater, leading to a decrease in salinity that is most pronounced at low tide. In many of the shallow bays that hold populations of this species, we have measured decreases in salinity (< 60% SW) for as long as 1.5 h before and after a low tide. In the same areas, low-salinity conditions are occasionally more severe and persist for longer periods during times of heavy runoff (Curtis and McGaw, unpubl. obs.). In the absence of physiological mechanisms to cope with hyposaline exposure, behavioral modifications may be used to exploit these habitats (Spaagaren, 1973; Jury *et al.,* 1994a; Wolcott and Wolcott, 2001).

Since *C. gracilis* cannot survive prolonged exposure to low salinity, it follows that these crabs should be able to detect changes in salinity and move away from such areas. When exposed to both a high and a low salinity gradient, *C. gracilis* individuals displayed a brief period of exploratory behavior before moving to the highest salinity offered. The weak osmoregulators *Cancer magister* (the Dungeness crab) and *Homarus americanus* (the American lobster) are also reported to favor the highest salinity offered (Jury *et al.,* 1994a; Curtis and McGaw, 2004). Correspondingly, *Homarus americanus* exhibits avoidance behavior in response to strong haloclines that develop after severe storms (Jury *et al.,* 1995). Nevertheless, one must be careful when extrapolating behavioral responses in the laboratory to movements in the field. Laboratory conditions usually involve sharp gradients over small spatial scales, whereas salinity changes in the field may be widespread, and therefore directional orientation may not be possible (Bell *et al.,* 2003).

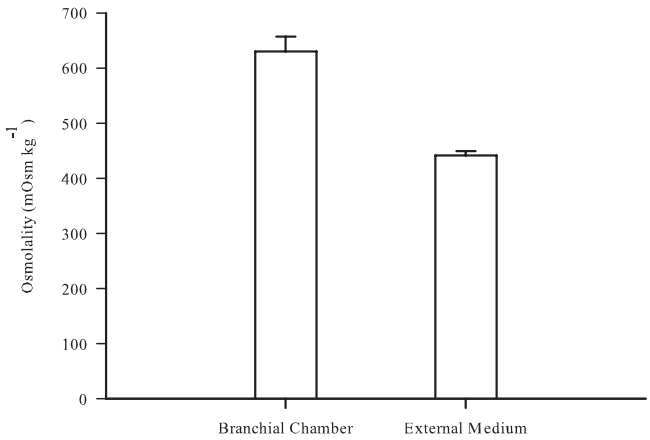

Figure 8. Osmolality of water from within the branchial chamber and the external medium (n = 15; mean ± SE) after 0.5-h exposure to 50% seawater.

Although most of the crabs in the salinity gradient moved toward the highest salinity, a number of individuals exhibited a different behavior. Crabs in salinities between 55% and 65% SW did not move to the highest salinity, but remained in the same area for the duration of the trial. This behavior was more evident when crabs were exposed to static hyposaline conditions: the crabs became quiescent, drawing in the chelae and walking legs, retracting the antennules, and tightly closing the mouthparts. During this closure response, the branchial chambers are apparently isolated. The closure response was initiated in all animals as salinity was decreased, and the threshold for this behavior was 60.3% ± 1.9% SW. Similar behavior has been described for *Cancer magister* when salinity drops below 50% SW (Sugarman *et al.,* 1983).

Although it has been suggested that the closure response may help crabs endure tidal-scale exposures to low salinity, there has been no evidence to suggest that this behavior is a means of ion conservation (Sugarman *et al.,* 1983). In the present study, specimens of *C. gracilis* isolated the branchial chambers as salinity was reduced, holding higher salinity water inside (Fig. 8). When higher salinity water is maintained inside the branchial chambers, the gradient for diffusive ion loss is less than if the water within the chamber were at equilibrium with the external medium. Similar iso-

lation behaviors to conserve ions during low-salinity exposure have been observed in relatively sedentary species of bivalves (Shumway, 1977; Djangmah *et al.,* 1979), cirripeds (Cawthorne, 1979), and gastropods (Shumway, 1979). However, the observations reported here represent a novel behavior for highly mobile invertebrates. Closure may be an effective means for osmoconformers to minimize the costs associated with locomotion (McMahon *et al.,* 1979) during avoidance behaviors in areas subject to tidal-scale salinity changes. When crabs displaying closure behavior were probed with a glass rod, they reacted vigorously and did not reassume the closed posture. It is therefore unlikely that the closure behavior observed here is the result of the crabs becoming incapacitated due to osmotic water onload (Prosser and Brown, 1961; Norfolk, 1978; Mantel and Farmer, 1983), as occurs in porcelain crabs (Davenport, 1972). It is also unlikely that crabs are reducing locomotor activity as a means of conserving energy for allocation to increased active ion transport or decreased permeability, because osmoconformers show no immediate changes in these parameters when exposed to dilute media (Lucu *et al.,* 2000; Rainbow and Black, 2001). The maintenance of higher salinity water in the branchial chamber, coupled with diffusive ion loss into the closed chamber, may allow *C. gracilis* to reduce the rate of diffusive ion loss and osmotic water onload (Cornell, 1973, 1974; McGaw and McMahon, 1996).

The closure response was closely linked to oxygen uptake. When exposed to low salinity, the crabs spent more time closed (Fig. 2), and there was a corresponding decrease in oxygen uptake (Fig. 6b). After closure, oxygen in the branchial chamber was depleted within a matter of minutes, and the P_{O_2} of the water in the branchial chamber fell to between 0 and 5 kPa. When crabs resumed activity, P_{O_2} within the branchial chamber rapidly returned to pretreatment levels. Thus, it appears that *C. gracilis* is using closure behavior as a means of exerting control over the rate of diffusive ion loss and osmotic water onload. This strategy may be advantageous in dealing with acute hyposaline exposure.

In addition to a short-term decrease in oxygen uptake, there was a longer-term bradycardia (Fig. 6a). Although changes in heart rate are thought to be closely related to locomotor activity (McGaw *et al.,* 1999), this does not seem to explain the bradycardia exhibited by *C. gracilis* in hyposaline conditions. After 2 h of exposure to 50% or 25% SW, crabs resumed pretreatment activity levels (Fig. 2), but without a corresponding increase in heart rate (Fig. 6a). The reduction in heart rate is coupled with a decrease in cardiac output (McGaw, 2006), which would ultimately lead to a decrease in hemolymph flow through the gills (McGaw *et al.,* 1999). The benefit of increasing the residence time of hemolymph in the gills could be a further reduction in the gradient for diffusive ion loss and osmotic water onload (Cornell, 1973, 1974; McGaw and McMahon, 1996). Heart rate and scaphognathite beat frequency remain depressed after oxygen uptake returns to pretreatment levels (McGaw, 2006), which suggests that the observed short-term decrease in oxygen uptake is attributable to changes in activity level (McMahon *et al.,* 1979) in the form of the observed closure behavior, independent of changes in ventilation and cardiac parameters. This is in contrast to *Homarus americanus,* where changes in oxygen uptake correspond to changes in heart rate and ventilation (Jury *et al.,* 1994b).

Since *C. gracilis* exhibited more than one behavioral strategy for coping with low salinity, a trade-off may be occurring between avoidance of low salinity and use of the closure response. Although a few individuals exhibited a brief closure response when exposed to salinities as high as 75% SW, the average salinity that initiated closure was 60.3% ± 1.9% SW. The salinities at which crabs displayed closure behavior when given a choice of salinities in a salinity gradient (Fig. 3a) approached the salinity tolerance for this species (55%–60% SW; Curtis and McGaw, unpubl. obs.). In *Cancer magister,* closure behavior is also displayed at salinities approaching the lethal limit (Sugarman *et al.,* 1983). Energetically, if episodes of low salinity are transient, it may be beneficial to wait for salinity to increase rather than enduring the added costs of locomotion (McMahon *et al.,* 1979). However, the exact interplay between avoidance and closure behavior in the field is yet to be determined.

Kinne (1966) reported that organisms respond to dilution of the medium with an increase, decrease, or no change in respiratory parameters. On the basis of this observation, it has been inferred that euryhaline organisms show an increase and stenohaline organisms show a decrease in respiratory parameters, but this hypothesis had been difficult to substantiate (Wheatly, 1988). As more information becomes available on the respiratory, cardiac, and behavioral responses of decapod crustaceans to hyposaline exposure, a general trend is developing. Efficient osmoregulators such as *Callinectes sapidus* and *Carcinus maenas* show an increase in heart rate, respiration, and locomotor activity with dilution of the medium, facilitating increased oxygen uptake and active ion transport (Taylor, 1977; McGaw and Naylor, 1992; Piller *et al.,* 1995; Hume and Berlind, 1976; McGaw and Reiber, 1998; McGaw *et al.,* 1999). Weak osmoregulators such as *Cancer magister* tend to show mixed responses: oxygen uptake is unaffected by dilution of the medium (Brown and Terwilliger, 1999); however, heart rate increases (McGaw and McMahon, 1996). There is also a transient increase in locomotion (McGaw *et al.,* 1999) that is sometimes followed by a closure response (Sugarman *et al.,* 1983). The lobster *Homarus americanus* shows a reduction in heart rate similar to that of osmoconformers (Cornell, 1973, 1974) when acutely exposed to low salinity (Dufort *et al.,* 2001). However, in contrast to *Cancer gracilis,* as

described here, there is an increase in oxygen uptake (Jury *et al.,* 1994b). The osmoconformer *Libinia emarginata* exhibits a decrease in heart rate and locomotion with hyposaline exposure (Cornell, 1973, 1974; McGaw *et al.,* 1999). The behavioral and physiological responses of *C. gracilis* to hyposaline exposure extend these findings and support our prediction that osmoconformers will exhibit a reduction in oxygen uptake and heart rate, which may serve to limit diffusive ion loss (Cornell, 1973, 1974; McGaw and McMahon, 1996) in the absence of efficient regulatory mechanisms.

The present study suggests a behavioral mechanism that allows control of physiological variables, further emphasizing the idea that in the absence of physiological adaptation, osmoconformers use behavior to cope with osmotic stress (Spaargaren, 1973; Wolcott and Wolcott, 2001). During hyposaline exposure, behavior exerts much more influence on the physiological parameters of *Cancer gracilis* than has previously been reported for the osmoconformer *Libinia emarginata* (McGaw *et al.,* 1999). The results of this study extend previous findings for weak osmoregulators and emphasize the vital link between behavior and physiology in osmoconforming decapods. By combining physiological and behavioral mechanisms, osmoconforming decapods may be able to exploit habitats that were previously thought to be the exclusive domain of osmoregulators. This work underscores the importance of an integrative approach when investigating how organisms that appear to be poorly adapted cope with environmental perturbations.

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