



# Effects of salinity on the juvenile crab physiology and agonistic interactions between two species of blue crabs, *Callinectes sapidus* and *C. similis* from coastal Louisiana

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## Abstract

*Callinectes sapidus* and *Callinectes similis* are found in estuaries along the northern Gulf of Mexico. Juvenile *C. sapidus* are heavier at similar carapace widths than juvenile *C. similis*. Juvenile *C. sapidus* and *C. similis* exhibit similar patterns of hemolymph osmolality, oxygen consumption, lactic acid concentration, and renal filtration and secretory rates along constant and fluctuating salinity gradients. When similar sized juveniles were present, significantly more *C. sapidus* were preyed upon at 30 PSU but not at 5 PSU. Juvenile *C. similis* are more vulnerable to predation by adult *C. sapidus* at low salinity, this increased predation by adult *C. sapidus* on juvenile *C. similis* at 5 PSU may contribute to limiting the presence of *C. similis* to higher salinity water along estuarine gradients.

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## 1. Introduction

The blue crab, *Callinectes sapidus*, and lesser blue crab, *Callinectes similis*, are found on the Atlantic and Gulf of Mexico coasts of North America. Both juveniles and adults are found in river mouths, estuaries and coastal waters (Cameron, 1978; Hsueh et al., 1993). As juveniles, the two species often compete for resources (Engel, 1977; Hsueh et al., 1992b). The similarity in diet between *C. sapidus* and *C. similis* indicates that there is a high percentage of overlap in their prey. Fish, bivalves, brachyuran, and gastropod remains constituted the four

most common prey items for both species from a mud-bottom habitat in Mobile Bay, Alabama (Hsueh et al., 1993). The two species inhabit similar environmental niches. Based on laboratory observations, *C. sapidus* appears more aggressive. This aggression may play a larger role in the local distribution of juveniles than previously thought, because *C. similis* is more likely to suffer from this competition.

While both species tolerate habitats with a wide range of salinities, *C. sapidus* inhabits a wider range, reportedly from 0 to 117 PSU than *C. similis*, generally above 15, (Williams, 1984). Hsueh et al. (1992a) reported 75% mortality in juvenile *C. similis* after seven days at 5 PSU. Guerin and Stickle (1992, 1997a,b) reported *C. sapidus* to be only slightly more tolerant of low salinity than *C. similis*. The 28 day LC<sub>50</sub> is less than 2.5 PSU for

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*C. sapidus* and is 2.6 for *C. similis*. Decreased growth, scope for growth and increased metabolic demands at salinities less than 10 PSU in laboratory experiments may limit juvenile *C. similis* to salinities greater than 10 (Guerin and Stickle, 1997a,b). Short term salinity fluctuation below 10 PSU may also limit the distribution of the lesser blue crab. The urine of adult *C. sapidus* adapted to fresh water is isosmotic with the hemolymph and the rate of urine production and  $\text{Na}^+$  and  $\text{Cl}^-$  efflux was estimated to represent 12.7% of the body weight and ion efflux per day (Cameron, 1978). Therefore, urinary losses could represent a substantial physiological stress on both species.

The effect of constant salinity gradients on juvenile *C. sapidus* and *C. similis* tolerance, osmoregulation and energy budgets, including oxygen consumption rates has been assessed by Guerin and Stickle (1992, 1997a,b). *C. sapidus* acclimatized to brackish (20–30 PSU) water had a 33% higher oxygen consumption rate on an ash free dry weight basis at 2.5 than at 25 PSU while *C. sapidus* acclimatized to hypersaline (30–45) waters had a 66% higher oxygen consumption rate at 2.5 than at 25 PSU. *C. similis* acclimatized to brackish waters had a 190% higher oxygen consumption rate on an ash free dry weight basis at 2.5 than at 25 PSU (Guerin and Stickle, 1992, 1997a,b).

Estuaries frequently exhibit tidally influenced fluctuating salinity cycles, thus it is important to understand the effect that a salinity cycle has on the physiology of juvenile blue and lesser blue crabs. Salinity variation has been monitored for 791 days in a number of locations in coastal Louisiana where the vast majority of the patterns are diurnal and the daily amplitude of fluctuation as high as 20 PSU occurred 11% of the time (Hewatt, 1951). Blue crabs are osmoregulators and compensate for the fluctuating salinities to which they are exposed via several physiological mechanisms (Findley et al., 1978a,b; Lovett et al., 2006; Sabourin, 1984; Guerin and Stickle, 1997b). Hemolymph osmolality and ion concentrations were consistently lower in *C. similis* than in *C. sapidus* at low salinities (Piller et al., 1994). Likewise, Guerin and Stickle (1997b) found juvenile *C. sapidus* to be a slightly better hyperosmotic regulator than *C. similis* at low salinity.

When aerobic biochemical pathways are no longer available or are unable to meet metabolic demands, decapod crab energetic demands are met by anaerobic glycolysis with L-lactate as the end product (Graham et al., 1983; Livingstone et al., 1990); the presence of L-lactate is an indicator of anaerobic stress.

Because *C. sapidus* does not osmoregulate completely across salinity gradients, lower hemolymph osmolality reduces the oxygen affinity of hemocyanin which lowers

the overall oxygen carrying capacity of the hemolymph (Mangum and Towle, 1977). Lactate also has a pronounced effect on the oxygen affinity of hemocyanin by causing a marked increase in oxygen affinity when bound to hemocyanin. These counteracting effects of decreased hemolymph osmolality and increased lactic acid concentrations on hemocyanin stabilize oxygen uptake at the gill, as opposed to a switch to anaerobic metabolism in the tissues (Graham et al., 1983).

The present study investigated the physiological and behavioral factors which affect the distribution of these two sympatric species, *C. sapidus* and *C. similis* along an estuarine gradient. The objectives of the study were to: (1) quantify the effects of steady state and fluctuating salinity on hemolymph osmolality, oxygen consumption rates, renal filtration and secretion rates (steady state only) and lactic acid content in juvenile *C. sapidus* and *C. similis* and (4) determine agonistic interactions between *C. sapidus* and *C. similis* at 30 and 5 PSU.

## 2. Methods

### 2.1. Collection and maintenance

Juvenile *C. sapidus* and *C. similis* were collected by dip net near the base of the bridge over Bayou LaFourche at Port Fourchon, Louisiana in June and July 1999 and July and August 2000 for the physiology experiments. The salinities at the times of collection were 28–32 PSU. Juvenile crabs were maintained in the laboratory in artificial sea water made from Instant Ocean sea salts and stepwise adapted at 2 PSU per day until the experimental salinity was reached and held at that experimental salinity for a minimum of 14 days before experiments were conducted. Crabs were held together in individual compartments made of PVC piping with the ends covered by Nitex screen in several large aquaria under 24 hour illumination at 22 °C.

Juvenile crabs of both species were collected by dip net from the base of the bridge over Bayou Fourchon near Port Fourchon on June 9 and June 24, 2003, September 10, 2004, and September 12 and 29, 2005 for the agonistic behavior experiments. Ambient salinity was 28–31 PSU. All crabs were returned to LSU where their carapace width and wet weight was determined. Weight to length relationships of both species were fit to a second degree polynomial equation. Crabs were held together in individual compartments made of PVC piping with the ends covered by Nitex screen in several large aquaria under 24 hour illumination at 17 °C. Juvenile crabs were maintained in the laboratory in artificial sea water made from Instant Ocean sea salts

and stepwise adapted at 2 PSU per day until the experimental salinity was reached then they were held at that experimental salinity for a minimum of 14 days before experiments were conducted.

## 2.2. Salinity

### 2.2.1. Steady state

A set of 5–8 crabs of each species were also stepwise adapted together to 2.5, 10 and 25 PSU and held for 14 days. These crabs provided acclimation data for the steady state salinities.

Oxygen consumption, hemolymph osmolality, and lactic acid production were determined at the three steady state salinities; 2.5, 10 and 25 PSU. Wet weights of *C. sapidus* were  $3.10 \pm 1.21$  (SEM) ( $N=8$ ),  $2.73 \pm 1.60$  (7), and  $2.69 \pm 1.43$  (8) g at 25, 10, and 2.5 PSU respectively, while wet weights of *C. similis* were  $2.72 \pm 1.32$  (7),  $3.06 \pm 1.43$  (8), and  $2.77 \pm 1.90$  (5) g at 25, 10, and 2.5 PSU. Crab wet weight did not vary significantly between species or among experimental salinities (two way ANOVA).

Renal filtration and secretion rates were also determined at 2.5, 10 and 25 PSU. *C. sapidus* wet weight was  $24.23 \pm 4.69$  ( $N=5$ ) at 25 PSU, and  $10.47 \pm 4.59$  (6) at 2.5 PSU (p-NS), data from the 10 PSU could not be used because bath radioactivity per ml was too low to accurately calculate renal filtration and secretion rates. *C. similis* wet weights were  $2.83 \pm 1.46$  (6) g at 25 PSU,  $2.71 \pm 1.23$  (6) g at 10 PSU and  $2.51 \pm 1.57$  (5) g at 2.5 PSU ( $p \leq 0.019$ ). Dry weight values for the same *C. sapidus* were  $7.40 \pm 1.20$  (5) g at 25 PSU and  $2.58 \pm 1.17$  (6) g at 2.5 PSU (p-NS), in *C. similis* these values were  $0.87 \pm 0.48$  (6) g at 25 PSU,  $0.78 \pm 0.34$  (6) at 10 PSU and  $0.74 \pm 0.55$  (5) g at 2.5 PSU (p-NS).

### 2.2.2. Diurnal fluctuations

A diurnal salinity cycle was established using a technique developed by Wells and Ledingham (1940) and modified by Stickle and Howey (1975) and Findley and Stickle (1978). We chose the diurnal 10→2.5→10 PSU pattern to represent salinity stress at an area in the upper estuary where *C. similis* would be expected to be excluded if salinity is the limiting environmental factor.

The diurnal salinity fluctuation pattern of 10→2.5→10 PSU was simulated by adding deionized water into a fixed volume aquarium over a 10 hour period at a flow rate calculated to lower the salinity, followed by a 2 hour simulated slack water period at 2.5 PSU. Then 12.5 PSU seawater was added to the system at the same flow rate as in the descending phase over another 10 hour period followed by another 2 hour simulated slack water period

at 10 PSU. Every 3 h ambient seawater and 6 juvenile *C. sapidus* and *C. similis* were sampled for hemolymph osmolality; 8–10 crabs of each species were sampled for oxygen consumption rate and lactic acid production.

## 2.3. Hemolymph osmolality

Hemolymph was obtained by blotting dry the membrane at the base of a swimming leg, puncturing the membrane with a glass capillary tube, and collecting 6–8  $\mu$ l of hemolymph. The hemolymph osmolality was determined with a Wescor vapor pressure osmometer (Model 5100 B). The osmolality of a sample of ambient seawater was determined with each set of hemolymph samples.

## 2.4. Oxygen consumption

Oxygen consumption rates were determined using a flow-through system described by Stickle et al. (1985). For each experimental exposure, 8–10 crabs were used. Individuals were placed into one of twelve glass chambers (~250 ml) through which water flowed by gravity via a manifold system. The two outermost chambers were used as blanks to read the partial pressure of oxygen in the control water. After the individuals were placed into their chambers, they were allowed to acclimate for 1 h. Oxygen partial pressure was determined with a calibrated Strathkelvin 781 oxygen meter connected to an oxygen electrode. After acclimation, flow rates were determined by collecting drip samples from the exit port of the individual glass chambers. For oxygen partial pressure determination, water samples were collected anaerobically from the exit port of each chamber using a glass syringe and immediately injected into an oxygen electrode maintained at 22 °C by a water jacketed flow-through housing chamber. In order to determine the amount of oxygen consumed by the individual crab, the following equation was used:  $\mu\text{l O}_2\text{h}^{-1} = (\text{PO}_2\text{ of control} - \text{PO}_2\text{ from animal chamber}) / \text{PO}_2\text{ from control} \times \text{flow rate} (\text{lh}^{-1}) \times 1000 \times \text{oxygen content in water at that salinity and temperature} (\text{mL O}_2 \cdot \text{L}^{-1})$ .  $\text{PO}_2$  is the partial pressure of oxygen in the water sample.

## 2.5. Lactic acid

After exposure to experimental conditions, juvenile crabs were frozen in liquid nitrogen and stored in an ultracold freezer (−80 °C) until homogenization. Whole crabs were weighed and homogenized in 10 times their wet weight (g) in ice cold perchloric acid (0.6 mol/L).

The homogenate was centrifuged for 15 min at 3000 g at 2 °C. The supernatant (4 ml) was transferred to a fresh tube where 0.01 ml methyl orange (0.05%, w/v) was added. The supernatant was neutralized by titration with 3 M KOH. A 1 ml aliquot of the neutralized supernatant was used for the assay sample. L-lactic acid assay was determined by the Franz Noll procedure (Bergmeyer, 1983). The final assay contained: 116 mmol/l glutamate, 0.93 mmol/l NAD, 1.3 kU/l ALT (GPT), and 4 kU/l LDH. L-lactic acid was used as the standard for this assay. The assay absorbance was read at 340 nm on a spectrophotometer after 2 h incubation at 25 °C.

### 2.6. Renal filtration and secretory rates

Both species were acclimated to 2.5, 10 or 25 PSU for two weeks. Each crab was injected with 10 µl containing 1 µCi <sup>14</sup>C-polyethylene glycol (PEG) and 1 µCi <sup>3</sup>H-*p*-aminohippuric acid (PAH) and allowed 1 h for equilibration of the isotopic markers in the hemolymph. PEG was selected as a marker for renal filtration and PAH was chosen for tracking renal secretion. Each crab was rinsed three times in the acclimation seawater and transferred to 30 ml of the appropriate medium. Bath samples were collected at time zero and 1 h later to determine the total radioactivity excreted by the animal. After the 1 hour sample, each crab was weighed and a hemolymph sample (200–300 µl) collected. The radioactivity in each sample was determined using a double label counting procedure with a Beckman 6000 Liquid Scintillation Counter. The <sup>14</sup>C-PEG lost from the hemolymph to the bath was assumed to represent the renal filtration rate of the kidney using the method of Dietz and Byrne (1999). The total disintegrations per minute (DPM) of PEG appearing in the bath was divided by hemolymph PEG specific activity (DPM/ml) to calculate filtration rate. Filtration rate was expressed as ml of hemolymph cleared of PEG per hour per kilogram wet and dry crab. PAH secretion was calculated by a similar method. PEG and PAH clearances measured simultaneously were compared for both species in the acclimation salinities.

### 2.7. Agonistic behavior

*C. sapidus* and *C. similis* were stepwise adapted to either 30 or 5 PSU at 16–17 °C water temperature. The crabs were then placed into aquaria with sand and *Rangia cuneata* shell substrate which approximates the substrate at the collection site of sympatrically collected juveniles. There was no sea grass at this collection locale. Groups consisted of different combinations of species and size. Size of crabs was initially determined

by carapace width; *C. sapidus* juveniles were considerably heavier than *C. similis* juveniles of the same carapace width. In order to study agonistic interactions among similarly sized juveniles (16–20 mm CW), four experimental replicates were run for each treatment at 5 and 30 SU. Sample size for each treatment was 14 crabs per replicate in a 38 l aquarium containing an under-gravel filter overlaid with 2.5 cm oyster chips covered with 2.5 cm sand and shells of *R. cuneata*. Abdomens of frozen shrimp were dissected and added to each experimental tank once a day to reduce hunger induced predation among juvenile crabs. The duration of each experimental period was 7 days after which surviving crabs were counted. Each experimental treatment was replicated 4 times. The experimental treatments were:

- C. similis* juveniles alone
- C. sapidus* juveniles alone
- C. similis* and *C. sapidus* together (28 total crabs)

Mortalities were analyzed by ANOVA and Tukey's Multiple Range Test was used to determine significant differences among treatment means if the ANOVA test indicated significant variation among treatment means.

Adult blue crabs (>104 mm CW) also served as predators at 30 and 5 PSU. Abdomens of frozen shrimp were dissected and added to each experimental tank once a day to reduce hunger induced predation among juvenile crabs. The number of juvenile crabs (7.4–24.8 mm CW mean for the 5 replicates per species) per experimental treatment was 12 and the duration of the experiment was 7 days after which surviving crabs were enumerated. Mortalities were analyzed by ANOVA and Tukey's Multiple Range Test was used in the juvenile agonistic experiment to determine significant differences among treatment means if the ANOVA test indicated significant variation among treatment means. *T*-tests were used to compare mortality rates of juvenile *C. sapidus* and *C. similis* in the presence of adult (>104 mm carapace width) *C. sapidus*.

### 2.8. Statistical analysis

All juvenile crabs from each species and collection were weighed wet, their carapace width measured and the data were fit to a second order polynomial equation with the PRISM Software package.

In order to remove the factor of body weight, all oxygen consumption rates and lactic acid concentrations were standardized to 1 g body weight by dividing by the wet weight in grams. One-way or two-way analysis of variance (ANOVA) was used after determining that the data were

normally distributed to identify significant differences among main effects at the 0.05 level of significance. If variation in the one way ANOVA of the main effect was significant, *a posteriori* Tukey's test was used to determine significant differences among treatment means. Student's *t*-tests were used to compare crab weights in the renal experiments and experimental survivals in each predation experiment at the 0.05 level of significance.

### 3. Results

#### 3.1. Juvenile recruitment

The relative composition of juvenile blue crabs and lesser blue crabs at the field site was predominantly blue crabs in June and predominantly lesser blue crabs in September. Equal carapace width juvenile blue crabs (17.5 mm carapace width) were 1.30–1.55 times heavier than lesser blue crabs. Second order polynomial equations fit the weight to carapace width (cw) of both species very well on the sampling dates for both species ( $r^2=0.9285-0.9891$ ): Data and a regression line for the June 9, 2003 collection are plotted in Fig. 1.

#### 3.2. Steady state salinity

Hemolymph osmolality of *C. sapidus* and *C. similis* varied directly with salinity across the 2.5, 10 and 25 PSU range (Table 1). There was a significant difference between the hemolymph osmolalities as a function of ambient salinity ( $p<0.003$ ) following acclimation to 2.5, 10 and 25 PSU. There was no significant difference in hemolymph osmolality between *C. sapidus* and *C. similis* ( $p<0.100$ ). There were no significant differences between species ( $p<0.260$ ) or between salinities ( $p<0.087$ ) in

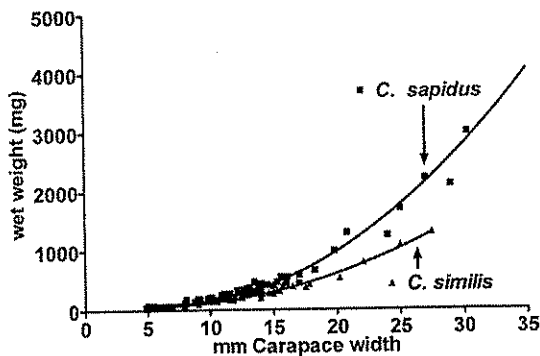


Fig. 1. Crab wet weight – carapace width data were fit to a second order polynomial equations for a collection on June 9, 2003. The equation for *C. sapidus* was  $\text{mg wet} = 307.2 - 64.66 \text{ mmCW} + 4.92 \text{ mm}^2$  ( $n=73$ ;  $r^2=0.9816$ ) and the equation for *C. similis* was  $\text{mg wet} = 124.2 - 25.66 \text{ mm} + 2.497 \text{ mm}^2$  ( $n=26$ ;  $r^2=0.9829$ ).

Table 1

Hemolymph osmolality, oxygen consumption and lactic acid body burdens of *Callinectes sapidus* and *C. similis* after acclimation to three steady state salinities

Variables/ species	2.5 PSU	10 PSU	25 PSU
Hemolymph osmolality (mOsM)			
<i>C. sapidus</i>	642±102 (6)	732±52 (6)	806±17 (6)
<i>C. similis</i>	690±30 (6)	794±20 (6)	908±30 (6)
Oxygen consumption ( $\mu\text{l O}_2 \cdot \text{g wet}^{-1} \cdot \text{h}^{-1}$ )			
<i>C. sapidus</i>	177±11 (10)	304±93 (10)	292±78 (10)
<i>C. similis</i>	222±70 (10)	451±127 (10)	321±43 (10)
Lactic acid concentration ( $\text{mmol lactate} \cdot \text{g wet}^{-1}$ )			
<i>C. sapidus</i>	2.237±0.20 (10)	1.755±0.32 (10)	2.939±0.47 (10)
<i>C. similis</i>	0.920±0.10 (10)	2.920±0.46 (10)	1.382±0.24 (10)

Mean±standard error ( $n$ =number of animals).

oxygen consumption rate (Table 1). There was a significant difference between species ( $p<0.0001$ ) in the lactic acid concentration. There were no significant differences between salinities ( $p<0.208$ ) in the lactic acid concentration of *C. sapidus* and *C. similis* acclimated to 2.5, 10 and 25 PSU (Table 1).

No significant difference occurred in renal filtration (PEG) and secretion (PAH) rates at 2.5 and 10, and

Table 2

Renal filtration (PEG) and secretion (PAH) rates are given for *Callinectes sapidus* and *C. similis* adapted to 2.5 or 25 PSU in  $\text{mls} \cdot \text{Kg wet}^{-1} \cdot \text{h}^{-1}$ ,  $\text{mls} \cdot \text{Kg dry}^{-1} \cdot \text{h}^{-1}$ , and Percent body water as  $\bar{x} \pm \text{SEM}$  (N)

Species/ variable	2.5 PSU	10 PSU	25 PSU	ANOVA
<i>C. sapidus</i>				
PEG-wet	43±19 (5)	ND	4±2 (5)	$p<0.07$
PEG-dry	159±51 (5) <sup>a</sup>	ND	17±9 (5) <sup>b</sup>	$p<0.03$
PAH-wet	344±146 (5)	ND	64±29 (5)	$p<0.10$
PAH-dry	1311±482 (5)	ND	259±135 (5)	$p<0.07$
Percent water	74.8±2.4 (5)	72.8±2.3 (5)	68.4±3.3 (5)	$p<0.15$
<i>C. similis</i>				
PEG-wet	41±5 (4)	90±22 (6) <sup>a</sup>	34±10 (5)	$p<0.59$
PEG-dry	225±64 (4)	344±74 (6) <sup>a</sup>	115±30 (5)	$p<0.14$
PAH-wet	298±88 (4)	621±130 (6) <sup>a</sup>	599±217 (6)	$p<0.31$
PAH-dry	1374±457 (4)	2850±175 (6) <sup>a</sup>	2425±691 (5)	$p<0.27$
Percent water	77.8±5.0 (4)	73.7±2.4 (6) <sup>a</sup>	70.6±2.0 (5)	$p<0.18$

<sup>ND</sup>Radioisotope DPM per milliliter was <50 after a 1 h incubation period, therefore filtration and secretion rates could not be calculated and percent body water data are not presented. Fisher's Protected LSD test was used to determine the statistical difference among treatment means if the ANOVA indicates significant variation; values sharing the same letter in a row are not significantly different from each other.

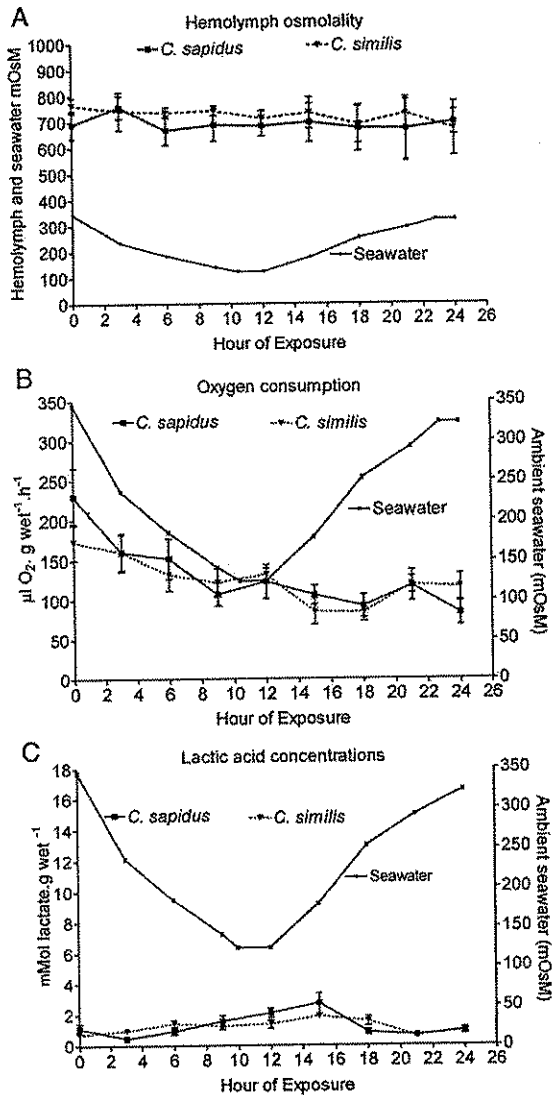


Fig. 2. Physiological responses of *Callinectes sapidus* and *C. similis* to a 10→2.5→10 PSU diurnal salinity fluctuation (A: Hemolymph osmolality; B: Oxygen consumption; C: Lactic acid levels). Data were plotted as the mean±SEM. The usual sample size for the hemolymph osmolality determinations was 6 and for oxygen consumption and lactic acid levels was 8.

25 PSU for *C. sapidus* and *C. similis* on a wet weight basis (Table 2). *C. sapidus* filtration of PEG in 25 PSU was significantly lower than at 2.5 PSU when calculated on a dry weight basis. Percent body water of neither species varied significantly as a function of salinity (Table 2).

### 3.3. Diurnal fluctuating salinity cycle

Under a diurnal fluctuating salinity regime, hemolymph osmolalities did not vary in a significant manner across the diurnal 10–2.5–10 PSU amplitude of

fluctuation ( $p < 0.276$ ) or between species ( $p < 0.984$ ) (Fig. 2A). Throughout the diurnal salinity fluctuation, oxygen consumption rates varied significantly among experimental salinities ( $p < 0.001$ ) and between *C. sapidus* and *C. similis* ( $p < 0.001$ ). The oxygen consumption rate of *C. sapidus* and *C. similis* decreased significantly ( $p < 0.05$ ) throughout the course of the cycle (Fig. 2B). Lactic acid concentration in the crabs varied significantly between species during the salinity fluctuation ( $p < 0.001$ ). Lactic acid concentration of *C. similis* did not vary significantly over the course of the diurnal salinity fluctuation ( $p < 0.094$ ) and was maximum at 15 h of the cycle ( $1.89 \pm 0.17$  mmol g wet<sup>-1</sup>) while lactic acid concentration of *C. sapidus* decreased during the first 3 h of declining salinity then increased significantly as salinity decreased to 2.5 PSU, increased again to  $2.72 \pm 0.68$  mmol g wet<sup>-1</sup> at 6.1 PSU by 15 h of the cycle ( $p < 0.001$ ), and subsequently decreased significantly as salinity increased ( $p < 0.01$ ) (Fig. 2C).

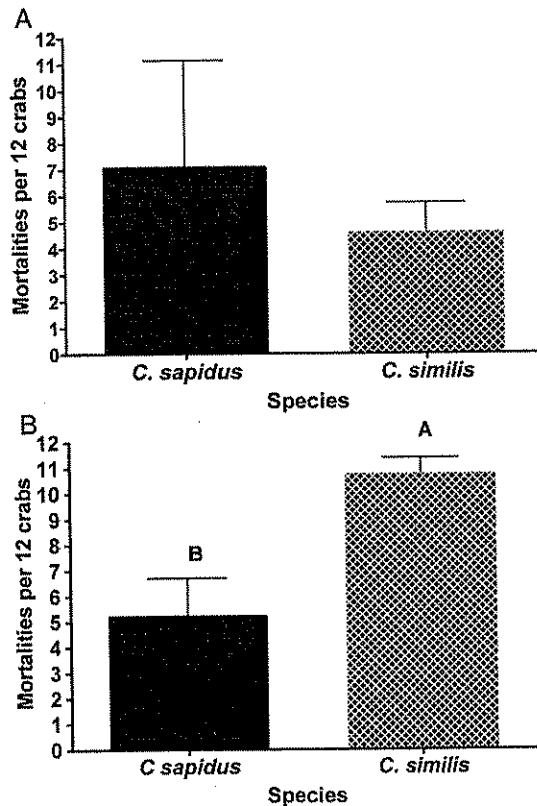


Fig. 3. A: Predation rates of adult (>104 mm CW) *Callinectes sapidus* on 12 juvenile *C. sapidus* and *C. similis* at 30 PSU. B: Predation rates of adult *C. sapidus* (>104 mm CW) on 12 juvenile *C. sapidus* and *C. similis* at 5 PSU. Significantly more *C. similis* than *C. sapidus* were preyed upon at 5 PSU. Data were plotted as mean±SEM.

3.4. Agonistic behaviors

The agonistic interactions between *C. sapidus* and *C. similis* indicate that adult blue crabs prey on significantly more juvenile *C. similis* than *C. sapidus* at 5 PSU and juvenile *C. sapidus* are significantly more cannibalistic on themselves alone than when both species are present together or only *C. similis* is present at 30 PSU. No significant difference in predation rates by adult blue crabs on juvenile *C. sapidus* and *C. similis* occurs at 30 PSU (Fig. 3A) but adult blue crabs prey on significantly more *C. similis* at 5 PSU (Fig. 3B). Juvenile *C. sapidus* prey on significantly more similar sized juvenile *C. sapidus* than on specimens of both species together, or juvenile *C. similis* among themselves at 30 PSU (Fig. 4A). No significant difference exists with respect to cannibalism within or among similar sized juveniles of the two species at 5 PSU (Fig. 4B).

4. Discussion

The lack of a significant difference in oxygen consumption rate in the steady state salinity data is contrary to the findings of Guerin and Stickle (1997b). They found significant differences based on steady state salinity experiments. Their data show an increased oxygen consumption rate at 2.5 PSU over that at 10 and 25 PSU. This discrepancy between the previous and present studies may occur because data are presented on a wet weight basis in the present study while Guerin and Stickle's data (1992, 1997b) are recorded on an ash free dry weight basis. Although there was no significant difference in the percent body water as a function of salinity in either species due to individual variability, the percent body water of both species varied inversely with ambient salinity which would tend to obscure any increase in the rate of oxygen consumption at low salinity (Table 1).

The filtration rate of PEG of both species tended to be higher in 2.5 PSU but was only significant in *C. sapidus* when calculated on a dry weight basis. Binns (1969) observed higher filtration rates in *Carcinus meanus* in 40% compared with 100% salinity. Our juvenile crabs were active during the kidney study, and motor activity reduces urine release in crustaceans (Riegel, 1960). In addition, urine is released from the bladder of *C. sapidus* intermittently as noted by the pulsatile release of Na and Cl (Cameron, 1978). The discontinuous release of urine likely accounted for the high variability in these studies. Filtration rate differs from urination rate depending on the amount of water reabsorbed. Our PEG filtration rate values are essentially the same as the urine production rate measured by Cameron (1978) suggesting that water reabsorption rate is small. The renal filtration rate is the same in both species of *Callinectes* so excretory function may not impose a species specific salinity restriction.

PAH clearance, from both species of juvenile crabs was about an order of magnitude higher than for PEG. This result would be expected because most of the PAH clearance is due to secretory mechanisms. PAH secretion would be more sensitive to the concentration of PAH in the hemolymph than changes observed for PEG filtration. However, variation in individual crab release of urine, as noted above, would mask rather modest changes in the secretion rate of PAH (Riegel, 1960; Cameron, 1978).

The two significant differences in response patterns between *C. sapidus* and *C. similis* exposed to a diurnal pattern of salinity fluctuation were variation in the lactic acid concentrations and oxygen consumption rates. Lactic acid is a known indicator of anaerobic stress

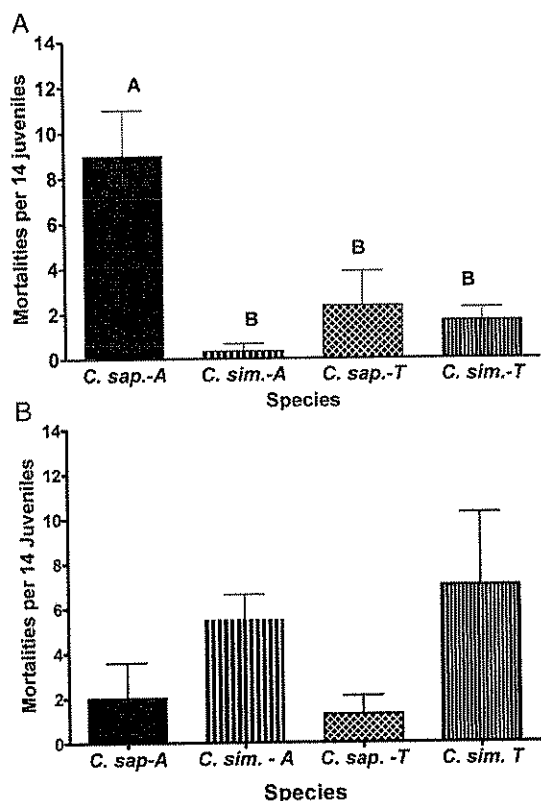


Fig. 4. A. Mortality was significantly higher after one week among 14 similar sized juvenile *Callinectes sapidus* than among juvenile *C. similis* or among both species in the same experimental aquarium (28 total crabs) at 30 PSU. B. No significant difference existed in the mortality of 14 juvenile *C. sapidus* or *C. similis* alone or together (28 total crabs) in the same experimental aquarium at 5 PSU.

in invertebrates (Graham et al., 1983). The initial responses of the two species were different from one another; *C. sapidus* exhibited a decrease in lactic acid concentration for the first three hours of the diurnal pattern of salinity fluctuation and then increased until hour 15 when ambient salinity had increased from 2.5 to 6.1 PSU, then the lactic acid concentration decreased for the remainder of the diurnal cycle. In contrast, the lactic acid concentration in *C. similis* was higher than in *C. sapidus* and it did not change significantly during the cycle. Although statistically significantly different, the concentrations of lactic acid found may not be biologically stressful to either species of blue crab. The lactic acid concentration of *C. sapidus* exposed to anoxia for 80% of their  $Lt_{50}$  at 22.5 °C was  $10.44 \pm 2.38$  mmol g wet<sup>-1</sup> (unpublished data). The maximum concentration of lactic acid,  $2.73 \pm 0.68$  mmol·g wet<sup>-1</sup> and  $1.89 \pm 0.17$  mmol g wet<sup>-1</sup>, occurred in *C. sapidus* and *C. similis* at hour 15 of the diurnal salinity cycle when ambient salinity was 6.1 PSU. These maximum lactic acid concentrations in *C. sapidus* juveniles exposed to fluctuating salinity only represent 26.1% of the lactic acid concentration accumulated after exposure to 80% of their  $Lt_{50}$  time at 22.5 °C.

Findley et al. (1978a,b) found that adult blue crabs exhibited a pattern of oxygen consumption rate that varied inversely with fluctuating salinity during a diurnal 30→10→30, 20→10→20, and 10→5→10 PSU cycle and that the hemolymph osmolality was hyperosmotic and varied little during the 30→10→30, and 20→10→20 PSU cycles. The blue crab rate of oxygen consumption dropped during the initial phase of declining salinity at a rate directly proportional to the rate of salinity decrease, perhaps a metabolic adjustment period of the adult crabs prior to the increase in oxygen consumption rate. In contrast, the rate of oxygen consumption of juvenile *C. sapidus* exposed to the diurnal 10→2.5→10 PSU cycle in this study decreased throughout the course of the cycle. Differences in response patterns of juvenile and adult *C. sapidus* may be due to the more extreme salinity stress to which the juvenile crabs were exposed or to the relative amount of gill surface area exposed to fluctuating ambient salinity in juvenile and adult blue crabs. Of particular note is the fact that changes of the gill Na<sup>+</sup>, K<sup>+</sup>-ATPase of *C. sapidus* as an ion transporter during the initial 24 h after transfer from 32 to 10 PSU involves modulation of existing enzyme or other mechanisms rather than an increase in the amounts of Na<sup>+</sup>, K<sup>+</sup>-ATPase enzyme but crabs exposed to dilute seawater over 18 days exhibited an increase in enzyme specific activity and protein level (Lovett et al., 2006). Modulation of existing enzyme

activity during tidal fluctuations of ambient salinity and hemolymph osmolality conserves the energetic cost of anisosmotic regulation.

In the agonistic behavior study, adult *C. sapidus* did not exhibit a preference for juveniles of either species at 30 PSU but preyed more effectively on juvenile *C. similis* at 5 PSU. This difference in the predation rate of adult blue crabs on juveniles is likely due to the reduced activity of juvenile *C. similis* at 5 PSU in comparison to the activity of juvenile *C. similis* at 30 PSU. Juvenile *C. similis* frequently swam in the aquarium and were often seen on the back of adult blue crabs at 30 PSU but the juvenile lesser blue crabs seldom swam or exhibited active movement at 5 PSU. Finally, similar size juvenile *C. sapidus* prey more effectively on themselves than on similar size *C. similis* at 30 PSU but this relationship disappeared at 5 PSU. These laboratory based agonistic behavior experiments in static aquaria indicate the potential role of predation in limiting the distribution of juvenile *C. similis* but require substantiation in mesocosm and/or field tethering experiments.

Do *C. similis* and *C. sapidus* exhibit different feeding behaviors in response to food stimuli in the water? Zimmer-Faust et al. (1984) report that marine decapods typically respond to weak chemical stimuli with near-field substrate probing, but when exposed to stronger chemical stimuli will locomote in search of food. Finelli et al. (2000) found that when *C. sapidus* is presented with wounded prey scent, it elicits an active search and upstream walking behavior. The system in which the behavior studies were run in the present study, was saturated with chemical stimuli when the predators were introduced to the prey. The responses to chemical stimuli between *C. sapidus* and *C. similis* should be investigated further.

*C. sapidus* appears to be much more aggressive than *C. similis*. *C. similis* will immediately attempt to flee when captured in a dip net, whereas *C. sapidus* will sit and exhibit aggressive displays from the bottom of the net. When trying to remove *C. sapidus* from the net, the aggression heightens. A possible explanation for the apparent aggression of *C. sapidus* may be its serotonin levels. Serotonin levels are known to increase the length of aggressive displays when injected into crustaceans by decreasing the likelihood of retreat (Huber et al., 1997).

Because these species obviously behave the same physiologically as a function of environmental salinity, except at the very lowest salinities, there is a possibility that their niche is separated in estuaries by several different factors, including tolerance to other environmental



factors to cannibalism by larger *C. sapidus* on juvenile *C. sapidus* and *C. similis*. Juvenile *C. sapidus* appears to be more cannibalistic on itself at high salinity. *C. sapidus* and *C. similis* include brachyurans in their diets (Hsueh et al., 1993). In some cases, the brachyurans in the diet included blue crabs, which supports the agonistic observations from this study and the contention that intrageneric predation and/or cannibalism is prevalent (Laughlin, 1982; Hines et al., 1990). Another factor may be the larger size of adult blue crabs and a faster growth rate of *C. sapidus*. *C. sapidus* typically grow less in carapace width per molt but molt more frequently than *C. similis* and adults are much larger than *C. similis* (Guerin and Stickle, 1997b). The fact remains that we are still unsure of the direct cause of the lack of juvenile *C. similis* in the low salinity region of estuaries, juvenile *C. similis* are certainly not physiologically limited by low salinity because they maintain a positive energy budget at 2.5 PSU. Different salinity tolerances of the megalopae larva of the two species may also contribute to their distributional separation in the lower end of the estuarine salinity gradient. Agonistic interactions between the species may be an important factor limiting the abundance of *C. similis* and other members of the “danea” species complex (Norse and Fox-Norse, 1982) in low salinity.

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