Respiratory and Circulatory Responses of the Blue Crab, Callinectes Sapidus (Rathbun), to Salinity and Naphthalene.

Thomas Donald Sabourin
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RESPIRATORY AND CIRCULATORY RESPONSES OF THE BLUE CRAB, CALLINECTES SAPIDUS (RATHBUN), TO SALINITY AND NAPHTHALENE

The Louisiana State University and Agricultural and Mechanical Col. Ph.D. 1981

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RESPIRATORY AND CIRCULATORY RESPONSES OF THE
BLUE CRAB, Callinectes sapidus (Rathbun),
TO SALINITY AND NAPHTHALENE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in
The Department of Zoology and Physiology

by
Thomas Donald Sabourin
B.A., University of Michigan, 1973
M.A., California State University Hayward 1977
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GLOSSARY OF TERMS

apnoea - Cessation of ventilation.

o/oo - Parts per thousand, standard units of expressing salinity (S) of sea water. Note: Some laboratories express salinity as "% sea water", but the salinity of full strength sea water does vary.

hemocyanin - Oligomeric copper-containing proteins (respiratory pigment) found in many arthropods and moluscs; oxygen binds to 2 copper atoms; polypeptide chain subunits about 75,000 daltons and form aggregates of hexamers.

hemolymph - Common term for hemocyanin-containing blood.

p50 - Estimate of oxygen affinity of respiratory pigment; oxygen tension where half of the pigment is bound to oxygen.

αwO₂ - Oxygen solubility coefficient; function of pressure, temperature, salinity.

PIO₂ - Oxygen tension (torr) of inspired water.

PEO₂ - Oxygen tension of expired water.

PAO₂ - Oxygen tension of postbranchial (analogous to arterial) hemolymph.

PVO₂ - Oxygen tension of prebranchial (analogous to venous) hemolymph.

CAO₂ - Oxygen content (vols %) of postbranchial hemolymph.

CVO₂ - Oxygen content of prebranchial hemolymph.

E, % - Percent extraction of oxygen from inspired water.

Eₜ₁₁, % - Percent extraction of oxygen from hemolymph by tissues.

VO₂ - Rate of oxygen uptake from ambient water.

Vₜ₉ - Volume of water ventilated across gills per unit time.
\( F_H \) - Heartbeat frequency.

\( SV \) - Stroke volume; volume of hemolymph pumped per heartbeat.

\( Q \) - Cardiac output; volume of hemolymph pumped per unit time, usually per min.

\( ppm \) - Parts per million; mg \( \cdot 1^{-1} \).

\( TL_m \) - Median tolerance limit; concentration of toxicant which is lethal to 50% of the test animals in a specified period of time.

water-soluble fraction - Portion of petroleum hydrocarbons which is dissolved in water.

naphthalene - dinuclear aromatic hydrocarbon; \( C_{10}H_8 \);

solubility in water = 20 ppm; present in crude and refined oils.
Adult intermolt blue crabs (Callinectes sapidus Rathbun) were stepwise acclimated to salinity at 22-25 °C. Respiratory and circulatory responses were investigated in steady state salinity (5, 10, 20 and 30 o/oo), diurnally fluctuating salinity (30-10-30 o/oo, 10-30-10 o/oo), and during 24-h exposure to water-soluble naphthalene at 0, 37.5% (0.9 ppm) and 75% (1.8 ppm) of the 24-h TLm in a flow through system at 10 and 30 o/ooS. No steady state salinity-related differences occurred in ventilation volume (Vw), oxygen extraction efficiency (E), oxygen uptake (VO₂), heartbeat frequency (ḞH), stroke volume (SV), cardiac output (Q), post- and pre-branchial oxygen tensions (PAO₂, PVO₂) and oxygen contents. Protein and copper concentrations, pH and in vitro hemolymph P₅₀ were higher in crabs at 10 o/oo than at 30 o/ooS. Responses to salinity fluctuation were affected by acclimation salinity. During 30-10-30 o/oo fluctuations little change occurred in blood flow and oxygen content. Vw and VO₂ changed inversely with salinity. PAO₂ and PVO₂ and hemocyanin oxygen affinity decreased. Salinity fluctuations of 10-30-10 o/oo elicited decreased ḞH, SV and Q as salinity was increased from 10 to 30 o/oo. After 96-h of fluctuation, mean ḞH, SV and Q were slightly elevated over 0-h controls. Percent oxygen
extraction, VO\textsubscript{2}, blood O\textsubscript{2} tension and content decreased during 10-30-10 o/oo fluctuations, while hemocyanin O\textsubscript{2} affinity remained constant. Protein and copper concentrations were unaltered during 96 hours of salinity fluctuation, regardless of direction.

No salinity related difference existed in the TL\textsubscript{m} of blue crabs exposed to naphthalene. The pooled salinity 24-h naphthalene TL\textsubscript{m} was 2.4 ppm. Responses to naphthalene differed with acclimation salinity. Crabs at 10 o/ooS exhibited increased V\textsubscript{w}, VO\textsubscript{2} and prebranchial pH, and decreased hemolymph O\textsubscript{2} tension in 37.5 and 75% TL\textsubscript{m}. SV, Q and body water increased, while hemolymph osmolality decreased 55-76 mOsm during exposure to 75% TL\textsubscript{m} - 10 o/ooS. In vitro oxygen affinity was increased at 37.5 and 75% TL\textsubscript{m} at 30 o/oo and 37.5% TL\textsubscript{m} at 10 o/oo. C. sapidus at 30 o/oo had increased V\textsubscript{w}, VO\textsubscript{2}, F\textsubscript{H}, SV and Q, and decreased PAO\textsubscript{2} and PVO\textsubscript{2} during exposure to 37.5 and 75% TL\textsubscript{m} of naphthalene. Dose-dependent alkalosis occurred in hemolymph of blue crabs acclimated to 30 o/ooS.
CHAPTER I

Title: Respiratory and Circulatory Responses of the Blue Crab, Callinectes sapidus to Salinity
INTRODUCTION

The blue crab, *Callinectes sapidus* Rathbun (1896), is a predominant member of estuarine communities along the Gulf of Mexico. In this region habitats for blue crabs range from fresh water to hypersaline lagoons (Powers, 1977). Tolerance to such a wide range of salinity has rendered *C. sapidus* the subject of numerous investigations of osmotic and ionic regulation (Mantel, 1967; Copeland and Fitzjarrel, 1968; Ballard and Abbott, 1969; Tagatz, 1971; Lynch et al., 1973; Towle et al., 1976; Findley and Stickle, 1978; Neufeld et al., 1980). Resistance to salinity change is a necessity for a swimming crab whose life history involves annual movements between estuarine and oceanic water masses.

In addition to differing ambient salinity conditions encountered due to migration patterns of the crab, the estuarine environment imposes altered salinity conditions on seasonal and daily bases. Rainfall, evaporation and river discharge contribute to seasonal or long-term salinity changes, while winds and tides are the causative agents for short-term fluctuations of salinity. All of the above physical factors influence salinity in bays and estuaries along the Louisiana coastline. Short-term salinity changes may have particular consequences on the metabolism of organisms dwelling in these waters. The existence of diurnal salinity
fluctuations in the estuaries of Louisiana is now well estab-
lished (Hewatt, 1954; Stickle and Howey, 1975; Sabourin and
Saintsing, unpublished).

Several studies have concerned the effects of semi-
diurnal and/or diurnal salinity fluctuations on marine and
estuarine fauna (for review see Stickle and Shumway, 1981).
Few have dealt with the osmoregulating crustaceans. Findley
and Stickle (1978) and Findley et al. (1978) investigated
the hemolymph composition and oxygen uptake (VO$_2$), respec-
tively, of C. sapidus during salinity fluctuations. Sabourin
and Stickle, (1980) observed osmotic regulation and oxygen
consumption of the striped hermit crab, Clibanarius vittatus.
Both species are ion and osmotic regulators below, and con-
form to salinities above, 25-27 o/oo. An inverse relation-
ship between VO$_2$ and either fluctuating or acclimated
salinity occurs for both species (Findley et al., 1978;
Sabourin and Stickle, 1980).

An inverse relationship between salinity and VO$_2$ was
also reported for the osmoregulating decapods C. sapidus
(King, 1965; Engel and Eggert, 1974), Carcinus maenas
(Taylor, 1977; Taylor et al., 1977a) and Crangon vulgaris
(Hagerman, 1970), using different techniques of salinity
adjustment. However, the lack of a salinity effect on VO$_2$
was demonstrated for the two portunids C. sapidus (Laird and
Haefner, 1976; Batterton and Cameron, 1978) and C. maenas
(Taylor et al., 1977a).
Oxygen uptake in crabs is the result of a complex physiological process involving the participation of a ventilatory pump (scaphognathite) and a cardiac pump (heart), which serve to deliver water and hemolymph to the respiratory site of gas exchange on the gill surface. Counter-current flow of the two fluids is established in the mode of forward ventilation. Forward ventilation is achieved by the creation of negative pressure in the branchial chamber via scaphognathite beats (Hughes et al., 1969), entry of water into the posterior gill chamber through the Milne-Edwards openings at the base of the first walking leg, and expulsion of water through the mouthparts via the exhalent aperture of the anterior branchial chamber. The respiratory pigment, hemocyanin, is present in variable titres in crab hemolymph and serves to facilitate oxygen loading and unloading at the gills and tissues. Johansen et al. (1970) and Mangum and Wieland (1975) determined that hemocyanin transports approximately 80-90% of the hemolymph oxygen that is delivered from gills to tissues in two brachyuran crabs *Cancer magister* and *C. sapidus*.

An understanding of the metabolic responses and adaptation to salinity in an osmoregulating crab requires a fundamental knowledge of the oxygen delivery process; however, there are some conflicting results for *C. maenas*. Taylor (1977) determined that an increased VO₂ after abrupt salinity transfer to low salinity (12 o/oo) was accompanied
by increased heart rate and ventilation volume. Likewise, Hume and Berlind (1976) found that heart rates increased upon transfer to any salinity between 20 and 75% sea water. Taylor et al. (1977a), working with the same species, did not find simultaneous increases in heart rate and ventilation volume with increased $V_{O_2}$ after transfer from 33.4 to 17 o/ooS.

A combination of extrinsic and intrinsic factors probably contribute to the differences observed. Mangum (1976) noted that the actual salinity may be important. A difference of 3-5 o/oo between "low salinities" may be critical in terms of rate functions governed by neural and hormonal systems. Collection, handling, feeding and salinity adjustment techniques vary considerably between laboratories. Subtle and even pronounced changes in physiological processes can be masked by these variations (Regnault, 1981; Stickle and Shumway, 1981; Bayne and Thompson, 1970). In addition, seasonality may be a very important determinate of both specific and generalized responses to salinity change in crustaceans (Sabourin and Stickle, 1980). Unfortunately, few authors mention the time of year during which individuals are collected and subjected to experimentation.

The present investigation concerns the response and adaptation of $C$. $sapidus$ to steady state and diurnally fluctuating salinity. Components of the respiratory and circulatory systems which serve to transport oxygen for
sustained metabolic performance under differing environmental conditions were studied.

MATERIALS AND METHODS

Collection and Maintenance

Stage C₁-C₄ intermolt adult blue crabs (Drach and Tchernigovtzeff, 1967) were trapped in pots or cages in the vicinity of Grand Isle, Louisiana, at various times of the year from 1979 to 1981. They were transported to LSU in Baton Rouge and placed in static or recirculating aquaria equipped with crushed oyster shell filters at the measured field salinity and room temperature (22-25°C). Instant Ocean sea salts (Aquarium Systems, Inc.) and deionized water were used to maintain and adjust salinities. The term "sea water" will refer to any prescribed mixture of these compounds.

Crabs were held in the laboratory under constant artificial illumination and were fed clams (Rangia cuneata) 3-4 times per week. Feeding was suspended three days before an experiment was initiated. Salinity of each aquarium was adjusted upward (addition of 40 o/oo sea water) or downward (addition of deionized water) by increments of 2 o/oo per day until the desired salinity was reached. In most cases crabs were maintained at the final salinity for 10-14 days before use in experiments. Crabs held under these conditions were considered salinity "acclimated" with respect to
the variables measured. Some crabs were held in the lab for a longer duration, but no individual held longer than 25 days at a salinity was used in experiments.

Most measurements taken in this study required some form of surgery. Crabs were held overnight before any post-surgery measurements were taken. During conditions where a time course of samples were required, the control determinations were made (hour 0). The time course was initiated immediately after the hour 0 sampling.

**Salinity Adjustments**

Ambient sea water salinity was monitored with a hand-held refractometer (American Optical Corp.) checked against a vapor pressure osmometer (Wescor Model 5100B). For experimental measurements 5-8 crabs were placed, unrestrained, in individual compartments of 2 x 0.3 x 0.25 meter plexiglass tanks holding 16-18 liters of the appropriate salinity. In the steady state salinity condition an additional 30-40 liters of sea water was continuously recirculated through each tank by a peristaltic pump (Cole Parmer Instrument Co.) at a flow rate of 5-10 liters per hour. Steady state salinities were 5, 10, 20 and 30 o/oo.

Salinity was fluctuated by the method of Stickle and Ahokas (1974). Blue crabs were acclimated to either high (30 o/oo) or low (10 o/oo) salinity and subjected to diurnal cycles of 30-10-30 or 10-30-10 o/oo, respectively.
Crabs were placed in a plexiglass tank held at fixed volume of 18 liters via a siphon. The equation of Wells and Ledingham (1940) was used to calculate the necessary flow rate \( F \) of either 40 o/oo sea water (change from 10 to 30 o/oo) or deionized water (change from 30 to 10 o/oo):

\[
F = \frac{V \cdot \ln(C_0 - C_i) \cdot \ln(C_o - C_f)}{(T_f - T_i)}
\]

where \( F \) = flow rate in ml·min\(^{-1}\); \( V \) = volume of tank in ml; \( T_i \) and \( T_f \) are the initial and final times; \( C_o \) is the salinity of water to be pumped into the tank; \( C_i \) and \( C_f \) are the initial and desired final salinity.

For a diurnal cycle the salinity was changed 20 o/oo in 10 hours, held constant for 2 hours (to simulate slack tide), changed 20 o/oo in 10 hours to return to the control salinity at hour 22, and held constant until hour 24. In some cases four cycles (96 hours) of salinity fluctuation were run. The peristaltic pumps moving 0 and 40 o/oo water were controlled by a programmable cycle timer (Chemical Data Systems, Inc. Model 400).

**Measurements and Analyses**

Heartbeat frequency was determined by implantation of 3-4 cm lengths of Intramedic Tubing housing bathythermograph wire (Sippican Corp.) into holes drilled through the carapace on both sides of the pericardial cavity (Figure 1). Care was taken so as not to penetrate the hypodermis. The tubes were held in place with dental wax (Lactona Surgident) and 5 minute epoxy glue (Scotch Brand). The copper wire served as an input into an impedance converter (Transmed
Figure 1. *Callinectes sapidus*. Diagram of a blue crab showing positions of mask and heart electrodes. Hemolymph samples were taken by syringe from the pericardial cavity and at the base of the walking legs.
Scientific Model 2991). Heartbeat frequencies were displayed on a Beckman Dynagraph Model 511A Recorder.

Measurement of ventilation volume and oxygen tensions of expired sea water were accomplished by fitting the crabs with a plastic mask. Urostomy bags (Hollister Inc., 9") were found to be best suited for this purpose since they are clear and, therefore, do not obstruct the vision of the blue crab. Masks were cut such that only the eyes and mouth-parts were enclosed, leaving the Milne-Edwards openings unobstructed. Masks were sealed with dental wax and 5 minute epoxy glue.

Two methods were used for the determination of ventilation volume ($V_w$). Initially, an overflow system was constructed such that a crab was restrained in a plexiglass experimental chamber, while a drain tube was connected to the mask. Ventilated sea water was collected in a graduated cylinder. Secondly, a blood flow transducer (In Vivo Metrics, Model K, 4 mm lumen diameter) was attached to the mask of an unrestrained crab. Ventilatory flow was recorded as mean flow with a Biotronix Model 610 Electromagnetic Flowmeter. Calibration was achieved by pumping sea water at known flow rates through the transducer assembly. Zero ventilation was set by placing a finger over the transducer lumen during a period of apnoea for each crab.
Oxygen tensions of expired sea water (PEO₂) were measured by drawing a sample from within the mask into a syringe equipped with a length of PE-90 tubing. The sample was injected into a Radiometer BMS3 Mk2 Blood Micro System and the resultant oxygen tension measured with an E5047 O₂ electrode read on an analog Radiometer PHM 71 Acid-Base Analyzer. The analyzer output was often displayed on an Omniscribe Strip Chart Recorder (Houston Instruments). Oxygen tensions of ambient sea water (PVO₂ inspired oxygen tension) were determined in a similar manner. Pre- and post-branchial hemolymph oxygen tensions (PVO₂, PAO₂) were determined by sampling with a 25-gauge needle connected to an iced syringe from the base of the 3rd or 4th walking leg and the pericardial cavity, respectively. These samples were immediately injected into the Radiometer Blood Micro System. Similar samples of pre- and post-branchial hemolymph were taken for pH determination (Radiometer pH electrode G299A). The clotting characteristics of blue crab hemolymph rendered this procedure a most difficult task. Precautions were taken to clean and recalibrate oxygen and pH electrodes immediately after reading each hemolymph sample.

Oxygen content of pre- and post-branchial hemolymph (CVO₂ and CAO₂) was determined by layering hemolymph samples under mineral oil in 0.4 ml polyethylene tubes and centrifuging for 5 minutes at 10,000 x g (Beckman Model
152 Microfuge). A 50 ul hemolymph sample was mixed with a solution of 0.2% potassium ferricyanide and the resultant oxygen tension measured as described by Laver et al. (1965).

Since the majority of the protein in crab hemolymph is hemocyanin (Horn and Kerr, 1969) and better than 93% of the copper is bound to hemocyanin (Martin et al., 1977) the levels of protein and copper were determined as an indication of hemocyanin concentration. Truchot (1978) determined that hemocyanin accounts for approximately 90% of the serum proteins of stage C₄ C. maenas adults. Hemolymph serum protein concentrations were assessed by diluting centrifuged samples 10-20 fold with Tris s-HCl buffer (pH 7.6) and mixing duplicate samples with Bio-Rad Protein Dye Reagent (Bio-Rad Laboratories). Bovine serum albumen (BSA) was used as a protein standard. Horn and Kerr (1963) stated that BSA and lyophilized hemocyanin had indistinguishable standard curves. Copper concentrations were measured with a Perkin Elmer Model 305B Atomic Absorption Spectrophotometer on centrifuged hemolymph serum samples which were diluted 41-fold with deionized water. Copper sulfate was used as a copper standard.

In some cases hemocyanin-oxygen dissociation curves were determined on hemolymph samples. The arthrodial membrane at the base of the swimmeret was punctured with an 18-guage needle and hemolymph was collected after the
clot was expressed through cheese cloth. The hemolymph sample (3-10 ml) was gently centrifuged. Required dilutions of the sample were obtained using 0.4-0.45 M NaCl. Dissociation was determined spectrophotometrically as the oxygen tension was gradually reduced in a vacuum manometer system. Complete dissociation was achieved by addition of a few grains of Na-dithionite to the hemolymph sample.

During the experiments where several hemolymph samples were taken over a time course two groups of crabs, of equal sample size, were bled in alternate hours. This reduced possible effects of decreased blood volume.

Calculations

Percent oxygen extraction from ambient sea water was calculated from inspired (PIO$_2$) and expired (PEO$_2$) oxygen tensions:

$$E, \% = \left\{(PIO_2 - PEO_2) \cdot \frac{1}{PIO_2}\right\} \cdot 100$$

Percent oxygen extraction from hemolymph by tissues was calculated from oxygen contents (CAO$_2$, CVO$_2$):

$$E_{hl}, \% = \left\{(CAO_2 - CVO_2) \cdot \frac{1}{CAO_2}\right\} \cdot 100$$

Oxygen uptake was calculated by the Fick principle from the inspired and expired oxygen tensions, ventilation volume and the oxygen solubility coefficient, $\alpha_{w02}$:

$$VO_2 = \left\{(PIO_2 - PEO_2) \cdot \alpha_{w02}\right\} \cdot V_w$$
where $a_{\text{w}O_2} (\text{ml}O_2 \cdot 1^{-1} \cdot \text{torr}^{-1})$ was derived by interpolation from the tables of Green and Carritt (1967): $P\text{IO}_2$ and $P\text{EO}_2 = 0.2 \text{ torr}$; $V_w = \text{ml} \cdot \text{min}^{-1} \cdot \text{crab}^{-1}$. Thus, $V\text{O}_2$ is in units of $\text{ml}O_2 \cdot \text{min}^{-1} \cdot \text{crab}^{-1}$. Weight-specific oxygen uptake includes wet and dry weight considerations.

Fick estimates of cardiac output were determined from oxygen uptake and oxygen content difference in post- and prebranchial hemolymph:

$$Q = \frac{V\text{O}_2}{C\text{A}_2 - C\text{V}_2}$$

where $V\text{O}_2$ is in $\text{ml}O_2 \cdot \text{kg wet wt}^{-1}$, $(C\text{A}_2 - C\text{V}_2)$ is in $\text{ml}O_2 \cdot \text{ml hemolymph}^{-1}$, and $Q$ in $\text{ml hemolymph} \cdot \text{kg wet wt}^{-1} \cdot \text{min}^{-1}$. A correction factor of 5% was subtracted from $V\text{O}_2$ prior to calculation to account for oxygen consumption by gill epithelia as cautioned by Burnett et al. (1980).

Stroke volume was determined from:

$$SV = \frac{Q}{F_H}$$

and has the units of $\text{ml hemolymph} \cdot \text{heartbeat}^{-1}$.

**Vital and Experimental Statistics**

Following each experiment sex, carapace width (cm), and wet weight was recorded for each crab. Each crab was then oven dried at $80^\circ \text{C}$ for 48 hours and dry weight was recorded.

All data in tables of this report are expressed as means $\pm$ standard errors of the mean. The Statistical Analysis System (SAS Institute Inc., 1979) GLM procedure
was used in this study as a package for statistical treatment of the data. Linear regressions were calculated for dependent variables on carapace width, wet weight and dry weight.

One-way analysis of variance (ANOVA) was used to determine the significance of variation among means for treatment groups. Duncan's multiple range test ($P < 0.05$) and Least Squares Means (LS MEANS) were used to ascertain significant groupings and estimate means during time course experiments. The difference probability option of LS MEANS, similar to the Least Significant Difference procedure (Steel and Torrie, 1960) was implemented to judge differences between means.
RESULTS

Pearson correlation coefficients for the carapace widths, wet weights and dry weights (including exoskeleton) of crabs used in this investigation were: carapace width/wet weight, 0.7955; carapace width/dry weight, 0.7423; wet weight/dry weight, 0.9062. All correlations were significant at the $P < 0.0001$ level. Carapace width ranged from 8.1 to 17.8 cm (mean ± s.e. = 14.6 ± 0.3). Wet weight ranged from 49 to 299 g (158.1 ± 8.7). Dry weight ranged from 11 to 64 g (39.8 ± 1.9). No statistically significant sex differences were found. In some cases too small a sample size made a testable hypothesis for sex impossible.

Steady State Salinity.

Linear and logarithmic regressions revealed that heartbeat frequency did not vary directly with wet or dry weight ($P > 0.05$ for individual treatments and combined data). Table 1 lists heartbeat frequencies for crabs acclimated to different salinities during different seasons. Spanning the acclimation range of 5 to 30 o/ooS, no significant differences in heart rate due to salinity occurred. Blue crabs acclimated to 10 o/ooS during February had reduced heartbeat frequencies compared to 10 o/oo acclimated crabs in June ($P < 0.05$, Duncan). The heart rates
Table 1

Callinectes sapidus. Heartbeat frequencies ($F_H$, beats·min$^{-1}$) recorded for steady state salinity acclimated crabs.

<table>
<thead>
<tr>
<th>Salinity (o/oo)</th>
<th>Month</th>
<th>n</th>
<th>$F_H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>February</td>
<td>8</td>
<td>$57 \pm 9$</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>5</td>
<td>$75 \pm 7$</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>5</td>
<td>$70 \pm 11$</td>
</tr>
<tr>
<td>20</td>
<td>August</td>
<td>5</td>
<td>$63 \pm 3$</td>
</tr>
<tr>
<td>10</td>
<td>February</td>
<td>6</td>
<td>$59 \pm 9$</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>5</td>
<td>$85 \pm 10$</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>6</td>
<td>$75 \pm 12$</td>
</tr>
<tr>
<td>5</td>
<td>August</td>
<td>5</td>
<td>$70 \pm 10$</td>
</tr>
</tbody>
</table>
recorded in December did not differ from either June or February. No seasonal changes in heartbeat frequency existed for crabs at 30 o/oo.

Ventilation volume (ml \cdot min^{-1}) varied significantly with both wet and dry weight according to the relationships:

\[ \log V_w = (0.71 \pm 0.41) + (0.513 \pm 0.201) \log \text{wet wt} \]

and

\[ \log V_w = (1.005 \pm 0.353) + (0.527 \pm 0.247) \log \text{dry wt} \]

No significant differences were found for ventilation volume between salinities and seasons (TABLE 2). The ventilation volume for an average sized crab of 120 g wet weight was \(60 \pm 4\) ml \cdot min^{-1}. The ventilation volumes recorded were similar for both overflow and flowmeter measurement techniques. Where heartbeat frequency and ventilation volume were recorded on the same crabs a significant correlation did not exist between the two variables (\(P > 0.38\), Pearson Correlation).

TABLE 3 summarizes the gas transport variables measured for different seasons and acclimation salinities. Oxygen extraction efficiency (E), postbranchial oxygen tension (PAO₂) and oxygen tension of the venous reserve (PAO₂ - PVO₂) were higher in crabs acclimated to 30 o/oo during November than February. Likewise, oxygen content (including both pigment-bound and dissolved oxygen) of
Table 2

*Callinectes sapidus*. Ventilation volumes (\(V_w\)) recorded for steady state salinity acclimated crabs.

<table>
<thead>
<tr>
<th>Salinity (o/oo)</th>
<th>Month</th>
<th>Method</th>
<th>n</th>
<th>Mean Wet Wt(g)</th>
<th>(V_w) (ml·kg wet(^{-1})·min(^{-1}))</th>
<th>Mean Dry Wt(g)</th>
<th>(V_w) (ml·g dry(^{-1})·min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>June</td>
<td>Flowmeter</td>
<td>5</td>
<td>114.3 ± 22.5</td>
<td>552 ± 62</td>
<td>29.4 ± 4.5</td>
<td>2.07 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>Dec</td>
<td>Overflow</td>
<td>5</td>
<td>123.0 ± 24.8</td>
<td>548 ± 16</td>
<td>26.4 ± 4.2</td>
<td>2.48 ± 0.53</td>
</tr>
<tr>
<td>10</td>
<td>June</td>
<td>Flowmeter</td>
<td>5</td>
<td>122.0 ± 16.9</td>
<td>530 ± 46</td>
<td>31.4 ± 2.4</td>
<td>2.01 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Dec</td>
<td>Overflow</td>
<td>6</td>
<td>120.1 ± 19.2</td>
<td>550 ± 95</td>
<td>25.2 ± 5.4</td>
<td>2.72 ± 0.52</td>
</tr>
</tbody>
</table>
Table 3

Callinectes sapidus. Values of measured respiratory and circulatory variables for steady state salinity acclimated crabs at 22-25 ºC.

<table>
<thead>
<tr>
<th>Salinity (o/oo)</th>
<th>Month</th>
<th>n</th>
<th>PIO2 (torr)</th>
<th>PEO2 (torr)</th>
<th>E (%)</th>
<th>PAO2 (torr)</th>
<th>PVO2 (torr)</th>
<th>PAO2-PVO2 (torr)</th>
<th>CAO2 (vols%)</th>
<th>CVO2 (vols%)</th>
<th>CAO2-CVO2 (vols%)^2</th>
<th>Ehl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>Feb</td>
<td>8</td>
<td>146</td>
<td>92±4</td>
<td>36.8±3.0</td>
<td>40±6</td>
<td>17±5</td>
<td>22±3</td>
<td>0.79±0.08</td>
<td>0.26±0.04</td>
<td>0.53±0.06</td>
<td>66.4±3.7</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(7)*</td>
<td>(7)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>5</td>
<td>142</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.35±0.12</td>
<td>0.75±0.07</td>
<td>0.61±0.09</td>
<td>44.2±4.3</td>
</tr>
<tr>
<td></td>
<td>Nov</td>
<td>6</td>
<td>145</td>
<td>68±9</td>
<td>53.3±6.5</td>
<td>58±6</td>
<td>20±3</td>
<td>38±7</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Aug</td>
<td>5</td>
<td>148</td>
<td>86±4</td>
<td>41.6±3.1</td>
<td>47±2</td>
<td>17±2</td>
<td>30±1</td>
<td>1.63±0.61</td>
<td>0.98±0.32</td>
<td>0.56±0.26</td>
<td>35.4±15.8</td>
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<td></td>
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<td></td>
<td>(3)</td>
<td>(3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Feb</td>
<td>6</td>
<td>146</td>
<td>74±6</td>
<td>49.4±4.1</td>
<td>44±5</td>
<td>16±2</td>
<td>29±4</td>
<td>0.74±0.19</td>
<td>0.26±0.05</td>
<td>0.48±0.17</td>
<td>60.0±8.1</td>
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<td></td>
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<td></td>
<td>(5)</td>
<td>(5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>5</td>
<td>141</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.71±0.17</td>
<td>0.75±0.12</td>
<td>0.96±0.16</td>
<td>55.7±6.3</td>
</tr>
<tr>
<td></td>
<td>Nov</td>
<td>6</td>
<td>147</td>
<td>74±5</td>
<td>49.8±3.5</td>
<td>46±5</td>
<td>14±2</td>
<td>33±4</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Aug</td>
<td>5</td>
<td>147</td>
<td>70±4</td>
<td>51.7±2.5</td>
<td>40±3</td>
<td>9±1</td>
<td>31±3</td>
<td>0.79±0.21</td>
<td>0.35±0.10</td>
<td>0.44±0.11</td>
<td>56.4±3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(4)</td>
<td>(4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Sample size is in parentheses if other than listed value for n.
pre- and postbranchial hemolymph were lower in February than June crabs at 30 o/oo, because percent hemolymph oxygen extraction by tissues was higher in February than June (P < 0.05, Student t-test). Pre- and postbranchial oxygen content (CAO₂, CV₀₂) and difference (CAO₂ - CV₀₂) were higher for June crabs than those taken in February for C. sapidus acclimated to 10 o/oo. Considering summer crabs only (June - August), oxygen content of pre- and postbranchial hemolymph was lower at 5 o/oo than 10, 20 and 30 o/oo. The highest mean tissue oxygen extraction from blood (Eₜₐₜ) was also recorded in 5 o/oo crabs.

Protein and copper concentrations were determined to assess the relative levels of hemocyanin at 30 and 10 o/oo. Protein and copper concentrations did not differ within a salinity between seasons (TABLE 4). Mean values for hemolymph serum protein and copper tended to be higher at 10 o/oo than 30 o/oo, but these differences were significant only for crabs sampled in November.

The oxygen uptake rate of blue crabs acclimated to 30 and 10 o/ooS during November-December was 495 ± 60 and 549 ± 39 μlO₂ · g dry wt⁻¹ · h⁻¹, respectively. These rates do not differ statistically. Fick estimates of cardiac output showed no acclimation salinity-related differences and Q varied from 189 - 378 ml · kg wet wt⁻¹ · min⁻¹ or 0.9 - 1.0 ml · g dry wt⁻¹ · min⁻¹. Cardiac output ranged from 23 - 45 ml · min⁻¹ · crab⁻¹. Considering
Table 4

*Callinectes sapidus*. Hemolymph serum protein and copper concentrations when acclimated to steady state salinity at 22-25°C. Sample size given in parentheses.

<table>
<thead>
<tr>
<th>Salinity (o/oo)</th>
<th>Protein (mg·ml⁻¹)</th>
<th>Copper (ug·ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>February</td>
<td>November</td>
</tr>
<tr>
<td>30</td>
<td>34±5 (8)</td>
<td>28±3 (10)</td>
</tr>
<tr>
<td></td>
<td>(15-53)*</td>
<td>(10-33)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>P &lt; 0.002</td>
</tr>
<tr>
<td>10</td>
<td>41±5 (6)</td>
<td>43±6 (9)</td>
</tr>
<tr>
<td></td>
<td>(23-57)</td>
<td>(15-58)</td>
</tr>
</tbody>
</table>

*Range of individual measurements located below mean and standard error.*
0.4 ml as the average heart volume of a crab weighing 125 g wet weight (approximated from deFur and Mangum, 1979), the heartbeat frequency should range from 57 - 112 beats \cdot min\(^{-1}\) to deliver 23-45 ml \cdot min\(^{-1}\) of hemolymph to the tissues. Calculated stroke volume at measured heart rates ranged from 0.27 - 0.76 ml \cdot beat\(^{-1}\) (mean \(\pm\) s.e. = 0.46 \(\pm\) 0.12) under steady state salinity conditions with no apparent salinity effect.

Pre- and postbranchial hemolymph pH differed under steady state salinity conditions at both 30 and 10 o/oo (TABLE 5). Recorded mean pre- and postbranchial pH was higher in crabs acclimated to 10 o/oo than those at 30 o/oo. The significance of this slight change in circulating body fluid pH is addressed in terms of oxygen delivery in a later section along with the response to salinity fluctuation.

**Fluctuating Salinity (30-10-30 o/oo Regime).**

When crabs were acclimated to 30 o/oo and subjected to diurnal, 30-10-30 o/oo, salinity fluctuations heart rates \((F_H)\) changed in an inverse manner with the salinity of the medium (FIGURE 2). ANOVA for time effects was 0.0063. Duncan's multiple range test showed that \(F_H\) at hour 12 and hour 36 was significantly higher than hour 0 and hour 16 through hour 24, but was not different from hour 48 and hour 96. Mean \(F_H\) recorded at hour 48 and hour 96 did not differ from control \(F_H\).
Table 5

_Callinectes sapidus_. Prebranchial and postbranchial hemolymph pH when acclimated to steady state salinity at 22-25 °C.

<table>
<thead>
<tr>
<th>Salinity (o/oo)</th>
<th>Month</th>
<th>n</th>
<th>Prebranchial pH</th>
<th>Postbranchial pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>June</td>
<td>11</td>
<td>7.58 ± 0.04</td>
<td>7.75 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>10</td>
<td>June</td>
<td>11</td>
<td>7.71 ± 0.04</td>
<td>7.83 ± 0.02</td>
</tr>
</tbody>
</table>
Figure 2. *Callinectes sapidus*. Time course of normalized heartbeat frequency during 30-10-30 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 5. Vertical lines represent standard errors of the plotted mean. Values above asterisks are statistical probability differences of the mean from hour 0. ANOVA for time (P<0.0063).
Callinectes sapidus

Heartbeat Frequency (% Control)

Salinity (%)
Transient changes occurred in ventilation volume, however, the change was not as pronounced as change in heartbeat frequency (FIGURE 3). \( V_w \) at hour 4 was significantly elevated compared to hour 14 through hour 20 \( (P < 0.05, \text{ Duncan}) \). Overall, the response of \( V_w \) to a 30-10-30 pattern of fluctuation was little change from control levels.

FIGURE 4 demonstrates time course changes in oxygen tension and oxygen content during 30-10-30 o/oo fluctuations. Oxygen tension of expired water consistently rose throughout the first 48 hours of the experiment and differed from control levels at hour 36 and hour 48. The value recorded at hour 96 did not differ from hour 0. These changes reflect the change in oxygen extraction, whereby a decrease in extraction efficiency of nearly 20% was recorded at low salinity which persisted at the high salinity period of the 2nd cycle (FIGURE 5). At the end of the 4th cycle (hour 96) \( E \) had risen to 42.7%, but remained lower than the control hour. The transient rise in \( E \) recorded at hour 4, although not differing from hour 0, differed significantly from \( E \) during all measurements after hour 8 \( (P < 0.05, \text{ Duncan}) \). The value at hour 4 corresponds to increased trends in heartbeat frequency and ventilation volume as the surrounding medium is diluted.

No change occurred in postbranchial oxygen content \((\text{CAO}_2)\) during 30-10-30 o/oo salinity alterations (FIGURE 4).
Figure 3. *Callinectes sapidus*. Time course of normalized ventilation volume during 30-10-30 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 5. Vertical lines represent standard errors of the plotted mean. ANOVA for time (P<0.1094).
Callinectes sapidus

Graph showing the effect of time on ventilation volume (% Control) and salinity (%) for Callinectes sapidus. The x-axis represents time in hours (0 to 96), and the y-axis for ventilation volume is labeled as % Control with values ranging from 30 to 140, while the y-axis for salinity is labeled as % with values ranging from 10 to 30. The graph shows fluctuations in both ventilation volume and salinity over time.
Figure 4. *Callinectes sapidus*. Time course of changes in oxygen tension of water and hemolymph and oxygen content of hemolymph during 30-10-30 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 5 for O$_2$ contents, 6 for O$_2$ tensions. Vertical lines represent standard errors of the plotted mean. Values above asterisks are statistical probability differences of the mean from hour 0. ANOVA for time (P <: PEO$_2$; 0.0002; PAO$_2$; 0.1051; PVO$_2$; 0.0250; CAO$_2$; 0.6087; CVO$_2$; 0.1323).
Figure 5. *Callinectes sapidus*. Time course of changes in percent oxygen extraction during 30-10-30 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 6. Vertical lines represent standard errors of the plotted mean. Values above asterisks are statistical probability differences of the mean from hour 0. ANOVA for time ($P < 0.0001$).
Oxygen content of prebranchial hemolymph (CVO\textsubscript{2}) was reduced by 57% as salinity decreased from 30 to 10 o/oo during the first 12 hours. A decrease of similar magnitude was recorded at hour 18 for the oxygen tension of prebranchial hemolymph, indicating that the lowered oxygen levels were due to an increased delivery to tissues from physical solution rather than via hemocyanin. Postbranchial oxygen tensions were lower than control between hour 12 and hour 48, but did not differ from control at hour 96. Similarly, prebranchial oxygen levels had returned to control levels by hour 96.

An initial rise in oxygen uptake was determined at hour 4 of the 30-10-30 o/oo fluctuation patterns (FIGURE 6). The VO\textsubscript{2} had returned to control level by hour 8 and remained at, or slightly below, the control rate for each of the remaining measurements through hour 96. Cardiac output and stroke volume were estimated at hours 0, 12, 36, 48, and 96. Neither variable was found to change at these sampling hours over the course of four 30-10-30 o/oo salinity cycles. Cardiac output and stroke volume ranged from 155 - 209 ml \cdot kg wet wt\textsuperscript{-1} \cdot min\textsuperscript{-1} and from 0.17 - 0.26 ml \cdot heartbeat\textsuperscript{-1}, respectively, for the average sized crab of 120 g wet wt.

A large degree of variability occurred in pre- and postbranchial hemolymph pH during the course of a diurnal 30-10-30 o/oo fluctuation (FIGURE 7). As a result, no significant, salinity-related change was observed in
Figure 6. *Callinectes sapidus*. Time course of normalized oxygen consumption rate during 30-10-30 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 6. Vertical lines represent standard errors of the plotted mean. Value above asterisk is statistical probability difference of the mean from hour 0. ANOVA for time ($P < 0.0736$).
Figure 7. *Callinectes sapidus*. Time course of changes in postbranchial and prebranchial hemolymph pH during 30-10-30 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 5 for each variable. Vertical lines represent standard errors of the plotted mean. Values above asterisks are statistical probability differences of the mean from hour 0. ANOVA for time (P<:post:0.3679; pre:0.0895).
postbranchial pH. As salinity reached 19 o/oo at hour 4 mean prebranchial pH had risen by 0.1 unit and remained at this level until the direction of salinity change was reversed after hour 12. pH values at hour 4 through hour 12 differ significantly from hour 0, but not hour 16 through hour 24 (P < 0.05, Duncan). The short duration of the diurnal cycle may not allow complete acid-base adjustments and, hence, lead to observed variability between individual crabs.

Fluctuating Salinity (10-30-10 o/oo Regime)

A pronounced change in heartbeat frequency occurred when crabs were subjected to 10-30-10 o/oo cycles of fluctuating salinity (FIGURE 8). The heart rate followed salinity in an inverse manner. Frequencies recorded at hour 6 through hour 10 and hour 36 showed a decline from control heartbeat frequencies. Additionally, the heart rates at hour 20 through hour 24, hour 48 and hour 96, where salinity returned to 10 o/oo, were significantly higher than the levels at hour 8, hour 10 and hour 36, where salinity reached the high point of a given cycle.

A significant time course change in ventilation volume occurred during a 10-30-10 o/oo salinity pattern (FIGURE 9; P < 0.05, ANOVA); although no mean rate differed statistically from control. The time course trend in ventilation volume was similar to that observed for heart
Figure 8. *Callinectes sapidus*. Time course of normalized heartbeat frequency during 10-30-10 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 5. Vertical lines represent standard errors of the plotted mean. Values above asterisks are statistical probability differences of the mean from hour 0. ANOVA for time ($P < 0.0013$).
Figure 9. *Callinectes sapidus*. Time course of normalized ventilation volume during 10-30-10 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 6. Vertical lines represent standard errors of the plotted mean. ANOVA for time ($P < 0.0365$).
Salinity (%o)

Ventilation Volume (% Control)

Callinectes sapidus

Time (h)

0 12 24 36 48 96

0 10 20 30
rate, $V_w$ cycled downward while salinity was raised to 30 o/oo and upward as salinity was lowered to 10 o/oo. The low values for $V_w$ found at hour 6 and hour 8 ($46 \pm 8 \text{ ml} \cdot \text{min}^{-1}$ for each hour) differ from the peak ventilation ($71 \pm 8 \text{ ml} \cdot \text{min}^{-1}$) recorded for crabs at hour 20 ($P < 0.05$, Duncan).

Expired water oxygen tension did not change during the first 12 hours of a 10-30-10 o/oo salinity change, but rose 23-28\% above control PEO$_2$ between hour 12 and hour 36 (FIGURE 10). FIGURE 11 underscores the decrease in oxygen extraction from the ambient water. This decrease was effected by salinity change only during the latter stages of the 1st and 2nd diurnal cycle. Percent oxygen extraction reached a minimum at hour 24, but equalled control by hour 48 and hour 96. Similarly, oxygen content and tension of pre- and postbranchial hemolymph decreased during 10-30-10 o/oo salinity fluctuations (FIGURE 10). Indeed, both prebranchial and postbranchial oxygen content remained significantly reduced from control levels at hour 96, indicating that less oxygen was binding to circulation hemocyanin molecules at the gills. Less oxygen was available to tissues for activity after hour 12 of the 10-30-10 o/oo cycles, as evidenced by the diminished venous reserve. The time course of the relations $\text{CAO}_2$-$\text{CVO}_2$ and $\text{PAO}_2$-$\text{PVO}_2$ attest to this fact. Furthermore, the time course changes indicate that dissolved oxygen was utilized by tissues primarily during the
Figure 10. *Callinectes sapidus*. Time course of changes in oxygen tension of water and hemolymph and oxygen content of hemolymph during 10-30-10 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 5 for $O_2$ contents, 6 for $O_2$ tensions. Vertical lines represent standard errors of the plotted mean. Values above asterisks are statistical probability differences of the mean from hour 0. ANOVA for time ($P < : PEO_2 : 0.0028; PAO_2 : 0.0155; PVO_2 : 0.0142; CAO_2 : 0.0001; CVO_2 : 0.0081$).
Callinectes sapidus

Oxygen Tension (torr)

Oxygen Content (Vols%o)

Salinity (%o)

Time (h)
Figure 11. *Callinectes sapidus*. Time course of changes in percent oxygen extraction during 10-30-10 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 6. Vertical lines represent standard errors of the plotted mean. Values above asterisks are statistical probability differences of the mean from hour 0. ANOVA for time (P < 0.0061).
Callinectes sapidus

Percent Oxygen Extraction vs. Time (h)

Salinity (%) vs. Time (h)
early stages of fluctuation, while hemocyanin bound oxygen was delivered disproportionately during later cycles.

A decline in both oxygen content and oxygen tension is reflected in the oxygen uptake rate (FIGURE 12). The VO\textsubscript{2} remained below control level between hour 4 and hour 36. Control rates were resumed by hour 48. Cardiac output decreased by 35% between hour 0 and hour 12 of a 10-30-10 o/oo salinity cycle (200 + 14 to 131 + 13 ml · kg wet wt\textsuperscript{-1} · min\textsuperscript{-1}), but returned to, and remained at, levels slightly elevated over control by hour 36 through hour 96 (224 + 18 - 261 + 24). Similarly, stroke volume showed a small, insignificant decline from 0.28 to 0.25 ml · beat\textsuperscript{-1} · crab\textsuperscript{-1} at hour 12 and was elevated to 0.39 ± 0.04 at hour 96. The change observed in cardiac output during the 10-30-10 o/oo fluctuating salinity regimes and the lack of change during the 30-10-30 o/oo regimes were due to alterations of both heartbeat frequency and stroke volume.

Prebranchial and postbranchial hemolymph pH did not change during a diurnal 10-30-10 o/oo salinity fluctuation (FIGURE 13). A significant difference between postbranchial and prebranchial hemolymph was lacking at hour 16, evincing the possible effect of the gradual salinity change on regulation of extracellular fluid acid-base balance.

Hemocyanin Concentration During Salinity Fluctuation

Prebranchial hemolymph protein and copper concentrations were determined at hour 0, 12, 24, 36, 48 and 96 of
Figure 12. *Callinectes sapidus*. Time course of normalized oxygen consumption rate during 10-30-10 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 6. Vertical lines represent standard errors of the plotted mean. Values above asterisks are statistical probability differences of the mean from hour 0. ANOVA for time ($P < 0.0049$).
Callinectes sapidus

Oxygen Consumption Rate (%Control)

Salinity (%o)

Time (h)
Figure 13. *Callinectes sapidus*. Time course of post-branchial and prebranchial hemolymph pH during 10-30-10 o/oo salinity fluctuation. Salinity is indicated by shading. Sample size is 8 for each variable. Vertical lines represent standard errors of the plotted mean. ANOVA for time ($P < \text{post:0.9055; pre:0.8501}$).
Callinectes sapidus

**Hemolymph pH**

- **Post**
- **Pre**

**Salinity (%)**

- 0 - 24 (Time h)
both 30-10-30 o/oo and 10-30-10 o/oo diurnal salinity fluctuation patterns. Levels of protein and copper were significantly higher for 10 o/oo acclimated crabs compared to 30 o/oo, indicating higher concentrations of hemocyanin. Protein and copper concentrations were $40 \pm 4 \text{ mg} \cdot \text{ml}^{-1}$ and $83 \pm 8 \mu \text{g} \cdot \text{ml}^{-1}$ at 10 o/oo. At 30 o/oo levels of 30 $\pm$ 3 and 54 $\pm$ 7 were determined for protein and copper, respectively. Concentrations of protein and copper, and therefore hemocyanin concentration, did not differ from control during 30-10-30 or 10-30-10 o/oo salinity regimes throughout either 96 hour experiment.

**Oxygen-Hemocyanin Dissociation**

Hemolymph of crabs acclimated to 10 o/oo had a lowered affinity for oxygen than hemolymph of crabs acclimated to 30 o/oo (FIGURE 14). The shift in in vitro dissociation, which occurs near the in vivo range of prebranchial hemolymph oxygen tension, is significant at the $P < 0.02$ level. TABLE 6 lists the $P_{50}$ of hemolymph samples taken at hour 0, 24 and 96 of inverse patterns of salinity fluctuation. In the 10-30-10 o/oo cycle no change was observed in oxygen affinity, whereas a decrease in affinity occurred between hour 24 and hour 96 of a 30-10-30 o/oo cycle.
Figure 14. *Callinectes sapidus*. *In vitro* oxygen-hemocyanin dissociation curves for hemolymph of blue crabs acclimated to 30 and 10 o/oos. Sample size is 5 in each case.
Callinectes sapidus

Oxygen Tension (torr)

% Oxy-Hemocyanin

30%
10%
Table 6

Callinectes sapidus. Oxygen affinity (P$_{50}$, torr) of hemocyanin when subjected to diurnal salinity fluctuations.

<table>
<thead>
<tr>
<th>Salinity Regime</th>
<th>Time</th>
<th>Salinity</th>
<th>P$_{50}$</th>
<th>n</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-10-30 o/oo</td>
<td>hour 0</td>
<td>30 o/oo</td>
<td>11.8 torr</td>
<td>5</td>
<td>7.57 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>30</td>
<td>11.7</td>
<td>3</td>
<td>7.60 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>30</td>
<td>13.5*</td>
<td>5</td>
<td>7.72 ± 0.03</td>
</tr>
<tr>
<td>10-30-10 o/oo</td>
<td>hour 0</td>
<td>10 o/oo</td>
<td>14.2 torr</td>
<td>5</td>
<td>7.78 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>10</td>
<td>14.7</td>
<td>3</td>
<td>7.67 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>10</td>
<td>14.5</td>
<td>4</td>
<td>7.75 ± 0.02</td>
</tr>
</tbody>
</table>

*P$_{50}$ at hour 96 is significantly different from hour 0 and hour 24 (P < 0.001).
DISCUSSION

This report documents the fact that ventilation volume of *C. sapidus* was unaffected by stepwise acclimation to 10 and 30 o/ooS. Dyer and Uglow (1980) reported increased scaphognathite beating rates at salinities between 22 and 14 o/oo compared to 34 o/oo controls for the shrimp, *C. crangon*. Batterton and Cameron (1978) determined mean ventilation volumes ranging from 123 to 179 ml · min\(^{-1}\) · 200 g wet wt\(^{-1}\) for *C. sapidus* and noted that *V_w* was unaffected by abrupt transfer to reduced salinity. Considerable variability in ventilation rate occurred in their study, perhaps masking any minor salinity effects. Mean ventilation volume in the present study was approximately 110 ml · min\(^{-1}\) · min\(^{-1}\) · 200 g wet wt\(^{-1}\). I found no seasonal change in ventilation volume. Johansen et al. (1970) stated that use of a flowmeter gave consistently higher values than employment of an overflow method of recording ventilation due to flow resistance. If this was the case in blue crabs, the actual ventilation volume would be greater during the winter.

During rapid ambient salinity alteration (0-10 min) ventilation volume changed inversely to salinity at 10 and 15 °C in *C. maenas* (Taylor, 1977; Taylor et al., 1977a), but no change occurred at 18 °C (Taylor et al., 1977a). Although a great deal of individual variability existed under gradual salinity change (10 h) the ventilation of blue crabs followed
salinity inversely. This response is transient, however, as no 12 hour interval denoting salinity minima and maxima differed significantly from control hour in either 30-10-30 o/oo or 10-30-10 o/oo salinity fluctuation pattern.

Oxygen uptake initially followed a pattern similar to ventilation volume as an initial rise occurred when salinity was lowered, followed by a decrease in VO$_2$ during a 30-10-30 o/oo salinity cycle. Overall, there was little change from control oxygen consumption rates. This result is similar to the findings of Findley et al. (1978) for the blue crab and Sabourin and Stickle (1980) for C. vittatus. In all three investigations the osmoregulating decapod in question responded to a 30-10-30 o/oo diurnal salinity fluctuation with a VO$_2$ averaged throughout the experiment which was not different from control VO$_2$. In the present investigation the VO$_2$ of C. sapidus averaged over the entire cycle was 99 $\pm$ 12% of control oxygen uptake. Conversely, acclimating crabs to 10 o/ooS and subjecting them to a 10-30-10 o/oo salinity alteration effected decreases in overall experimental VO$_2$ versus control for both C. sapidus and C. vittatus (Findley et al., 1978; Sabourin and Stickle, 1980; present data). The present results verify that, in the two subtropical species investigated thus far, ambient salinity fluctuation leads to decreased or unchanged oxygen uptake rates. In the former case control VO$_2$ levels are resumed within 48 to 96 hours under continual diurnal salinity fluctuation.
The rapid metabolic adaptation to salinity fluctuation and the lack of a salinity effect on stepwise acclimated crabs support the results of Laird and Haefner (1976) who infer that $\text{VO}_2$ of $\text{C. sapidus}$ is little affected by salinity between 10 and 30 o/oo. The results of Dehnel (1960), Taylor et al. (1977a) and Sabourin and Stickle (1980) indicate that seasonal interactions occur between acclimation temperature, salinity and oxygen uptake rates. Seasonal interactions could be attributed to as yet undetermined changes in the metabolic cost of osmotic regulation (Dehnel, 1962; Ballard and Abbot, 1969; Wieland and Mangum, 1975). The results of Dehnel (1960), Laird and Haefner (1976), and Taylor et al. (1977a, 1977b) indicate that we know very little about the effects of the phenomenon of seasonality upon metabolism of osmoregulating crabs.

Differences in metabolic responses observed between studies on closely related species are also related to experimental technique differences. Salinity adjustment techniques were reviewed by Stickle and Shumway (1981). The $\text{VO}_2$ of blue crabs reported in this study is higher than that reported by Findley et al. (1978), but in line with $\text{VO}_2$ recorded for $\text{C. sapidus}$ in other investigations (Laird and Haefner, 1976; Batterton and Cameron, 1968). Differences, which are not substantial as they fall within the same order of magnitude, are due to nutritive history, stress of restraint and handling stress. In my investigation
crabs were fed on a routine schedule and maintained in unrestrained conditions. However, the stress of attached electrodes, masks and periodic blood sampling is unavoidable in a study of this nature. The range of \( \text{VO}_2 \)'s reported by different investigators for blue crabs under steady state conditions is approximately 150-500 \( \mu \text{L O}_2 \cdot \text{g dry wt}^{-1} \cdot \text{h}^{-1} \). Considerable scope for activity remains when \( \text{VO}_2 \) lies within this range as evinced by the ample venous reserve recorded for steady state salinity crabs in my investigation. McMahon et al. (1979) determined that \( \textit{C. magister} \), which had previously undergone surgery similar to that of the present study, responded to 20 min of enforced activity by increasing \( \text{VO}_2 \) 2.3-fold. The range of measured \( \text{VO}_2 \) for \( \textit{C. sapidus} \) found by different investigators probably reflects the \( \text{O}_2 \) uptake fluctuation during normal activities. For this reason, the rates reported in this study should be considered routine metabolic rates (Newell, 1979).

Repetitive blood sampling and disturbance could result in significant deviation from control values late in an experiment. This would lead to a misinterpretation of results obtained from an experimental group. No such change occurred in steady state salinity controls of the present investigation (see Chapter II).

Decapod crustaceans exhibit a wide range of oxygen extraction efficiency, from lows of 15 to 30% for rock crabs and lobsters (Johansen et al., 1970; McMahon et al.,
1970; McMahon and Wilkens, 1975) to a high reported for
*C. sapidus* of approximately 50% (Batterton and Cameron,
1978). Several fishes have been reported to extract 60-80%
of the available oxygen from fully oxygenated water (Piiper
et al., 1977; Wood et al., 1979). In the present study
mean percent oxygen extraction ranged from 37-53. The
winter (February) blue crabs stepwise acclimated to 30 o/oo
had a significantly lower efficiency of oxygen extraction
than those acclimated to 10 o/oo and 30 o/oo acclimated
crabs in November. Otherwise, no salinity acclimation or
seasonal effects were noted. Unlike the crabs sampled
during other seasons, the crabs collected in February
appeared sluggish upon entering the laboratory. Blue crabs
normally do not begin to leave the muds and move up into
the estuaries until late February to early March in Louisiana.

Crabs acclimated to 10 o/oo and sampled in Febru-
ary did not demonstrate characteristically different
respiratory and circulatory measurements from those sampled
in other seasons. The 30 o/oo acclimated group not only
exhibited a lowered oxygen extraction efficiency, but also
a decreased venous reserve, and higher percent hemolymph
oxygen extraction by the tissues. Ventilation was not
measured at this time, but heart rates were relatively low.
The respiratory pigment appears to have delivered propor-
tionately more oxygen during this period.
Extraction efficiency, E, did not change from control levels following rapid salinity decreases and increases in C. maenas, regardless of temperature (Taylor, 1977; Taylor, et al., 1977a). Similarly, no significant change occurred in E during the first 12 hours of a 10-30-10 o/oo cycle and 24 hours of a 30-10-30 o/oo cycle in the present study. However, decreases in percent extraction had occurred by the next sampling period in each case. These decreases in E were generally reflected in the patterns of oxygen consumption for each regime of salinity fluctuation.

Dissolved oxygen in postbranchial and prebranchial hemolymph was unaffected by salinity acclimation between 5 and 30 o/oo. Taylor (1977) arrived at the same conclusion for C. maenas between 12 and 28 o/oo. The range of values recorded in the present study were not appreciably higher than the PAO₂ and PVO₂ reported by Mangum and Wieland (1975) of 35 ± 4 and 14 ± 2 torr, when blue crabs were acclimated to 22.5 o/oo at 22 °C. Wieland and Mangum (1975) detected a slight, but ungraded, rise in prebranchial oxygen tension upon acclimation of C. sapidus to salinities lower than 15 o/oo. Oxygen content and, therefore, the amount of oxygen transported to tissues via hemocyanin also was little affected by acclimation salinity. Differences were found in CAO₂ and CVO₂ which correspond to season as summer oxygen content was generally higher than winter. The venous reserve remained relatively constant by
salinity and season. Slight seasonal changes in hemolymph oxygen carrying capacity could influence \( V_O^2 \) and explain the results of seasonal differences in \( V_O^2 \) reported by Eggert (1974) and Findley et al. (1978). Spoek (1974) determined that \( V_O^2 \) of lobsters, as well as heart rates, increased with decreased hemocyanin concentration in the blood. This might lead to lowered scope for activity. Change in hemocyanin concentrations were not found in this study. However, a definitive answer can only be realized by sampling monthly throughout the year.

The efficiency of oxygen removal by tissues showed considerable variability. As reported previously (Mangum, 1977), extraction of oxygen from blood at the tissue level was higher than extraction from water at gill epithelia. The highest tissue extraction efficiency (60-66%) was recorded during February. Slightly reduced heart rates, and possibly blood flow, may have contributed to a longer circulation time and slightly elevated extraction efficiencies. Lower or unchanged oxygen extraction from water by the gills could be attributed to steady or slightly increased ventilation rate.

During both patterns of salinity fluctuation, time course decreases occurred in oxygen tension of pre- and postbranchial hemolymph. By hour 96 all variables had returned to control values. The decrease was more pronounced during the 10-30-10 o/oo regime. The decrease
in hemolymph PO$_2$ corresponds to lowered oxygen uptake during both cycles. Oxygen content during a 30-10-30 o/oo salinity fluctuation was essentially unaltered, whereas during a 10-30-10 o/oo regime CAO$_2$ and CVO$_2$ were significantly lower after hour 36. It appears that an increasingly greater role in oxygen delivery to tissues was played by hemocyanin during the latter stages of the 10-30-10 o/oo cycle, while the relative delivery via physical solution and pigment did not change appreciably over the course of four simulated tidal cycles of 30-10-30 o/oo. Since protein and copper levels did not change during either cycle, the adjustments can be attributed to cardiac and ventilatory pumping rates and altered hemocyanin-oxygen binding characteristics.

Protein and copper concentrations in hemolymph serum were higher for 10 o/oo than 30 o/oo acclimated crabs, indicating that hemocyanin levels were also higher. Several workers have demonstrated recently that blood proteins in osmoregulating crustaceans vary inversely with ambient salinity (Gilles, 1977; Pequeux, et al.; 1979) and stage of molt cycle (Truchot, 1978). Mean protein and copper concentrations double between stages $C_1$ and $C_4$ in C. maenas (Truchot, 1978). Boone and Schoffeniels (1979) inferred that hemocyanin has an osmoregulatory role concerning free amino acid metabolism during hypo- and isoosmotic medium-blood conditions. They determined that incorporation of labelled amino acid precursors into hemocyanin synthesis
occurred within 24 hours after transfer of C. maenas from 32 to 16 o/oo. My results do not corroborate these findings. Blue crabs subjected to four diurnal high-low-high and low-high-low salinity cycles did not exhibit a time course of hemolymph protein and copper concentrations which approached intermediate values between 10 and 30 o/oo. The crabs were not fed during the experimental fluctuations, which would account for the lack of exogenous nitrogen. However, the premise of Boone and Schoffeniels (1979) is based on an endogenous amino acid source along with copper stores within the digestive gland (Djangmah, 1970).

Horn and Kerr (1963) reported hemolymph protein and copper concentrations of C. sapidus taken directly from the field or held in running sea water for less than twelve hours, however, ambient salinity was not reported. Serum proteins and copper ranged from 8.8 to 132 mg·ml⁻¹ and 8 to 176 ug·ml⁻¹, respectively, with means of 52-63 mg·ml⁻¹ and 70-84 ug·ml⁻¹. Although they lie within both ranges, the mean values that I observed for total protein are slightly lower. There are two possible explanations for this discrepancy. First, the crabs in my investigation were maintained in the laboratory for 1-3 weeks before protein determination. The possibility of lowered protein levels due to laboratory stress cannot be discounted. Secondly, copper:protein ratios of about 0.13 were found by Horn and Kerr (1963), whereas, in the present study the
average ratio was 0.18-0.19. A copper:protein ratio of 0.2 is considered the approximate value for nearly 100% of hemolymph protein being hemocyanin (Truchot, 1978). Since intermolt crabs are characterized by having hemolymph in which about 90% of the protein is hemocyanin, the copper and protein levels reported here are reasonable.

An augmented P\textsubscript{50} (decreased affinity) of \textit{C. sapidus} hemolymph in vitro occurs upon acclimation to lower salinity. This result is similar to the data of Truchot (1973) for \textit{C. maenas}. In the steady state salinity condition, assuming the in vitro data represents the in vivo situation, more oxygen would be released to tissues at 10 o/oo than 30 o/oo, even though oxygen tensions and oxygen contents do not differ statistically. The reason for this shift is believed to be due to the dilution effect on hemocyanin at the lower salinity (Mangum, 1976). Hemolymph of blue crabs is reduced some 150-200 m\textsuperscript{0}sm between environmental salinities of 30 and 10 o/oo (Findley and Stickle, 1978). The dilution effect on oxygen-hemocyanin binding properties is opposed by allosteric effects of pH on the oxygen binding site. Pre- and postbranchial pH are higher at lower salinity, a factor which leads to increased affinity of the respiratory pigment for oxygen. Mangum et al. (1976) concluded that the observed pH increase at low, compared to high, salinity is due to increased ammonia circulating in the hemolymph. Hemolymph ammonia levels increased 6-7 fold one day after transfer
of C. sapidus from 100 to 50% sea water (Gerard and Gilles, 1972). Truchot (1981) determined from base flux studies that another component is involved, which is additional to ammonia, in the elevation of pH. This component is as yet unknown.

During the declining salinity phase of a diurnal 30-10-30 o/oo salinity fluctuation a pH increase was recorded in prebranchial hemolymph, which would correspond to possible increased blood ammonia levels. Little is known of the body fluid ammonia changes in fluctuation salinity.

Findley and Stickle (1978) determined that NPS changed in a direct manner with salinity in a 30-10-30 o/oo fluctuation, but did not change during a 10-30-10 o/oo cycle. The contribution of ammonia to the measured NPS was not determined during the experiments of Findley and Stickle (1978). However, their results and the record of pH during inverse salinity fluctuation patterns in the present study suggest that the hemolymph composition changes during the 30-10-30 o/oo cycles such that a base excess exists (FIGURE 6, hour 4 through hour 12; TABLE 6, hour 96) as salt levels are decreased. The overall result is a decrease in hemocyanin oxygen affinity.

Conversely, no significant pH and \( P_{50} \) changes occurred during low-high-low salinity fluctuations. These contrasting responses may have decisive effects on the in vivo oxygenation properties of the blood and might explain
some of the differences observed in oxygen uptake and circulating oxygen levels when crabs are subjected to salinity change in opposing directions (Findley et al., 1978; Sabourin and Stickle, 1980; Stickle and Shumway, 1981; present study).

Heart rate, stroke volume and resultant cardiac output were unaffected by acclimation salinity over the range of 5-30 o/oo. This is consistent with the data for the heart rate of C. sapidus reported by deFur and Mangum (1979), which shows only a slight increase at 5 o/oo recorded 2-4 days after salinity change. Following abrupt transfer of crabs to low salinity Taylor et al. (1977a) determined no alteration in heartbeat frequency accompanied an increase in VO$_2$ of C. maenas. The lack of change in post- and prebranchial oxygen content difference led the authors to suggest elevated cardiac output via an increased stroke volume was exhibited. Hume and Berlind (1976) and Taylor (1977) found increased heartbeat frequency in hyposmotic media for C. maenas. Spaargaren (1974) and Taylor (1977) attributed increased cardiac output to heart rate rather than stroke volume in C. maenas transferred to dilute seawater.

During both 30-10-30 and 10-30-10 o/oo salinity fluctuations the heart rate of C. sapidus cycled inversely to salinity change. Acclimation to high or low salinity and the direction of the salinity change effected slightly different responses. No overall change in cardiac output
and stroke volume occurred during a 30-10-30 o/oo fluctuation, however, there was significant variability in these data. The pattern of oxygen consumption (FIGURE 5) reflects this variability. Since ventilatory changes were minimal during 30-10-30 o/oo cycles, changes in heartbeat frequency were likely opposed by compensatory changes in stroke volume to maintain a constant cardiac output and VO₂.

The pronounced pattern of decreased heartbeat frequency during the first 12 hours of 10-30-10 o/oo regimes was accompanied by a slight decline in stroke volume and, hence a decrease in cardiac output. Alterations in cardiac output during gradual salinity fluctuation appear to be related primarily to heartbeat frequency. Interestingly, stroke volume has been implicated as the main influence upon temporal variations in cardiac output of resting, sea water acclimated C. sapidus (Burnett et al., 1979).
SUMMARY AND CONCLUSIONS

Acclimation of *C. sapidus* to a range of salinities from 5-30 o/oo has little effect on many variables of the oxygen delivery system. Differences, related to salinity of the medium, are found in the blood chemistry. Hemocyanin concentrations, pH and $P_{50}$ vary inversely with salinity. Compounded with salinity-related differences observed in this study were several seasonal effects (e.g., $O_2$ content varies with season).

Gradual salinity change induces many compensatory adjustments. Ventilation volume and heart rate, noted by some researchers to be linked in performance, showed similar responses during salinity fluctuations which were inversely related to the direction of fluctuation. The mode and degree of compensation for other variables of aerobic metabolism is dependent upon the acclimation salinity (in this instance, 10 and 30 o/ooS). Thus, responses to salinity fluctuation are affected by acclimation salinity.

Acclimating crabs to 30 o/oo and subjecting them to 30-10-30 o/oo salinity fluctuations has no effect on blood flow characteristics, but leads to eventual base excess and probable decrease in hemocyanin oxygen affinity. Despite an initial increase in oxygen uptake rate, little overall change was registered in oxygen consumption. In fact, a decrease was observed in the time course of gill oxygen extraction.
efficiency after 36 hours of salinity fluctuation. Significant change of the \textit{in vivo} acid-base state occurred, which led to a decreased \textit{in vitro} oxygen affinity.

\textit{C. sapidus} acclimated to low salinity (10 o/oo) and exposed to a 10-30-10 o/oo diurnal pattern of ambient salinity fluctuation responded with an overall decreased rate of aerobic metabolism. Branchial oxygen extraction efficiency and oxygen uptake declined due both to decreased cardiac output and gill ventilation. Hemolymph oxygen content and oxygen tension were diminished. After 48-96 hours of salinity fluctuation the role of hemocyanin in tissue oxygenation had increased.

\textit{C. sapidus} maintains a dual-role system for oxygen delivery. First, and energetically costly, the branchial and cardiac pumps serve to extract oxygen from the medium and move it to cellular mitochondria. These pumps have finite limitations and appear to be utilized maximally only for brief periods or under extreme stress. The second component is the respiratory pigment. Hemocyanin concentration and contribution to oxygen transport changes depending upon the ion and acid-base status of the hemolymph. Whether oxygen is delivered to respiring cells chiefly by physical solution or via hemocyanin is inconsequential. Once the respiratory pigment is synthesized, the oxygen carrying capacity of \textit{C. sapidus} blood is raised to a level which affords routine activity, such as swimming, at relatively
little energetic cost. The integration of the dual-role system and altered contribution of hemocyanin aid in explaining why blue crabs adapt so well to a wide range of constant and changing salinities.
CHAPTER II

Title: Respiratory and Circulatory Responses of the Blue Crab to Naphthalene and the Effect of Acclimation Salinity
INTRODUCTION

Estuaries are characterized by continuous change in abiotic factors. Blue crabs have successfully adapted to the estuarine habitat by maintaining an internal osmotic, ionic and acid-base environment which is only slightly variable over a wide range of change in the surrounding medium. This ability is achieved when internal conditions counteractively change, resulting in overall stability, and has been termed enantiotasis (Mangum and Towle, 1977). The onset of toxic effects of introduced foreign substances upon crustaceans may be the result of disruption of this state of balance.

Estuarine regions are subjected to relatively high input of various pollutants. Petroleum hydrocarbons have received considerable attention as agents of lethal and sublethal toxicity to aquatic fauna. Sheltered tidal flats and salt marshes, habitats where blue crabs are commonly found, are considered extremely vulnerable to oil spills (Gundlach and Hayes, 1978). The present investigation assesses the effect of a petroleum-derived hydrocarbon, naphthalene, on the aerobic metabolism of the blue crab Callinectes sapidus (Rathbun).

Anderson et al. (1974b) determined the specific hydrocarbon content of the water-soluble fraction of a 10%
solution of South Louisiana crude oil. Naphthalene comprised 0.12 ppm and total naphthalenes 0.3 ppm of a total aromatic fraction of 13.9 ppm, which was 59% of the dissolved hydrocarbons measured. The fate of spilled oil via weathering processes has been thoroughly studied and is a process of complex physical and biological interactions (Haegh, 1980). Riley et al. (1981) recently determined that 7-55% of the original naphthalene of Prudhoe Bay crude oil remained in tanks after 24 days of "weathering". The degree of volatilization reflected the harshness of experimental weathering. Cox et al. (1975) found that the peak concentration of total naphthalenes, 0.3 ppm. was reached two days after an experimental #2 fuel oil spill in a shrimp pond. The naphthalenes (including naphthalene + alkynaphthalene) concentration steadily declined, reaching low limits after 96 days. The naphthalene exposure concentrations used in the present experiment, 0.9 and 1.8 ppm, exceed naphthalene concentrations in crude oil, but are within the range of values for some refined oils (Anderson et al., 1974b). In addition, the concentrations are well within the range of values reported for total aromatics.

Lee et al. (1976) demonstrated that petroleum-derived hydrocarbons are rapidly taken up, metabolized and excreted by blue crabs with little retention. These results corroborated the earlier findings of Corner et al. (1973) with *Maia squinado*. Pearson and Olla (1980) recently
discovered that \textit{C. sapidus} behaviorally detects naphthalene in sea water at concentrations lower than $10^{-7}$ ppm. The detection threshold of a more complex mixture of petroleum hydrocarbons was hypothesized to be lower than that of the pure compound, naphthalene, in blue crabs and demonstrated to be lower in \textit{Cancer magister} (Pearson et al., 1980). Branchial cavity detection of a water soluble fraction of crude oil was found to induce bradycardia of at least one minute's duration in \textit{Pugettia producta}, a response which naturally occurs when food is present in the water column (Zimmer et al., 1979).

These responses occur in exposure concentrations well below lethal levels. Relatively little is known of the respiratory and circulatory responses of crustaceans to short-term sublethal petroleum hydrocarbon exposure. Most investigators have reported a general increase in oxygen consumption rate to occur upon exposure to oil fractions (Lee et al., 1978; Neff et al., 1976). The complexity of gas exchange systems in crustaceans is evidenced by additional reports of decreased oxygen uptake during oil exposure (Neff et al., 1976). One shrimp, \textit{Crangon crangon}, exhibited a 32% increase in heart rate after 12 hours of exposure to the water-soluble fraction of North Sea crude oil (Edwards, 1978).

A more in-depth understanding of the changes in hemolymph oxygen transport associated with the reported
alterations of oxygen consumption and heart rate in crustaceans exposed to petroleum-derived hydrocarbons is clearly necessary. One natural stressor in the estuarine environment is seasonal and daily alterations of salinity (see Chapter I). Blue crabs maintain an osmotic gradient between extracellular fluids and the ambient water below 27 o/ooS (Findley and Stickle, 1978). The present study was undertaken to assess the responses of the oxygen transport system to naphthalene in C. sapidus acclimated to high and low salinity.

MATERIALS AND METHODS

Intermolt adult blue crabs (Stage C1-C4, Drach and Tchernigovtzeff, 1967), of weight range 50-227 g wet, were trapped in pots or cages in the vicinity of Grand Isle, Louisiana, during the spring, 1981. They were transported to LSU in Baton Rouge and placed in static or recirculating aquaria equipped with crushed oyster shell filters at the measured field salinity and room temperature (22-23°C). Instant Ocean sea salts (Aquarium Systems, Inc.) and deionized water were used to maintain and adjust salinities. The term "sea water" will refer to any prescribed mixture of these compounds.

Crabs were held in the laboratory under constant artificial illumination and were fed clams 3-4 times per week. Feeding was suspended two days before an experiment
was initiated. Salinity of aquaria was adjusted upward (additions of 40 o/oo sea water) or downward (addition of deionized water) by increments of 2 o/oo per day until the desired final salinity of 10, 20 or 30 o/oo was reached. Crabs were maintained at the final salinity for 7-14 days before use in experiments.

Heartbeat frequency was determined by implantation of 3-4 cm lengths of Intramedic Tubing (PE-90), housing bathythermograph copper wire (Sippicon Corp.), into holes drilled through the carapace on both sides of the pericardial cavity. Care was taken not to penetrate the hypodermis. The tubes were held in place with dental wax (Lactona Surgident) and 5 min epoxy glue (Scotch Brand). The copper wire leads passed to an impedance converter (Transmed Scientific Model 2991) and a Beckman Dynagraph 511A recorder.

For measurement of ventilation volume and oxygen tensions of expired water, crabs were fitted with masks (9" Urostomy bags, Hollister Inc.). Masks were cut such that only the eyestalks and mouthparts were enclosed, leaving the Milne-Edwards openings unobstructed. The masks were sealed with dental wax and 5 min epoxy glue.

Ventilation volume was determined by affixing a flow transducer (In Vivo Metrics Model K, 4 mm lumen diam.) to the mask of an unrestrained crab. Ventilatory water flow was recorded as mean flow with a Biotronix Model 610
Electromagnetic Flowmeter. The instrument was calibrated by pumping sea water of known flow rates through the transducer assembly. Zero ventilation was set by placing a finger over the transducer lumen during a period of apnoea for each crab.

A Radiometer BMS3 Mk2 Blood Micro System and PHM 71 Acid-Base Analyzer were used to determine oxygen tension and oxygen content (Radiometer E5047 O2 Electrode and the pH (Radiometer pH Electrode G299A) of various samples. Oxygen tension of expired sea water was measured by drawing a water sample from within the mask into a syringe equipped with a length of PE-90 tubing. Pre- and post-branchial hemolymph samples were obtained with a 25-gauge needle attached to an iced syringe from the base of the 3rd or 45th walking leg and the pericardial cavity, respectively. Postbranchial hemolymph samples were used for the determination of oxygen tension and oxygen content, while prebranchial hemolymph was taken for oxygen tension, oxygen content, serum protein, pH and osmolality determination.

All oxygen tension measurements were obtained immediately following the drawing of a water or hemolymph sample. Oxygen content was determined by layering hemolymph samples beneath mineral oil in 0.4 ml polyethylene tubes and centrifuging for 5 minutes at 10,000 x g. A 50 μl hemolymph sample was mixed with a solution of 0.2%
potassium ferricyanide and the resultant oxygen tension measured as described by Laver et al. (1965).

During each sample period an additional 75-150 μl of prebranchial hemolymph was centrifuged prior to measurement of pH, osmolality (Wescor Model 5100B Vapor Pressure Osmometer) and protein concentration. For protein analysis hemolymph was diluted 10-20 fold with Tris-HCl buffer (pH 7.6) and duplicate samples were mixed with Bio-Rad Protein Dye Reagent (Bio-Rad Laboratories). BSA was used as a protein standard.

At the end of each experimental treatment hemocyanin-oxygen dissociation curves were determined on the hemolymph samples. The arthrodial membrane at the base of the swimmeret was punctured with an 18-gauge needle and 3-10 ml of hemolymph was collected after the clot was expressed through cheese cloth. Required dilutions were obtained with 0.4-0.45 M NaCl. Pigment dissociation was determined spectrophotometrically as the oxygen tension was gradually reduced in a vacuum manometer system. Complete dissociation was achieved by addition of a few grains of Na-dithionite to the hemolymph sample.

Percent oxygen extraction (E, %) from ambient sea water, percent oxygen extraction from hemolymph by tissues (Ehl%), oxygen consumption rate (VO₂), stroke volume (SV) and cardiac output (Q) were calculated as outlined in Chapter I.
Naphthalene stock solutions were prepared by pumping sea water of the appropriate salinity through glass bottles containing reagent grade naphthalene crystals and glass wool. Flow rates of the stock solution and sea water diluent were adjusted to achieve desired test concentrations in a 20 liter plexiglass tank. Concentrations were determined by scanning UV spectrophotometry in the region of naphthalene's absorbance maxima (220 nm) by the method of Neff and Anderson (1974). Inflowing water was well aerated but no aeration was provided in the covered experimental tank. Measured oxygen levels in the tank ranged from 84-94% saturation for all experimental periods. Flow rates of toxicants + diluent summed to approximately 200 ml·min⁻¹, which resulted in a circulation time of 100 min.

Standard bioassays of 24 hours were conducted on blue crabs acclimated to 10, 20 and 30 o/ooS. The sample size for each dose was 10 crabs. Five dose levels were used at each salinity in order to calculate TLₘ's. From the bioassay data experimental dose levels of approximately 0, 37.5 and 75% TLₘ for 24 hours were established for crabs acclimated to 10 and 30 o/ooS. For each of the six treatment groups eight crabs were placed in a 20 liter compartmentalized plexiglass tank with 60-80 liters of continuously recirculated sea water and held overnight. The following day measurements were taken which constituted hour 0.
(hour 0) of the experimental period. Crabs were then transferred to a second tank containing the particular test concentration of naphthalene and further measurements were taken at hour 6, 12 and 24.

Sex, carapace width (cm) and wet weight were recorded after hour 24. Each crab was then oven dried to constant weight for 48 hours at 80 °C. Wet and dry weight relationships were used to calculate body water levels.

Data are presented as means ± standard errors of the mean. The Statistical Analysis System general linear models (GLM) procedure (SAS Institute Inc., 1979) was used as a package for all statistical treatment of the data, including analysis of variance (ANOVA), Duncan's multiple range test (Duncan), least squares means and probability differences (LS MEANS) and Probit Analysis (used in determination of TLₘ's).
RESULTS

TABLE 1 summarizes the results of naphthalene bioassays completed with Callinectes sapidus. Although sensitivity to naphthalene appeared to increase with increased acclimation salinity, considerable overlap of the fiducial ranges occurred. As a result, the data of all three salinities were pooled and a mean 24-h TL\textsubscript{m} of 2.40 ppm was established as 100% TL\textsubscript{m} for further investigation.

Actual naphthalene concentrations in the exposure chambers deviated only slightly from target values (TABLE 2). For practical purposes, the target dose levels of 0, 37.5 and 75% TL\textsubscript{m} will be nominally referred to in the following discussion.

Hemolymph serum of crabs acclimated to 30 o/oo and exposed to naphthalene was isoosmotic to ambient sea water (TABLE 3). Considerable variability, common for estuarine invertebrates, existed but no time or naphthalene dose effects were recorded. Crabs acclimated to 10 o/oo regulated hyperosmotically. Osmolality did not change over the 24-h time course for control and 37.5% TL\textsubscript{m} groups, however, a significant decline was evidenced by hour 6, which persisted through hour 24, in the 75% TL\textsubscript{m} group (P < 0.02, LS MEANS). Coincidentally, the high dose at 10 o/oo induced a significant rise in tissue hydration. Mean body water levels at the end of the experimental period were 5% higher than controls.
Table 1

*Callinectes sapidus*. Mean 24-h \( TL_m \) (ppm) and 95% fiducial limits (FL) of probit analysis for exposure to naphthalene.

<table>
<thead>
<tr>
<th>Salinity o/oo</th>
<th>( TL_m )</th>
<th>Range of 95% FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1.98</td>
<td>1.50 - 2.59</td>
</tr>
<tr>
<td>20</td>
<td>2.25</td>
<td>0.68 - 2.78</td>
</tr>
<tr>
<td>10</td>
<td>3.12</td>
<td>2.24 - 3.40</td>
</tr>
<tr>
<td>Pooled Data</td>
<td>2.40</td>
<td>2.06 - 2.69</td>
</tr>
</tbody>
</table>
Table 2

Naphthalene average dose levels (ppm) for 24-h exposures with 95% confidence limits. 100% TL<sub>m</sub> was established as 2.40 ppm. Naphthalene concentrations were determined 6-10 times during each 24-h period.

<table>
<thead>
<tr>
<th>Salinity (o/oo)</th>
<th>Target %TL&lt;sub&gt;m&lt;/sub&gt;</th>
<th>Average Dose</th>
<th>Actual TL&lt;sub&gt;m&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>0.7 ± 0.1</td>
<td>29 ± 4</td>
</tr>
<tr>
<td></td>
<td>75.0</td>
<td>1.8 ± 0.1</td>
<td>75 ± 4</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>0.9 ± 0.2</td>
<td>38 ± 8</td>
</tr>
<tr>
<td></td>
<td>75.0</td>
<td>1.9 ± 0.3</td>
<td>79 ± 12</td>
</tr>
</tbody>
</table>
Table 3

*Callinectes sapidus.* Hemolymph osmolality (mOsm/kg) of control and naphthalene-exposed crabs. Percent body water (B.W.) was recorded at hour 24. Sample size = 6-8.

<table>
<thead>
<tr>
<th>Salinity (o/oo)</th>
<th>%TL&lt;sub&gt;m&lt;/sub&gt;</th>
<th>SW</th>
<th>Hour 0</th>
<th>Hour 6</th>
<th>Hour 12</th>
<th>Hour 24</th>
<th>% B.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0</td>
<td>863</td>
<td>861 ± 12</td>
<td>849 ± 8</td>
<td>853 ± 7</td>
<td>848 ± 14</td>
<td>71.89 ± 1.66</td>
</tr>
<tr>
<td>37.5</td>
<td>873</td>
<td>849 ± 11</td>
<td>856 ± 6</td>
<td>875 ± 13</td>
<td>887 ± 9</td>
<td>67.63 ± 1.35</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>880</td>
<td>862 ± 14</td>
<td>830 ± 14</td>
<td>831 ± 21</td>
<td>69.57 ± 1.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>295</td>
<td>687 ± 11</td>
<td>667 ± 15</td>
<td>691 ± 6</td>
<td>680 ± 13</td>
<td>69.13 ± 1.71</td>
</tr>
<tr>
<td>37.5</td>
<td>293</td>
<td>682 ± 9</td>
<td>659 ± 19</td>
<td>682 ± 11</td>
<td>70.78 ± 1.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>292</td>
<td>706 ± 23</td>
<td>646 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>630 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>651 ± 11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.90 ± 1.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Differs significantly from hour 0 (P < 0.05).

<sup>b</sup>Differs significantly from 0% TL<sub>m</sub> value (P < 0.026).
Ventilation volume was unaffected by acclimation salinity. Mean $V_w$ for control hours was $80 \pm 23$ ml·min$^{-1}$·123 g wet wt$^{-1}$ at 30 o/ooS and $60 \pm 7$ ml·min$^{-1}$·123 g wet wt$^{-1}$ at 10 o/ooS. Ventilation volume in the 75% TL$_m$ group was higher than control $V_w$ at hour 6 at 30 o/oo (FIGURE 1; $P < 0.01$, LS MEANS) and at hour 6 and hour 12 at 10 o/oo ($P < 0.01$ for both times, LS MEANS). $V_w$ of crabs in each salinity returned to control levels by hour 24. Mean ventilation averaged over the 24-h time course was 70% higher than that of controls for the 10 o/oo - 75% TL$_m$ group ($103 \pm 7$ ml·min$^{-1}$·123 g wet wt$^{-1}$, $P < 0.0001$, LS MEANS), but did not differ statistically from controls in the 30 o/oo - 75% TL$_m$ group. $V_w$ at 37.5 TL$_m$ was not different from control at any time for either salinity, although recorded means were always intermediate between 0 and 75% TL$_m$.

Periods of apnoea lasting several seconds were common for control crabs at both salinities, however, exposure to either low or high naphthalene concentrations led to constant ventilation for the 24-h duration of recordings.

Little change was recorded in percent oxygen extraction from water by the gills (TABLE 4). A significant increase in $E$ was registered over the time course of exposure to 37.5% TL$_m$ in the 30 o/oo salinity group (ANOVA, $P < 0.0001$). However, the values did not differ from either 0 or 75% TL$_m$ treatments at hour 24.
Figure 1. *Callinectes sapidus*. Time course of normalized ventilation volume of crabs acclimated to 10 o/oo (upper panel) and 30 o/oo (lower panel). Symbols represent naphthalene dose levels: ○ = 0% TL$_m$, ★ = 37.5% TL$_m$, ■ = 75% TL$_m$. Vertical lines represent standard errors of the plotted mean. Sample size is 5 in each case.
Callinectes sapidus

Ventilation Volume (% Control)

Time (h)

10%

30%
Table 4

*Callinectes sapidus*. Percent oxygen extraction (E) from water. Sample size is 5.

<table>
<thead>
<tr>
<th>Salinity % TL&lt;sub&gt;m&lt;/sub&gt;</th>
<th>30 o/oo</th>
<th>10 o/oo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47 ± 3</td>
<td>49 ± 5</td>
</tr>
<tr>
<td>37.5</td>
<td>40 ± 3</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>75</td>
<td>54 ± 3</td>
<td>44 ± 5</td>
</tr>
<tr>
<td>0</td>
<td>46 ± 3</td>
<td>48 ± 4</td>
</tr>
<tr>
<td>37.5</td>
<td>37 ± 4</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>75</td>
<td>50 ± 3</td>
<td>48 ± 4</td>
</tr>
<tr>
<td>52 ± 2</td>
<td>53 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>53 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55 ± 3</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>75</td>
<td>55 ± 3</td>
<td>55 ± 4</td>
</tr>
<tr>
<td>54 ± 3</td>
<td>57 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46 ± 7</td>
</tr>
<tr>
<td>57 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58 ± 2</td>
<td>41 ± 6</td>
</tr>
<tr>
<td>75</td>
<td>37 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Differs significantly from Hour 0 (P < 0.05).
Oxygen consumption rates at 10 o/oo were 80 ± 7 ml·kg wet wt⁻¹·h⁻¹ or 265 ± 23 ul·g dry wt⁻¹·h⁻¹ and at 30 o/oo were 72 ± 13 ml·kg wet wt⁻¹·h⁻¹ or 239 ± 44 ul·g dry wt⁻¹·h⁻¹. These values were not statistically different. For blue crabs acclimated to 10 o/oo the rate of oxygen uptake during naphthalene exposure followed a pattern similar to that of $V_w$ (FIGURE 2). Only the 75% $T_{Lm}$ dose group had normalized $V_O_2$'s which differed from controls during a particular hourly period and $V_O_2$ in this case was not different from control at hour 24. Naphthalene exposed crabs at 30 o/oo had a $V_O_2$ which was nearly double the control levels at hour 24, regardless of dose level. Therefore, exposure to 37.5 and 75% $T_{Lm}$ in both salinities led to an overall increase in $V_O_2$, but $V_O_2$ only remained elevated for crabs acclimated to high salinity.

Heartbeat frequency of control individuals averaged 83 ± 8 and 88 ± 7 beats·min⁻¹, respectively, at 10 and 30 o/oo. Brief pauses in $F_H$ of a few seconds duration occasionally occurred in control crabs. The changes in $F_H$ associated with exposure to naphthalene again followed dissimilar patterns for each acclimation salinity (FIGURE 3). However, at each salinity, periods of bradycardia lasting 30 sec - 1 min were common at hour 6, but few to no pauses were observed at hour 12 and hour 24. At 10 o/oo a slight
Figure 2. *Callinectes sapidus*. Time course of normalized oxygen consumption rate of crabs acclimated to 10 o/oo (upper panel) and 30 o/oo (lower panel). Symbols represent naphthalene dose levels: ○ = 0% $TL_m$, ● = 37.5% $TL_m$, ■ = 75% $TL_m$. Vertical lines represent standard errors of the plotted mean. Sample size is 5 in each case.
Figure 3. *Callinectes sapidus*. Time course of normalized heart rate of crabs acclimated to 10 o/oo (upper panel) and 30 o/oo (lower panel). Symbols represent naphthalene dose levels: o = 0% TL<sub>m</sub>, • = 37.5% TL<sub>m</sub>, • = 75% TL<sub>m</sub>. Vertical lines represent standard errors of the plotted mean. Sample size is 5 in each case.
Callinectes sapidus

Heart Rate (% Control)

0 6 12 24
Time (h)

10%

30%
increase in the $F_H$ of the 37.5% $TL_m$ group occurred by hour 6 and persisted at hour 12 ($P < 0.05$, LS MEANS). Conversely, the 75% $TL_m$ exposure induced a decline in $F_H$ of 21%. At hour 24 the heart rate was virtually identical for all three groups.

At 10 o/oo stroke volume was not altered in either the control or 37.5% $TL_m$ group (TABLE 5). Increased stroke volume was recorded at hours 6, 12, and 24 of the 75% $TL_m$ exposure. This resulted in increased cardiac output during the last three sampling periods of the 75% $TL_m$ at 10 o/oo (TABLE 6). The increased ventilation observed at the high dose was accompanied by increased perfusion, resulting in an increased $VO_2$. The increased blood flow was due to changes in the heart stroke volume, not frequency of beats.

*C. sapidus* acclimated to 30 o/oo demonstrated a different response to naphthalene exposure. Increased $F_H$ was exhibited at hour 24, but not before this time. The increase was dose-dependent (FIGURE 3). At both 37.5 and 75% $TL_m$ stroke volume was elevated significantly during hour 6 and hour 12 (TABLE 5; $P < 0.0001$, LS MEANS). Stroke volume remained high at hour 24 in the 37.5% $TL_m$ group, but had returned to control in the 75% $TL_m$ dose. Cardiac output followed the same general pattern, erratically increasing by hour 6 (TABLE 6). Stroke volume changes again were implicated in altered cardiac output, while heart rate remained stable until the later stages of the
Table 5

*Callinectes sapidus*. Stroke volume (SV, ml · beat⁻¹ · 123 g wet wt⁻¹). Sample size is 5.

<table>
<thead>
<tr>
<th>Salinity (o/oo)</th>
<th>% TLₘ</th>
<th>Hour 0</th>
<th>Hour 6</th>
<th>Hour 12</th>
<th>Hour 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0</td>
<td>0.37 ± 0.29</td>
<td>-</td>
<td>-</td>
<td>0.24 ± 0.14</td>
</tr>
<tr>
<td>37.5</td>
<td>0.30 ± 0.04</td>
<td>0.51 ± 0.09⁺</td>
<td>0.81 ± 0.20⁺</td>
<td>0.63 ± 0.19⁺</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>0.32 ± 0.05</td>
<td>0.50 ± 0.06⁺</td>
<td>0.88 ± 0.24⁺</td>
<td>0.38 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0.19 ± 0.02</td>
<td>0.21 ± 0.03</td>
<td>0.27 ± 0.01⁺</td>
<td>0.24 ± 0.06</td>
</tr>
<tr>
<td>37.5</td>
<td>0.32 ± 0.13</td>
<td>0.28 ± 0.07</td>
<td>0.32 ± 0.10</td>
<td>0.35 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>0.18 ± 0.06</td>
<td>0.64 ± 0.31⁺</td>
<td>0.54 ± 0.13⁺</td>
<td>0.37 ± 0.11⁺</td>
<td></td>
</tr>
</tbody>
</table>

⁺Differs significantly from value at hour 0 (P < 0.05).
Table 6

*Callinectes sapidus.* Cardiac output (Q, ml. min⁻¹. 123 g wet wt⁻¹).

Sample size is 5.

<table>
<thead>
<tr>
<th>Salinity</th>
<th>30 o/oo</th>
<th>10 o/oo</th>
</tr>
</thead>
<tbody>
<tr>
<td>%TLₘ</td>
<td>0</td>
<td>37.5</td>
</tr>
<tr>
<td>Hour 0</td>
<td>28 ± 20</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>Hour 6</td>
<td></td>
<td>51 ± 19</td>
</tr>
<tr>
<td>Hour 12</td>
<td></td>
<td>63 ± 20</td>
</tr>
<tr>
<td>Hour 24</td>
<td>28 ± 15</td>
<td>43 ± 5</td>
</tr>
</tbody>
</table>

*Differs significantly from Hour 0 (P < 0.05).*
experimental dosing period. At this point, gill perfusion by blood was due to elevated $F_H$ and $SV$. The slight increase in these blood flow variables accounts for the persistence of a nearly 2-fold increased $VO_2$ at hour 24.

In order to obtain more information on the oxygen transport characteristics of blue crab hemolymph during naphthalene exposure in vitro oxygen-hemocyanin binding properties were examined as well as oxygen levels and hemolymph pH. TABLE 7 demonstrates the change in oxygen affinity ($P_{50}$) as a function of naphthalene exposure and ambient salinity. Under control conditions the oxygen affinity of hemocyanin is greater at 30 o/oo than 10 o/oo (see Chapter I). A decreased hemolymph $P_{50}$ occurred after both exposure doses at 30 o/oo. When crabs were acclimated to 10 o/oo and subjected to a 37.5% TL$_m$ naphthalene dose level, the $P_{50}$ decreased nearly 4.0 torr, a large magnitude considering the normal range of prebranchial oxygen tension. However, no change occurred in oxygen affinity when crabs were exposed to 75% TL$_m$ at 10 o/oo. The presence of naphthalene and naphthalene metabolites, and the altered internal milieu which these induce, in the blood and tissues appear to alter the characteristics of hemocyanin in vitro, such that oxygen is more tightly bound.

The concentration of hemocyanin, per se, likely did not change. Hemolymph serum protein concentrations at 10 o/oo ($44 \pm 5$ mg·ml$^{-1}$) and 30 o/oo ($35 \pm 3$ mg·ml$^{-1}$) were
Table 7

Callinectes sapidus. *In vitro* oxygen affinity of hemocyanin as a function of naphthalene dose following following 24 hours of exposure.

<table>
<thead>
<tr>
<th>Salinity (o/oo)</th>
<th>Dose (%TL&lt;sub&gt;m&lt;/sub&gt;)</th>
<th>P50 (torr)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>8.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>9.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>9.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>12.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>P50 of controls at 10 o/oo significantly higher than controls at 30 o/oo (P < 0.0001).

<sup>b</sup>P50 differs significantly from 0% TL<sub>m</sub> (P < 0.001).
unaltered during the 24-h time course of any one of the six treatments. Approximately 90% of the protein in intermolt crab hemolymph is hemocyanin protein (Truchot, 1978; Horn and Kerr, 1969). This finding does not rule out the possibility of induced changes in hemocyanin subunits upon exposure to naphthalene.

Oxygen content of pre- and postbranchial hemolymph during all phases of the experiments is displayed in TABLE 8. At 30 o/oo a slight decrease occurred in mean CAO$_2$ and CVO$_2$ during the time course of 37.5 and 75% TL$_m$ exposure. No change occurred in hemolymph oxygen content of crabs acclimated to 10 o/oo and exposed to a dose of 37.5% TL$_m$, whereas a large decrease occurred in CAO$_2$ of the group exposed to 75% TL$_m$.

During all naphthalene exposures, regardless of salinity, prebranchial oxygen tension declined (TABLE 9). The general trend for the decline was evident by hour 6 (P < 0.05 in all 4 naphthalene-exposed groups at hour 12, LS MEANS) and continued through hour 24. Slight decreases were recorded in mean PAO$_2$ during exposure to naphthalene, which were in some cases correlated with similar declines in CAO$_2$.

Exposure to naphthalene induced alkalosis in blue crabs adapted to both 10 and 30 o/oo (FIGURE 4). Increased prebranchial pH of exposure groups occurred by hour 6 at 30 o/oo (P < 0.0001) and hour 12 at 10 o/oo (P < 0.02).
<table>
<thead>
<tr>
<th>Salinity (o/oo)</th>
<th>ITL_m</th>
<th>Hour 0</th>
<th>Hour 6</th>
<th>Hour 12</th>
<th>Hour 24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CAO₂</td>
<td>CVO₂</td>
<td>CAO₂</td>
<td>CVO₂</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>1.18±0.13</td>
<td>0.39±0.07</td>
<td>1.29±0.18</td>
<td>0.37±0.14</td>
</tr>
<tr>
<td>37.5</td>
<td>1.23±0.15</td>
<td>0.60±0.11</td>
<td>0.98±0.09</td>
<td>0.37±0.08²</td>
<td>0.98±0.06</td>
</tr>
<tr>
<td>75</td>
<td>1.29±0.14</td>
<td>0.50±0.10</td>
<td>1.25±0.07</td>
<td>0.41±0.12</td>
<td>1.20±0.23</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>1.38±0.11</td>
<td>0.42±0.09</td>
<td>1.44±0.12</td>
<td>0.48±0.12</td>
</tr>
<tr>
<td>37.5</td>
<td>1.20±0.17</td>
<td>0.33±0.07</td>
<td>1.24±0.09</td>
<td>0.37±0.10</td>
<td>1.14±0.10</td>
</tr>
<tr>
<td>75</td>
<td>1.45±0.11</td>
<td>0.39±0.07</td>
<td>1.04±0.10²</td>
<td>0.32±0.14</td>
<td>0.97±0.14²</td>
</tr>
</tbody>
</table>

²Differ significantly from recorded value at hour 0 (P < 0.05).
Table 9

*Callinectes sapidus*. Prebranchial and postbranchial hemolymph oxygen tensions (torr). Sample size ranged from 5 to 8.

<table>
<thead>
<tr>
<th>Salinity</th>
<th>% TL&lt;sub&gt;m&lt;/sub&gt;</th>
<th>30 o/oo</th>
<th>75</th>
<th>10 o/oo</th>
<th>37.5</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hour 0</strong></td>
<td>PAO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>47 ± 3</td>
<td>49 ± 3</td>
<td>46 ± 2</td>
<td>47 ± 2</td>
<td>48 ± 5</td>
</tr>
<tr>
<td></td>
<td>PVO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>15 ± 2</td>
<td>18 ± 2</td>
<td>14 ± 2</td>
<td>14 ± 2</td>
<td>17 ± 2</td>
</tr>
<tr>
<td><strong>Hour 6</strong></td>
<td>PAO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>41 ± 5</td>
<td>40 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47 ± 5</td>
<td>41 ± 2</td>
<td>42 ± 2</td>
</tr>
<tr>
<td></td>
<td>PVO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>14 ± 3</td>
<td>11 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11 ± 1</td>
<td>15 ± 2</td>
<td>13 ± 2</td>
</tr>
<tr>
<td><strong>Hour 12</strong></td>
<td>PAO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>46 ± 3</td>
<td>44 ± 2</td>
<td>39 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43 ± 4</td>
<td>40 ± 2</td>
</tr>
<tr>
<td></td>
<td>PVO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>13 ± 2</td>
<td>11 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15 ± 2</td>
<td>10 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Hour 24</strong></td>
<td>PAO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>44 ± 2</td>
<td>42 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48 ± 5</td>
<td>44 ± 3</td>
</tr>
<tr>
<td></td>
<td>PVO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>13 ± 2</td>
<td>9 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19 ± 2</td>
<td>12 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Differs significantly from recorded value at Hour 0 (P < 0.05).
Figure 4. *Callinectes sapidus*. Time course of change in prebranchial hemolymph pH of crabs acclimated to 10 o/oo (upper panel) and 30 o/oo (lower panel). Symbols represent naphthalene dose levels: o = 0% TL<sub>m</sub>, • = 37.5% TL<sub>m</sub>, ■ = 75% TL<sub>m</sub>. Vertical lines represent standard errors of the plotted mean. Sample size is 6-8.
Callinectes sapidus

10%

30%
By hour 24 the mean values for 10 o/oo crabs were higher than, but not different from, control pH. The 30 o/oo acclimated crabs exhibited a dose-dependent alkalosis at hour 24.

**DISCUSSION**

The pooled-salinity 24-h TL$_{m}$ to naphthalene for *C. sapidus* is 2.40 ppm. During bioassays blue crabs subjected to naphthalene doses above the 24-h TL$_{m}$ exhibited considerable hyperactivity within the first two hours. Pearson and Olla (1980) reported similar observations of *C. sapidus*. Kinter and Pritchard (1977) attributed convulsive movements of individuals exposed to chlorinated hydrocarbons to changes in ion permeability of nerve membranes. Naphthalene appears to exert a similar effect, but this effect is only evident at lethal concentrations. The grass shrimp, *Palaemonetes pugio*, and brown shrimp, *Penaeus azteca*, had 24-h TL$_{m}$'s of 2.4 and 2.5 ppm, respectively (Neff et al., 1976; Anderson et al., 1974a).

Previous investigations have dealt with metabolic rate functions of marine invertebrates and fishes exposed to petroleum hydrocarbons for acute-short (min - h), acute-long (h - days), and chronic (days - weeks) durations. The present investigation falls into the acute-long category. During acute-short term petroleum hydrocarbon exposures, at or near the 24-h TL$_{m}$, Neff et al. (1976) reported
increased VO₂ in *Mysis almyra* and decreased VO₂ in *P. pugio*. The VO₂ pattern of *C. sapidus* followed that of *M. almyra* demonstrating a rise during the first 12 hours. Crabs at 10 o/oo did not exhibit VO₂ differences between exposures and controls at hour 24. This pattern is quite similar to that found for pink salmon fry, *Oncorhynchus gorbuscha* exposed to naphthalene (Thomas and Rice, 1979). Likewise, 32 days of exposure to dimethylnaphthalene did not induce significant elevation of VO₂ in *P. pugio* (Dillon, 1981). Thomas and Rice (1979) hypothesized that an increased extraction efficiency might have occurred in the fishes. Blue crab extraction efficiency from both water and hemolymph was essentially unchanged. Additionally, ventilation of 10 o/oo crabs followed a pattern similar to VO₂. Altered hemolymph oxygen levels and hemocyanin binding characteristics occurred at hour 24.

The often observed trend of the respiratory components to return to control levels by hour 24 might be attributed to either 1) habituation to the naphthalene stress or 2) a decrease or plateau of the tissue burden of naphthalene. Singer and Lee (1977) documented the existence of mixed-function oxygenase (MFO) activity in various tissues of the blue crab, which was particularly high during intermolt. The time course of metabolite formation due to baseline and induced MFO activity might approximate the 24-h time course of the present study. Tissue burden
of benzo(a)pyrene in blue crabs levelled off after 2 days of exposure, even though uptake from the water column persisted (Lee et al., 1976). The detoxification system of C. sapidus might be implicated in the observed stabilization of oxygen transport variables in some cases after 24 hours of naphthalene exposure.

Crabs acclimated to 30 o/oo had an increased VO\(_2\) upon exposure to 37.5 and 75% TL\(_m\) naphthalene at hour 24. Salinity does not affect VO\(_2\) of stepwise acclimated blue crabs (see Chapter I). Laughlin and Neff (1979) found an increased VO\(_2\) upon 5 day polychlorinated naphthalene exposure of juvenile mud crabs, Rhithropanopeus harrisii, which was enhanced at low salinity. The increased VO\(_2\) reported in the present study corresponded to slightly elevated mean extraction efficiencies of tissues. Percent tissue extraction of oxygen from hemolymph increased from 51 ± 4 (hour 0) to 61 ± 6 (hour 24) at 37.5% TL\(_m\) - 30 o/oo and from 64 ± 3 (hour 0) to 71 ± 3 (hour 24) at 75% TL\(_m\) - 30 o/oo. Therefore, the salinity-related differences observed in VO\(_2\) may not relate to cell osmotic regulation but to another priority, such as acid-base equilibrium.

Changes in specific activity of several glycolytic enzymes have been related to crude oil, cadmium and salinity stress in crustaceans (Chambers et al., 1978; Gould, 1980). The level of anaerobic metabolism associated with changes in enzyme activities at differing salinities undoubtedly
plays a major role in animal responses to petroleum-derived hydrocarbons. Truchot (1980) has recently shown that lactate, the primary anaerobic end product in decapods, increases the oxygen affinity of the hemocyanin of the crabs, *Carcinus maenas* and *Cancer pagurus*. Future investigations of anaerobic metabolism related to oil stress will be informative.

A paucity of literature exists concerning cardiac responses of crustaceans (Edwards, 1978; Zimmer et al., 1979) and fishes (Anderson et al., 1977; Wang and Nicol, 1977) to oil exposure. The general response of heartbeat frequency is an initial, possibly behaviorally related, reduction (Wang and Nicol, 1977; Zimmer et al., 1979). In the present study no records were taken until hour 6. Different patterns of $F_H$ were demonstrated during naphthalene exposure at high and low salinity. Little overall change in $F_H$ occurred at low salinity, while heart rate had increased to 46 and 89% above controls in the 37.5 and 75% $T_{LM}$ doses, respectively, at the 24 h point of 30 o/ooS exposure. The increase in $F_H$ at 30 o/oo corresponds with a slight increase reported in $F_H$ of *C. crangon* at 35 o/oo after 12 h of exposure to a crude oil water-soluble fraction (Edwards, 1978).

No previous reports document blood flow characteristics in crabs subjected to petroleum hydrocarbon stress. The mean stroke volume determined for steady state 30 and 10 o/oo acclimated *C. sapidus*, ranged from 0.18 to 0.37
ml·beat\(^{-1}\). 123 g wet wt crab\(^{-1}\). These values are within the range of measured heart volumes of similar sized *C. sapidus* (deFur and Mangum, 1979). Stroke volume and, consequently, cardiac output increased significantly during all naphthalene exposures except 10 o/oo, 37.5% TL\(_m\). The increased blood flow occurred during a time when postbranchial and prebranchial oxygen tensions were reduced. However, the reduced PA\(_O_2\) (ca. 40 torr) still allowed the respiratory pigment to be fully oxygenated at the gill. The lowered PVO\(_2\) facilitated delivery of oxygen to the tissues via hemocyanin. This response occurred concomitantly with altered hemocyanin-oxygen binding characteristics. Increased oxygen affinity, induced by blood alkalosis produced additional stress upon the circulatory system. This was particularly evident at 30 o/oo (FIGURE 4).

*P. pugio* exposed to dimethylnaphthalene exhibited reduced rates of nitrogen excretion (Dillon, 1981). Hemo-lymph ammonia levels were not measured by Dillon, but an increase might lead, in part, to the observed increase in pH of *C. sapidus* hemolymph (Mangum et al., 1976; Truchot, 1981). Blue crabs exposed to sublethal chlorine produced oxidants exhibited a similar reduction of ammonia excretion to that observed for the grass shrimp (Vreenegoor et al., 1977).

The high dose of naphthalene at 10 o/oo did not induce a downward shift of the hemocyanin P\(_{50}\). This can
be attributed to a decrease in blood ion concentrations and increased water content. Both a dilution effect and lowered extracellular chloride levels would enhance the unloading of oxygen.

Although the acclimation of blue crabs to steady state salinities between 10 and 30 o/oo has no effect on the sensitivity to naphthalene, underlying responses of the respiratory and circulatory system to naphthalene are different at 10 and 30 o/oo. Low salinity exposure is characterized by comparatively higher ventilation volume and less efficient osmotic regulation. High salinity exposure induces persistent increases in oxygen consumption, heart rate, stroke volume, and cardiac output, while hemolymph exhibits gradual, dose-related alkalosis.
SUMMARY AND CONCLUSIONS

Resistance of *C. sapidus* to naphthalene does not change with high or low salinity acclimation. However, interactions between salinity and naphthalene occur which induce different physiological responses at 30 and 10 o/ooS. These differences are related to maintainence of 1) osmotic gradients at low salinity and 2) acid-base balance at high salinity.

During exposure, crabs acclimated to either high or low salinity exhibited transiently increased gill ventilation and increased blood perfusion of gill surface. Actual oxygen extraction efficiency was unchanged, indicating that possible pathological effects might have occurred at the gill epithelia. Additionally, control crabs showed brief pauses in ventilation and heartbeat, while constant ventilation and heartbeat occurred in naphthalene-exposed individuals. Coincident with elevated ventilation and perfusion were lowered prebranchial oxygen tensions.

Oxygen uptake rate is not a viable indicator of condition or toxic effects in *C. sapidus*. The increased VO₂ upon naphthalene exposure reported here was not dose-dependent. Likewise, ventilation volume and heartbeat frequency vary as components of the enantiostatic system referred to earlier. The anaerobic metabolism of crabs
might be of considerable importance, especially in accounting for transient changes in the components of aerobic metabolism studied in this thesis.
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Dissertation Title: "Respiratory and Circulatory Responses of the Blue Crab, Callinectes sapidus (Rathbun), to Salinity and Naphthalene" (W. B. Stickle, advisor)
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"Effects of South Louisiana Crude Oil Instrusion on Oysters and Benthic Food Webs in a Louisiana Estuary" (Co-investigators: W. B. Stickle, and J. W. Fleeger) -- Approved, Funds Pending


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G. Publications

1. Abstracts


2. Journal and Book Publications


EXAMINATION AND THESIS REPORT

Candidate: Thomas Donald Sabourin

Major Field: Physiology

Title of Thesis: Respiratory and Circulatory Responses of the Blue Crab, Callinectes sapidus (Rathbun), to Salinity and Naphthalene

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

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