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Sodium/proton exchange in the gills of three species of crabs : *Libinia dubia*, *Callinectes sapidus*, and *Menippe mercenaria*

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Abstract

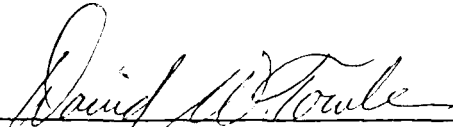
Na^+/H^+ exchange was studied in plasma membrane vesicles from gills of three species of crabs: Callinectes sapidus (blue crab), Libinia dubia (spider crab), and Menippe mercenaria (stone crab). Spider crabs and stone crabs were acclimated to full (35ppt) seawater, while blue crabs were acclimated to both dilute (5ppt) and full strength seawater. Membrane vesicles were prepared by sucrose density centrifugation. Na^+/H^+ exchange was analyzed using acridine orange fluorescence. Male C. sapidus showed the most exchange activity (per g gill tissue), followed by M. mercenaria, female C. sapidus and Libinia dubia. No difference in exchange activity was evident when blue crabs were acclimated to both full and dilute seawater. The Na^+/H^+ exchange stoichiometry was larger than one for both C. sapidus and M. mercenaria, but approximately one for L. dubia. C. sapidus is an osmoregulator and can move from high salinities to reduced; its exchange mechanism may be more efficient in obtaining Na^+ from lower salinities, compared to the stenohaline L. dubia. The control of ionic exchange in gills of M. mercenaria has not been studied. However, a stoichiometry as well as exchange rate similar to C. sapidus would suggest that some regulation is possible.

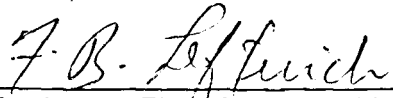
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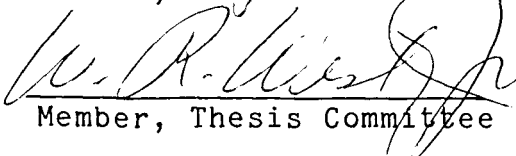
By

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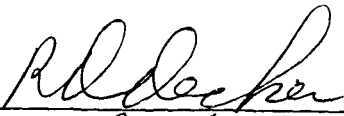

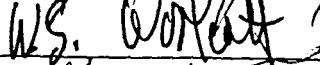
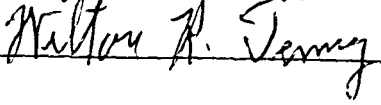
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CRABS: LIBINIA DUBIA, CALLINECTES SAPIDUS, AND MENIPPE
MERCENARIA

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B.S., University of Richmond, Va. 1986

A Thesis

Submitted to the Graduate Faculty

of the University of Richmond

in Candidacy

for the degree of

Master of Science

in

Biology

August, 1988

Richmond, Virginia

Acknowledgements

I would like to thank Dr. David W. Towle for his guidance, insight, and friendship throughout the course of this thesis. Thanks are also due to Dr. Francis B. Leftwich and Dr. Warwick R. West, Jr. for their suggestions concerning improvement of this work. I would also like to thank Robert E. Shetlar for his assistance in the laboratory. Thanks are also given to my parents, Nicholas, Sr. and Ernestine Maiolo, for their inspiration and financial support. Also a special note of thanks goes to my girlfriend, Nancy Wilbon, for her understanding, support, and devotion for the last three years.

Finally, I would like to thank the biology faculty at the University of Richmond for their guidance throughout my college career.

Table of Contents

Introduction.....	1
Materials and methods.....	3
Results.....	7
Discussion.....	11
References.....	20
Figures.....	24
Vita.....	50

Introduction

The relationship that aquatic organisms have between the blood and external medium can be divided into two major categories: osmoconformation and osmoregulation. An osmoconformer has an internal osmotic concentration that follows that of the surrounding medium (Kirschner, 1979). Osmoregulators are able to maintain a relatively constant internal osmotic concentration, either higher or lower than that of the surrounding medium. If the external medium changes, these organisms are able to survive by regulating what ions are released or retained (Lockwood, 1977). Some organisms are stenohaline, and unable to withstand wide variations in salinity. Others are euryhaline, and can tolerate a broad salinity range. Among these euryhaline conformers are the spider crabs (Libinia dubia, Libinia emarginata); these animals remain isosmotic to the medium (Mantel and Landesman, 1977). Some organisms are conformers to a point and then begin to regulate, as has been shown with the blue crab (Callinectes sapidus). It is isosmotic at high salinities and a strong hyperregulator at low salinities (Mantel and Farmer, 1983). Ionic exchange in Menippe mercenaria has not been studied.

Figure 1 illustrates the common patterns of response of membrane ionic exchange in conformers and regulators.

The isosmotic line is a reference point where external salt concentration equals blood concentration. Spider crabs are found where external salt concentration as well as blood concentration are high, along the isosmotic line. Blue crabs range from high salinity, along the isosmotic line, then regulate when the environment becomes dilute, deviating to the left of the isosmotic line (Mantel and Farmer, 1983).

In crabs, the plasma membrane of gills is responsible for maintaining a specific ionic composition in relation to their surrounding environment. It acts as a selectively permeable barrier whose permeability is highly specific. In order for materials to move into or out of the cell, associated transport molecules must be present within the plasma membrane. Ions (such as sodium, hydrogen, and potassium) and small molecules (sugars, amino acids) have been shown to be transported by specific membrane proteins, processes in which different animal cell types have particular transport proteins (Darnell et al., 1986). Among the many membrane proteins involved with salt transport is the Na^+/H^+ exchanger. In blue crabs and green shore crabs (*Carcinus maenas*), this protein is suspected to be associated with Na^+/K^+ ATPase in transepithelial movement of sodium (Shetlar and Towle, 1986). It has also been shown to be responsible for the bulk of sodium, bicarbonate

and water reabsorption in the proximal tubules of mammalian kidneys, as well as pH regulation in epithelial cells (Friedrich et al., 1986; Boron, 1986). Understanding of a mechanism such as this is important when studying membrane physiology as it allows a determination of whether or not animals with different salinity requirements contain the same transport mechanisms. One could determine then if both stenohaline and euryhaline organisms possess the sodium-proton exchanger, or if it is exclusive to organisms that are euryhaline (i.e. strong regulators), as has been found in blue and green crabs (Shetlar, 1987).

The present study was performed to determine if three species of crabs, including a strong regulator and a strict conformer, contain the Na^+/H^+ exchanger, and secondly, to compare and contrast activity and stoichiometry of the exchanger in each species that contains it.

Materials and Methods

Female and male blue crabs (Callinectes sapidus) were obtained from Virginia or Florida seafood suppliers. Stone crabs (Menippe mercenaria) and spider crabs (Libinia dubia) of both sexes were shipped from Gulf Specimen Co., Panacea, FL. All crabs were acclimated to high salinity (35 ppt) in artificial seawater (Instant-Ocean). In addition, blue

crabs were acclimated to low salinity (5 ppt). A 12 hour light/dark cycle was maintained. The seawater was recirculated through gravel filters by non-metallic pumps. Animals were allowed to acclimate to their specific salinity for at least 1 week prior to experimental use. Commercial squid was fed to all crabs at least once weekly.

Membrane vesicles were prepared by the following procedure: Crabs placed on ice for at least 30 min, were sacrificed by bisection. In blue crabs, posterior gills 6-7 were placed in ice cold homogenizing medium [0.25 M sucrose, 6 mM disodium ethylenediaminetetraacetic acid (EDTA), 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), adjusted to pH 6.8 with tris(hydroxymethyl)aminomethane (Tris)]. Gills 6-7 were selected because they have been shown to be rich in $\text{Na}^+\text{+K}^+\text{ATPase}$ activity (Neufeld, et al, 1980). Gill tissue was blotted, weighed, and placed in fresh homogenizing medium (5 ml/g tissue) containing 0.1% sodium deoxycholate, and homogenized in a Teflon glass homogenizer. Six to 8 ml of homogenate were layered over a 32-ml continuous sucrose density gradient (10-40%, wt/wt) containing 6 mM EDTA, 20 mM HEPES, pH 6.8 with Tris. Gradients were centrifuged in a Beckman SW27 swinging bucket rotor at 25,000 rpm for 30 min. Each gradient was visually inspected and the whitish membrane band located at the upper third of the gradient was

removed. The membrane fraction was diluted 10-fold with dilution-loading-resuspension medium [56 mM sucrose, 20 mM HEPES, 100 mM sodium gluconate, pH 6.8 with Tris], and centrifuged in a Beckman 50.2Ti rotor at 30,000 rpm for 60 min to pellet the membranes. Supernatants were removed from the centrifuge tubes and the tube interiors were dried by wiping the inside with a Kimwipe covering a cotton-tipped applicator. Membrane pellets were resuspended in dilution-loading-resuspension medium (at 5-10 mg protein/ml) using a Teflon microhomogenizer (Shetlar, 1987; Towle and Hølleland, 1987).

Membrane vesicles were stored on ice and assayed for total protein by the method of Bradford (1976). Membrane vesicles for spider and stone crabs were prepared similarly. However, all gills were utilized as $\text{Na}^+\text{K}^+\text{ATPase}$ activity in both anterior and posterior gills in spider crabs have been shown to be about equal (Mantel and Landesman, 1976), and no data on stone crabs have been located. Membrane purity was determined by measuring cytochrome oxidase and $\text{Na}^+\text{K}^+\text{ATPase}$ activities in homogenate and membrane fractions (Towle and Hølleland, 1987).

Sodium-hydrogen exchange was analyzed in an Aminco-Bowman spectrophotofluorometer or a Gilford Fluoro IV spectrofluorometer attached to a chart recorder

(excitation 493 nm, emission 525 nm), using the pH sensitive dye, acridine orange (Shetlar and Towle, 1986; Shetlar, 1987). Ten microliters of sodium-loaded vesicles were injected into 2 ml sodium-free incubation medium [10 μ M acridine orange, 36 mM sucrose, 100 mM tetramethylammonium (TMA) gluconate, 20 mM HEPES, pH 6.8 with Tris]. Following the initial quench and allowing for equilibration, 100 μ l 1.5M sodium gluconate (75 mM final concentration) were added to the cuvette which collapsed the pH gradient that had developed across the membranes, yielding a relief in fluorescence quench (Figure 2). Rates were measured by drawing a tangent line on this curve 2 sec after addition of sodium gluconate and activities were recorded (fluorescence/sec/mg protein or fluorescence/sec/g gill tissue).

Stoichiometry of the exchanger was determined by adding sodium gluconate to the incubation medium, using increasing amounts to correspond to a range of 7.5 mM to 75 mM Na^+ final concentration. These data were plotted as rate versus substrate concentration (Shetlar and Towle, 1987; Shetlar, 1987). Kinetic data were determined by using membrane vesicles from one preparation or 'pooled' vesicles from a series of preparations from the same species acclimated to a particular salinity. Coupling ratios (the number of Na^+ ions transported for each H^+)

were determined using Hill plots and regression analysis of the data. Hill plots are used when kinetic data reveal sigmoidal (S-shaped) rather than hyperbolic graphs of substrate concentration versus rate. Coupling ratios can be determined directly by taking the slope from the Hill plot.

Amiloride inhibition was analyzed by adding 0.25 mM (final concentration) amiloride to the incubation medium followed by addition of membrane vesicles. The pH gradient was collapsed by adding 7.5 mM sodium gluconate. Data were plotted and compared/contrasted for each species studied.

Statistical analysis was performed using Student's t-test with significance set at $p < 0.05$.

Results

All animals studied possessed the Na^+/H^+ exchanger. A typical quench - relief curve is illustrated in Figure 3. Injection of Na^+ -loaded vesicles into a Na^+ -free incubation medium resulted in an immediate decrease (quench) in fluorescence (Figure 3a). A pH gradient was established ($\text{pH out} > \text{pH in}$) and caused the weak base acridine orange (AO) to be incorporated into the acidic vesicular space. After 2 min, steady state was reached (Figure 3b) and sodium gluconate was added to the incubation buffer. This

led to an increase in AO fluorescence (relief of quench) (Figure 3c). In all experiments, complete relief of fluorescence quench did not occur.

Comparative analysis illustrated that specific activities calculated on the basis of mg protein were 33-74 fluorescence units/sec/mg protein larger in M. mercenaria than any other species studied. Libinia dubia and C. sapidus males had similar activities ranging from 46-52 fluorescence units/sec/mg protein. Female blue crabs had the lowest specific activity (Figure 4).

No significant difference in exchanger activity was evident in response to salinity change. Both male and female blue crabs had similar exchange rates with respect to high and low salinity. Male blue crabs, however, were significantly different from female blue crabs in both environments. In high salinity, exchange activity in males was 70% greater (Figure 4). Male blue crabs were not significantly different from spider crabs, but female blue crabs were ($p < 0.005$).

In order to determine the amiloride-sensitive portion of the exchanger, 0.25 mM amiloride was incorporated into the incubation buffer before injection of Na^+ -loaded vesicles. Rates were taken from the relief curve as described above, and utilized for comparison among the species. The effect of amiloride is depicted in Figure 5.

It is evident that when Na^+ is injected into the incubation medium amiloride affects the relief; this is also seen during the initial quench.

Amiloride-sensitive Na^+/H^+ exchange (per mg protein) was most active in Libinia, followed by Mennipe, male Callinectes, and female Callinectes (Figure 6). Spider crabs had an exchange rate twice that of stone crabs. However, this difference was not statistically significant. Male blue crabs were 2.6-fold higher in activity when compared to females; this difference was statistically significant ($p < 0.005$). Stone crabs were 4.6-fold more active than female blue crabs (level of significance ($p < 0.005$)). Exchange activity in male blue crabs was significantly higher than spider crabs ($p < 0.05$) and stone crabs ($p < 0.01$) (Figure 6).

When comparing activities with different species, one must be careful since the calculation for specific activity involves the amount of protein present. For instance, the activity from a gill with low protein would be much greater than one that had a higher protein content. Thus, if protein concentrations are compared and activity per mg protein is calculated actual similarities or differences among species might not be adequately illustrated. Total protein concentrations for each species is illustrated in Figure 7. Female blue crabs had a larger concentration

than males in both high and low salinity. Male blue crabs, however, contained more protein (4.67mg) than that found in the stone crab (3.31mg), and the spider crab (2.25mg).

Because there is such a difference with total protein among the species used here, calculations of exchange activity were expressed per amount of gill tissue (fluorescence units/sec/g gill tissue; Figure 8). No significant difference existed within either male or female blue crabs when acclimated to different environments, as had been shown above. However, using gill weight as the basis of comparison, male blue crabs had the most activity, followed by stone crabs, and finally spider crabs and female blue crabs. Exchange activity in male blue crabs was statistically higher than stone crabs ($p < 0.05$) as well as spider crabs ($p < 0.005$), and female blue crabs ($P < 0.005$). No difference existed between female blue crabs and spider crabs. Stone crabs were almost twice as active in the Na^+/H^+ exchanger as were spider crabs and female blue crabs (statistical difference $p < 0.005$, $p < 0.025$).

Likewise, amiloride sensitivity was determined using the amount of gill tissue present (Figure 9). Most of the species studied utilizing this calculation had similar inhibition by amiloride. The only statistical differences existed between male blue crabs and spider crabs ($p < 0.025$) and stone crabs and spider crabs ($p < 0.05$)(Figure 9).

Sodium/proton stoichiometry was analyzed using Hill plots and regression analysis of the data to determine coupling ratios for each species was conducted. Sodium/proton exchange was larger than 1.5 for male and female blue crabs, as well as stone crabs (Figures 10 and 11). The coupling ratio for L. dubia was approximately one (Figure 10a). Spider crabs exhibited normal Michaelis-Menten kinetics. However, stone crabs and blue crabs displayed sigmoid kinetics (Figures 12 and 13).

Discussion

Isolated membrane vesicles have been shown to be a useful tool in studying membrane transport. They allow the study of these functions without being affected by other complexities of the cell. Furthermore, the intravesicular content can be changed at will, which allows different mechanisms to be studied while using the same experimental design. Isolated membrane vesicles have been useful in determining many characteristics of membrane transport, such as $\text{Na}^+\text{K}^+\text{ATPase}$ in gills of the blue crab (Towle and Hølleland, 1987), Na^+/H^+ exchange in canine sarcolemmal tissue (Pierce and Philipson, 1985), and bicarbonate-chloride transport in the blue crab (Lee and Pritchard, 1985).

The utilization of the fluorescent dye, acridine orange, has been shown to be quite helpful in the study of pH changes that develop in both the exterior and interior of the vesicular space (Reenstra, et al., 1981; Shetlar and Towle, 1986; Shetlar, 1987). By using such a dye, the Na^+/H^+ exchanger can be manipulated such that pH gradients are formed and then collapsed as the exchanger is reversed (Figure 2). The present study utilized these characteristics of membrane vesicles and the pH-sensitive fluorescent dye to study Na^+/H^+ exchange in crabs with different patterns of membrane ionic exchange.

Osmoconformers are at the mercy of their environment. Stenohaline osmoconformers can tolerate very little salinity change, therefore only inhabit areas of specific salt concentrations. Libinia dubia is a euryhaline osmoconformer as it is capable of acclimating to lower salinities (Mantel and Landesman, 1976). Because it is a conformer, salt concentration in the blood is similar to that in the surrounding medium.

Euryhaline osmoregulators are more flexible in their environment. They have the ability to enter dilute waters and survive due to the ability to produce a dilute urine and/or to increase activities in specific membrane transport mechanisms in the gill.

Past studies have shown that osmoregulating male and

female blue crabs inhabit different salinities throughout their life cycle, but are unable to produce a dilute urine (Mantel and Farmer, 1983). Larvae develop and hatch in high salinity then migrate to estuarine waters of lower salinity to develop further (McKensie, 1970). In order to be able to do this, certain mechanisms must be present to control salt balance within the cell. Two of these mechanisms have been shown to be the $\text{Na}^+\text{K}^+\text{ATPase}$ system and the Na^+/H^+ exchange system (Shetlar, 1987; Towle and Hølleland, 1987; Neufeld et al., 1980).

Male blue crabs have been shown in the present study to possess the Na^+/H^+ exchanger, which was also found by Shetlar, 1987. When animals were acclimated to dilute seawater, activity (per gram gill tissue), compared to full strength sea water, was not statistically different. This finding deviates from past studies that have shown that a difference exists between low and high salinity acclimated animals (Shetlar, 1987). It has been demonstrated that male blue crabs increase their $\text{Na}^+\text{K}^+\text{ATPase}$ activity when introduced into lower salinities, thus the blue crab may be using some other transport system to aid in uptake of salts in dilute media. Male blue crabs had the highest total Na^+/H^+ exchange. This seems reasonable as male blue crabs tend to spend most of their lives in environments with salt concentrations less than full strength sea water.

Female blue crabs had a significantly lower activity of the Na^+/H^+ exchanger per gram gill tissue than any other species studied. These crabs also had the highest protein concentrations (8.5-11 mg/ml). This may indicate that female blue crabs have more protein in their plasma membranes when compared to the other animals studied. Female blue crabs also showed no significant difference in activity rates of the exchanger when introduced into lower salinities. Thus, it seems that both male and female blue crabs do not increase in Na^+/H^+ exchange activity when introduced into dilute environments, as has been shown with Na^+/K^+ ATPase activity (Neufeld et al., 1980).

When activity of the Na^+/H^+ exchanger was compared among the osmoconforming spider crab and the osmoregulating blue crabs, rates found in the spider crab were similar to those found in the female blue crab, but significantly different from the male blue crab. A possible reason may be that both Libinia and female Callinectes are found mostly in high salinity waters. In this environment, female blue crabs are osmoconformers as are spider crabs (Mantel, 1986). It is therefore reasonable to suggest that both species are utilizing the mechanism in the same manner. Male blue crabs were significantly different than spider crabs. This may also be due to the environment each is found in; the male blue crab need not return to full

strength sea water as does the female, therefore inhabits more dilute media. As a result the Na^+/H^+ exchanger may be utilized by the male blue crab *moreso*, even in higher salinities, than spider or female blue crabs in order to tolerate the salinity fluctuations encountered during its life cycle.

In blue crabs, males had a higher exchange rate than females possibly because the males need not return to sea water as do females. Females must return to sea water in order to release fertilized eggs. If this is the case, then males are continuously faced with low salinities and therefore must have a means to cope. This is consistent with the finding that Na^+/K^+ ATPase is also higher in the males versus the females (Neufeld et al., 1980).

Data on membrane ionic exchange in gills of *Menippe mercenaria* are lacking. Thus, the stone crab was analyzed only in high salinity. Transport activities without the addition of amiloride were found to be less than the blue crab, but higher than the others studied. Protein concentrations were quite low (2-4 mg/ml) as opposed to Na^+/H^+ activity: the low protein concentration may represent functional protein characteristic to this species. Nevertheless, it is significant that the stone crab was found to possess the Na^+/H^+ exchanger.

The amiloride-sensitive portion of the exchanger was

found to be largest in L. dubia and female C. sapidus. This coincides with the total activity of the exchanger; both spider crabs and female blue crabs had similar activities, probably due to the similar environment inhabited. Stone crabs and male blue crabs showed less amiloride sensitivity which is opposite to that found in total activity without amiloride. Male blue and stone crabs had higher activities than female blue and spider crabs. This seems to indicate that although Libinia and female Callinectes have a lower activity compared to the others, a larger percentage of that activity is sensitive to amiloride. This may indicate that only one Na^+ binding site exists on the exchanger for these animals while male blue crabs and stone crabs contain multiple binding sites. In order to get a better understanding on the binding characteristics of Na^+ , stoichiometries of the exchanger were determined using Hill plots.

Stoichiometries of the exchanger ranged from 1 in the osmoconforming spider crab to 2 in the osmoregulating male blue crab. The female blue crab was found to have a coupling ratio between the male blue crab and the spider crab. The coupling ratio of 1, indicating 1 Na^+ per 1 H^+ involved in transport would seem reasonable as Libinia has an abundant supply of salt in its environment. A direct relationship would be anticipated since salt uptake would

not seem to be a problem. Male and female blue crabs, however, are faced with more dilute salt concentrations thus obtaining salt would be more difficult than in the spider crab. As a result, stoichiometries of greater than one may be expected for osmoregulators. As the other data have shown, female blue and spider crabs show some similar trends. Females had a ratio between that of the male blue crab and the spider crab. It was higher than the osmoconformer which was expected; however, it was less than the male of the same species. Possibly, male blue crabs are genetically better fit to inhabit dilute media, and therefore have become better adapted to cope with salt change: a higher activity rate of the Na^+/H^+ exchanger as well as a 2:1 Na^+ to H^+ relationship. Female blue crabs are different from spider crabs in that they move into dilute salinities to mate. However, because most of their time is spent in high salinity waters, it is not surprising that the stoichiometry of the exchanger is similar to that in spider crabs.

Stone crabs had a stoichiometry also near 2, similar to the male blue crab. Amiloride sensitivity as well as total activity of the exchanger was similar to the male blue crab. Therefore it is quite possible that the stone crab has some regulatory capabilities. Further studies must be performed to confirm this.

Only Libinia dubia seemed to exhibit normal Michaelis-Menten kinetics. This would indicate that the Na^+/H^+ exchanger has only one Na^+ binding site. This was confirmed by using Hill plots (coupling ratio=1). This was not the case in the other animals studied. Normal hyperbolic curves were not found. Past studies have shown that the male blue crab displayed sigmoid kinetics (Shetlar, 1987). Through the use of Hill plots it was determined that two Na^+ ions were involved in exchange for every H^+ (Shetlar, 1987). This was confirmed in the present study. It was also shown that female blue crabs and stone crabs also had exchange ratios larger than one. This implies that the exchanger possesses additional binding sites for Na^+ . Because more than one ion binding site is present, there is the possibility of interactions between the binding sites during the binding process (Palmer, 1981). In the male blue crab, it has been suggested that positive cooperativity is occurring (Shetlar, 1987). Cooperativity occurs when more than one ligand-binding site exists on a protein and the binding of one molecule to one site increases the probability that further molecules will be bound by the remaining sites. This is in contrast to negative cooperativity where binding of one ligand molecule decreases the probability of binding by further ligand molecules. Sigmoid binding curves, like

those found in C. sapidus and M. mercenaria, are characteristic of positive cooperative binding (Lehninger, 1982). For the Na^+/H^+ exchanger 1 Na^+ ion would increase the affinity of the exchanger for other Na^+ ions. Cooperative Na^+ binding by the Na^+/H^+ exchanger would be a physiological advantage because it provides a mechanism by which more Na^+ could be taken into or removed from gill tissue. Cooperativity would therefore have a direct role in the capability of organisms such as blue crabs to move from high salinities to dilute salinities or vice versa.

The purpose of this study was to determine if species with different modes of dealing with the ionic environment possess the Na^+/H^+ exchanger. It is evident that all species studied possessed it. A second goal was to determine stoichiometries and activities of the exchanger and compare and contrast them with the various species studied. This study has shown that differences do exist in exchange activity, as well as the ratio of ions exchanged, among crab species with different modes of response to the ionic environment.

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Figure 1. Patterns of electrolyte regulation among marine animals (From Kirschner, 1979).

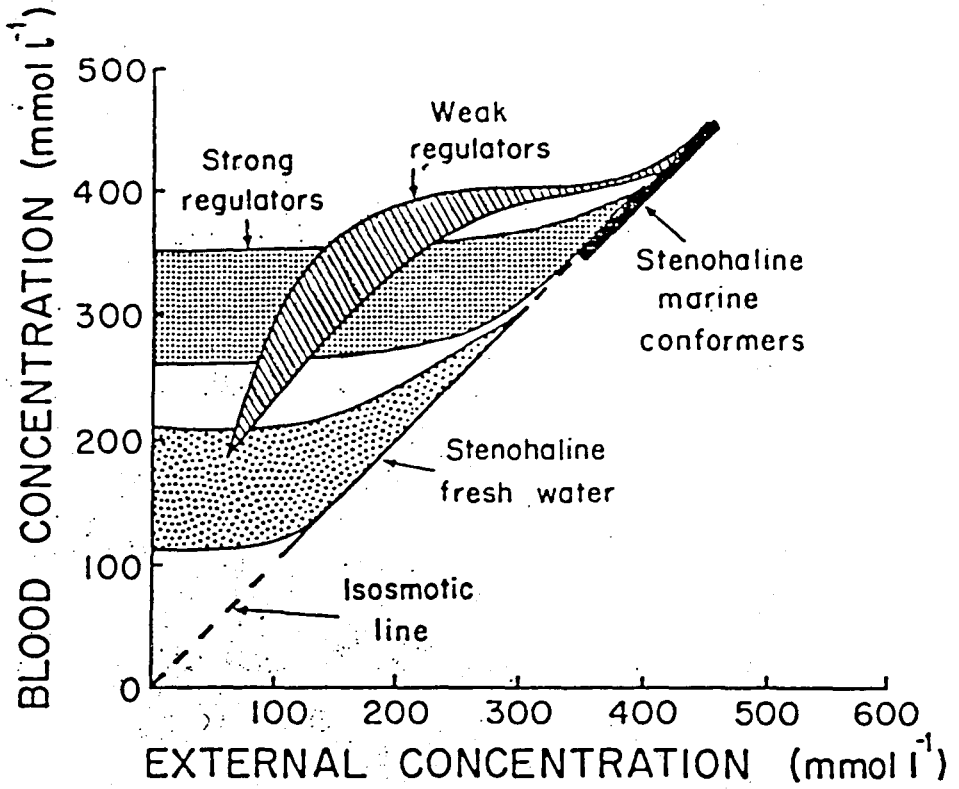
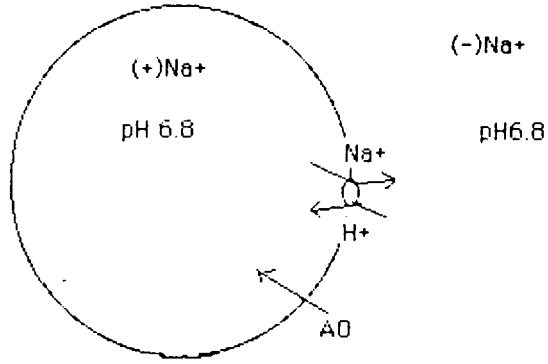
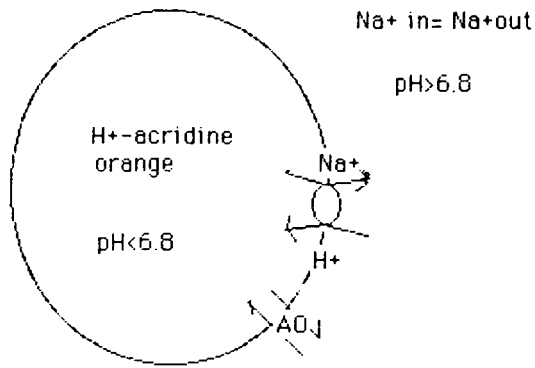


Figure 2: The use of acridine orange in studying Na^+/H^+ exchange. Sodium-loaded vesicles are released into a Na^+ -free medium causing H^+ to enter the cell and acridine orange to become incorporated (a). Following formation of the pH gradient where $\text{Na}^+_{\text{in}} = \text{Na}^+_{\text{out}}$, $\text{pH}_{\text{in}} < \text{pH}_{\text{out}}$ (b), the gradient is collapsed with the addition of sodium gluconate (75mM final concentration) (c).

a Na⁺ loaded vesicles injected into Na⁺ free incubation medium



b pH gradient forms



c Na⁺ injected into incubation medium

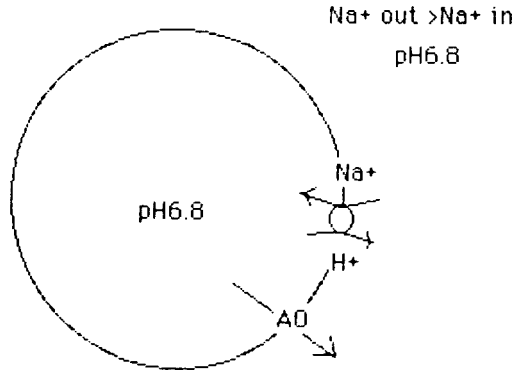


Figure 3: Typical quench-relief curve for Callinectes
sapidus. Vesicles are injected and an initial quench
results at (A). A pH gradient develops and equilibrates at
(B). Excess sodium gluconate is added at (C) resulting in
a relief of fluorescence quench.

F L U O R O I V

SAMPLE: **C. aspidua - m**

CODE 2-10-88
 TIME DRIVE 2.53 min
 TIME SCALE 0.17 min/cm
 EXCITATION 493 nm
 EMISSION 525 nm
 CALIBRATION 100.0
 HIGH VOLTAGE 316 V
 BLANK 4.3
 FACTOR 1.04
 RESPONSE 0.5 sec

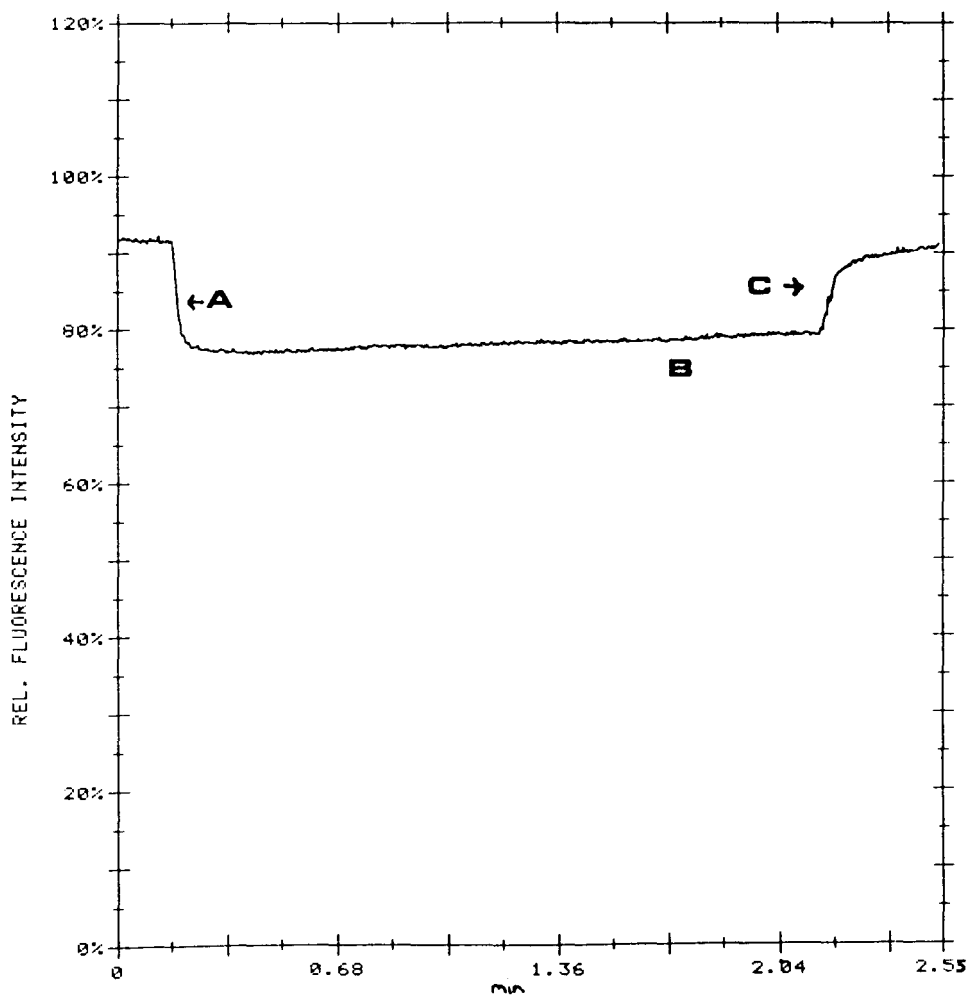


Figure 4: Na^+/H^+ exchange in Callinectes sapidus females [Cs(f)] and males [Cs(m)] in low (5 ppt.) and high (35 ppt) salinities, as well as Libinia dubia (Lib.) and Menippe mercenaria (Men.). Activities were calculated per mg protein. Statistical groupings (A, B, or C) indicate significant differences.

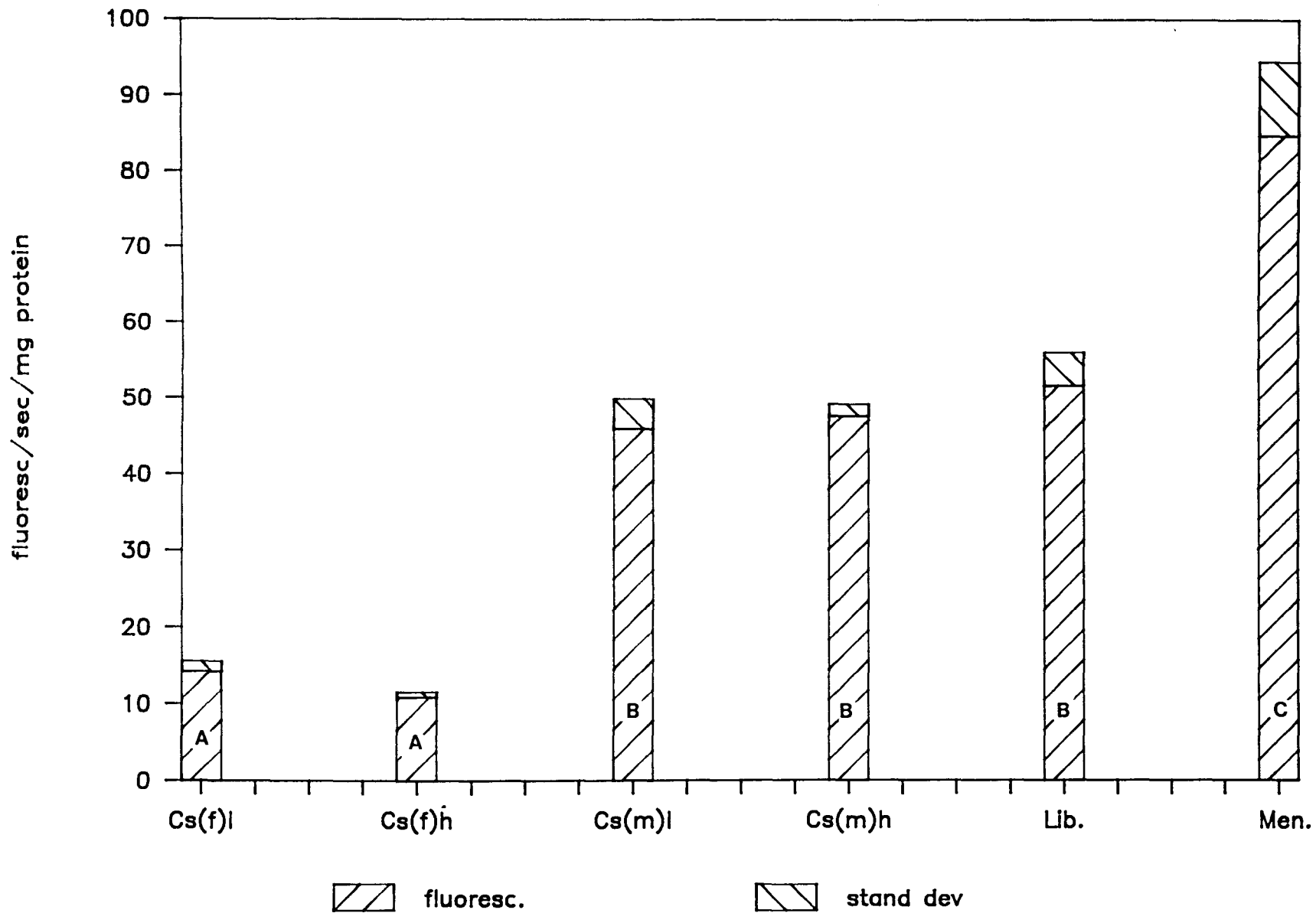


Figure 5: The effect of 0.25mM (final concentration) amiloride on Na^+/H^+ exchange in Callinectes sapidus female. In (A) no amiloride is present. In (B) amiloride is present. This causes the relief curve to bend toward the right versus the relatively steep curve in (A).

FLUORO IV

SAMPLE: **C. sapidus-f**

CODE	2-10-88
TIME DRIVE	2.55 min
TIME SCALE	0.17 min/cm
EXCITATION	493 nm
EMISSION	525 nm
CALIBRATION	100.0
HIGH VOLTAGE	316 V
BLANK	4.3
FACTOR	1.04
RESPONSE	0.5 sec

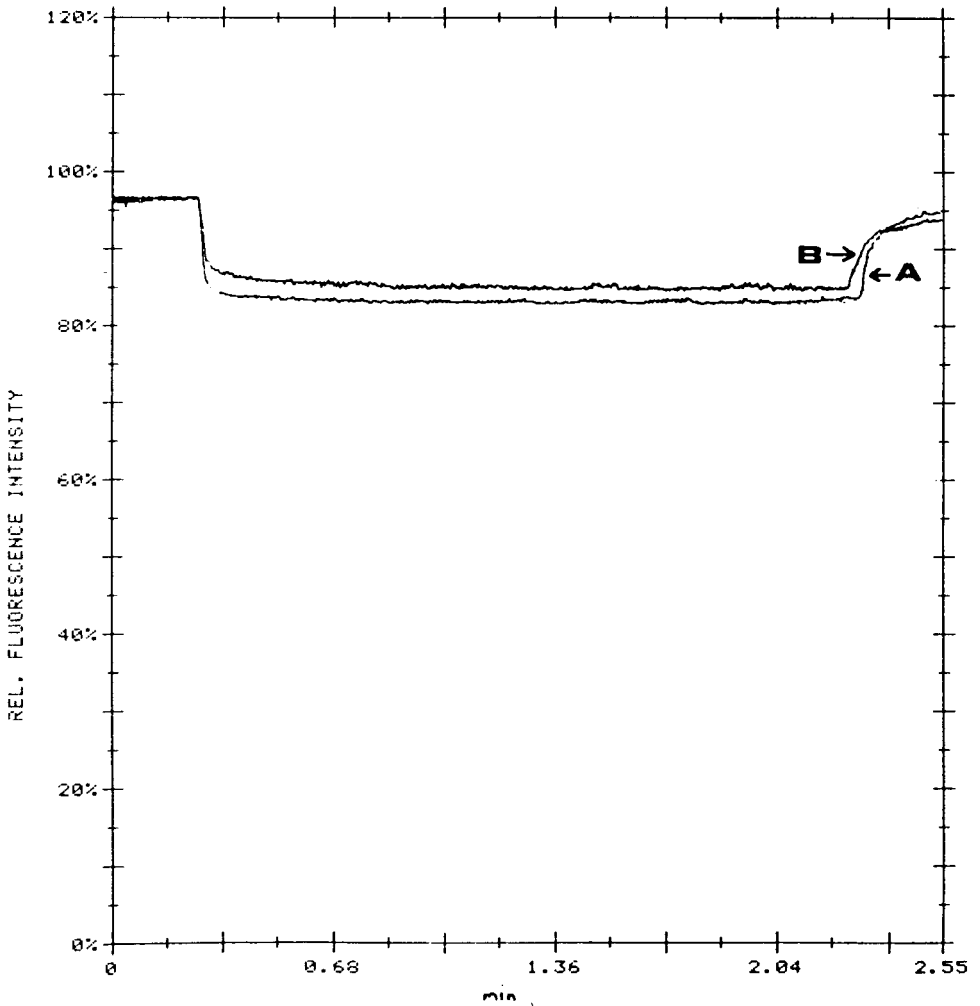
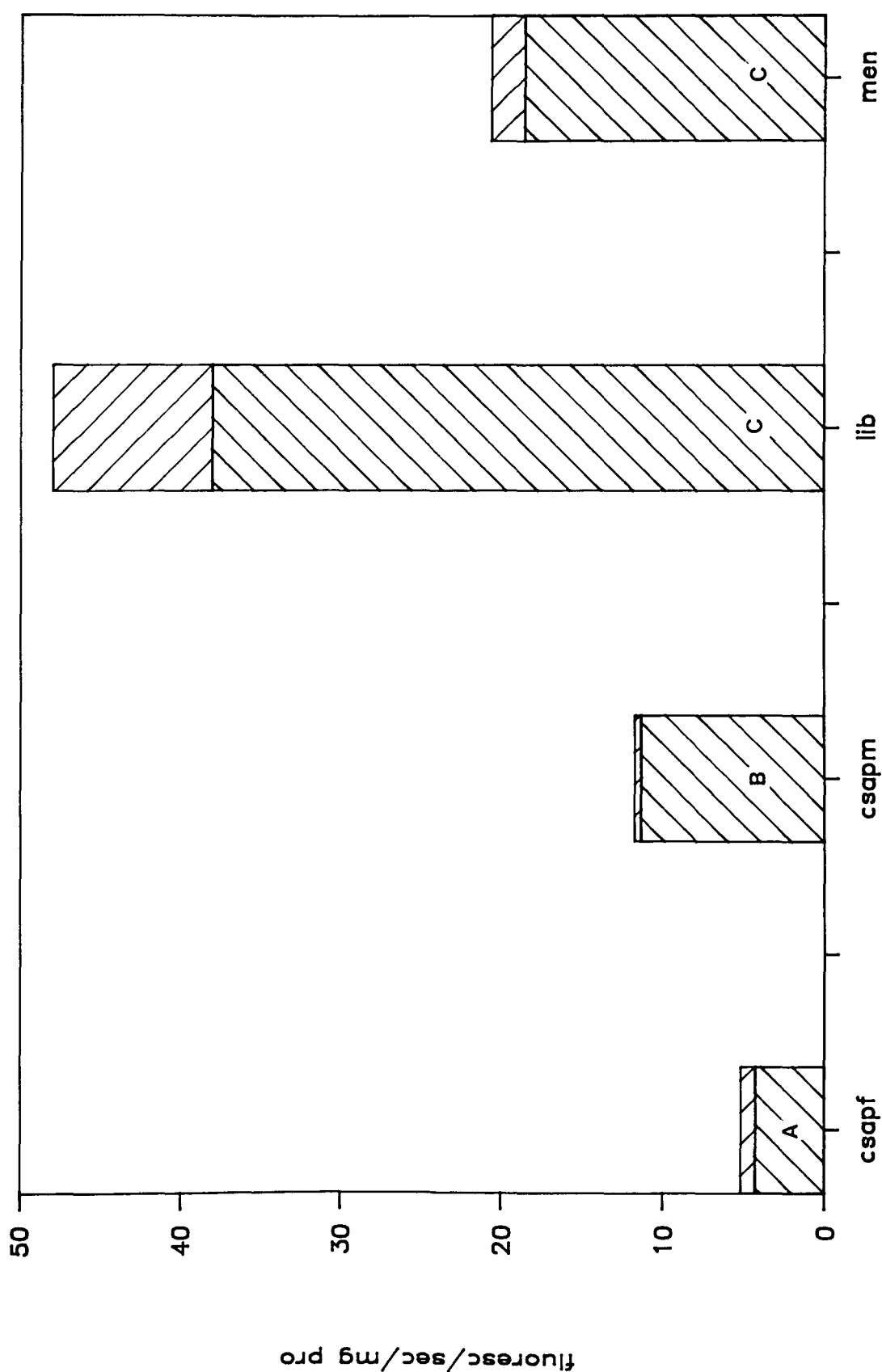


Figure 6: The effect of 0.25mM (final concentration) amiloride on Na^+/H^+ exchange in Callinectes sapidus females (csapf) and males (csapm), as well as Libinia dubia (lib) and Menippe mercenaria (men). All animals are acclimated to full strength sea water (35 ppt). Rate is determined per mg protein. Statistical groupings (A, B, or C) indicate significant differences.



 fluoresc.
  Animals acclimated to high salinity stand dev.

Figure 7: Total protein concentrations in vesicle preparations from Callinectes sapidus females [csap(f)] and males [csap(m)] (in high = H and low = L salinities), as well as Libinia dubia (L.dub) and Menippe mercenaria (M.merc). Standard for determining protein concentrations was bovine albumin (5mg/ml).

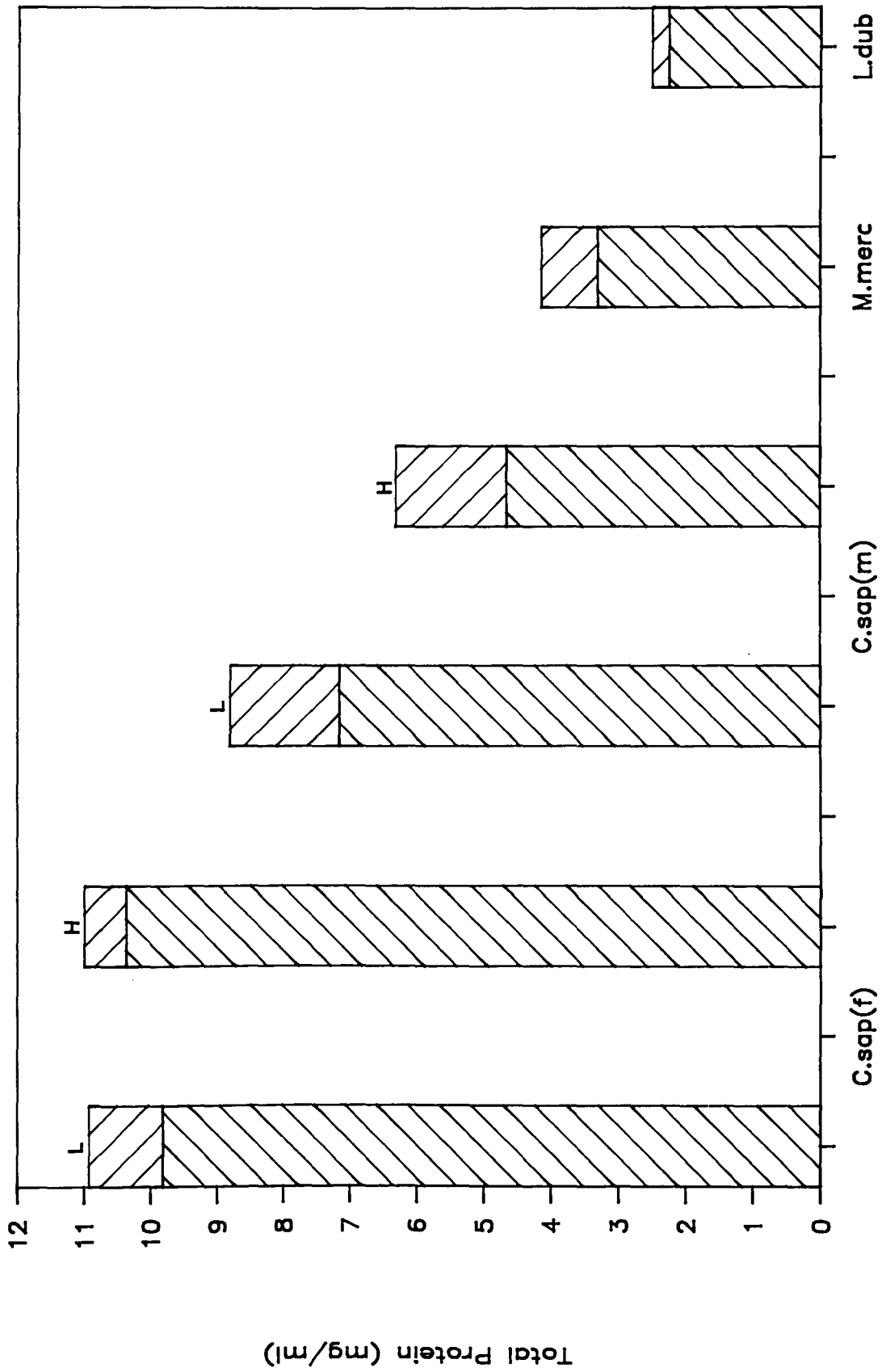


Figure 8: Na^+/H^+ exchange in Callinectes sapidus females [Cs(f)] and males [Cs(m)] in low (5 ppt) and high (35 ppt) salinities, as well as Libinia dubia (Lib.) and Menippe mercenaria (Men.). Activities were calculated per gram gill tissue. Statistical groupings (A, B, or C) indicate significant differences.

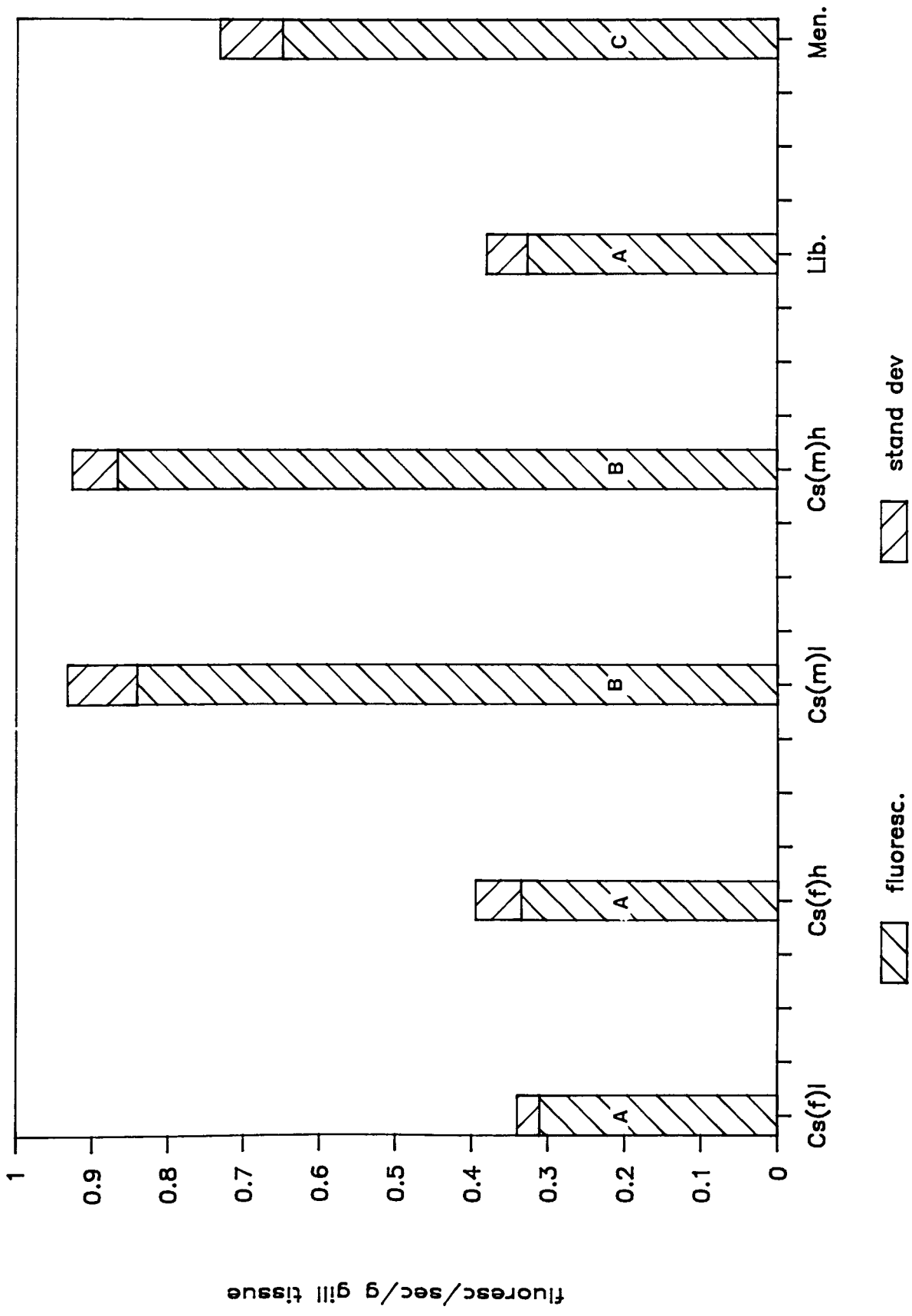
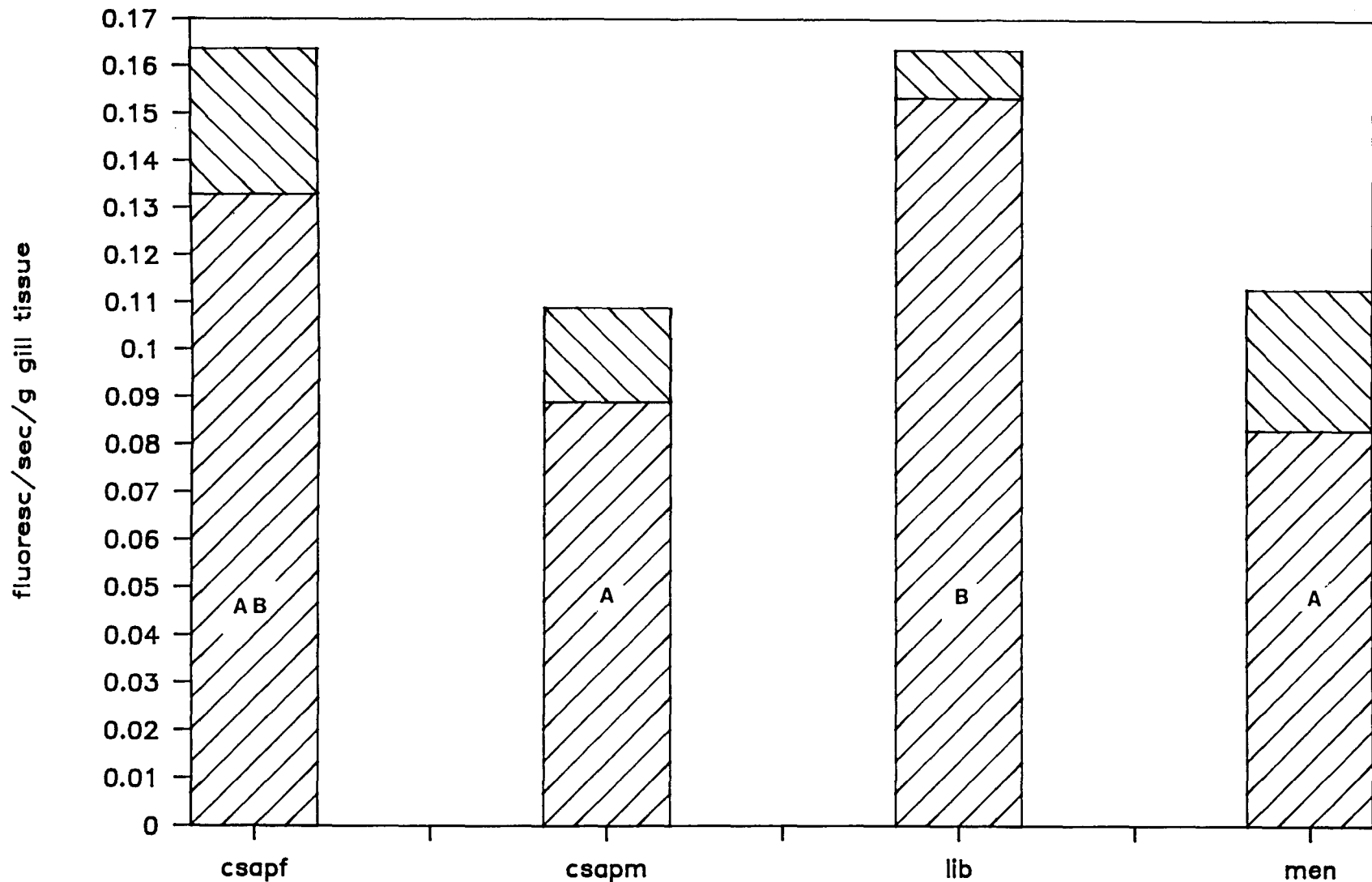


Figure 9: The effect of 0.25mM (final concentration) amiloride on Na^+/H^+ exchange in Callinectes sapidus females (csapf) and males (csapm), as well as Libinia dubia (lib) and Menippe mercenaria (men). All animals are acclimated to full strength sea water (35 ppt). Rate is determined per gram gill tissue. Statistical groupings (A, B, or AB) indicate significant differences.

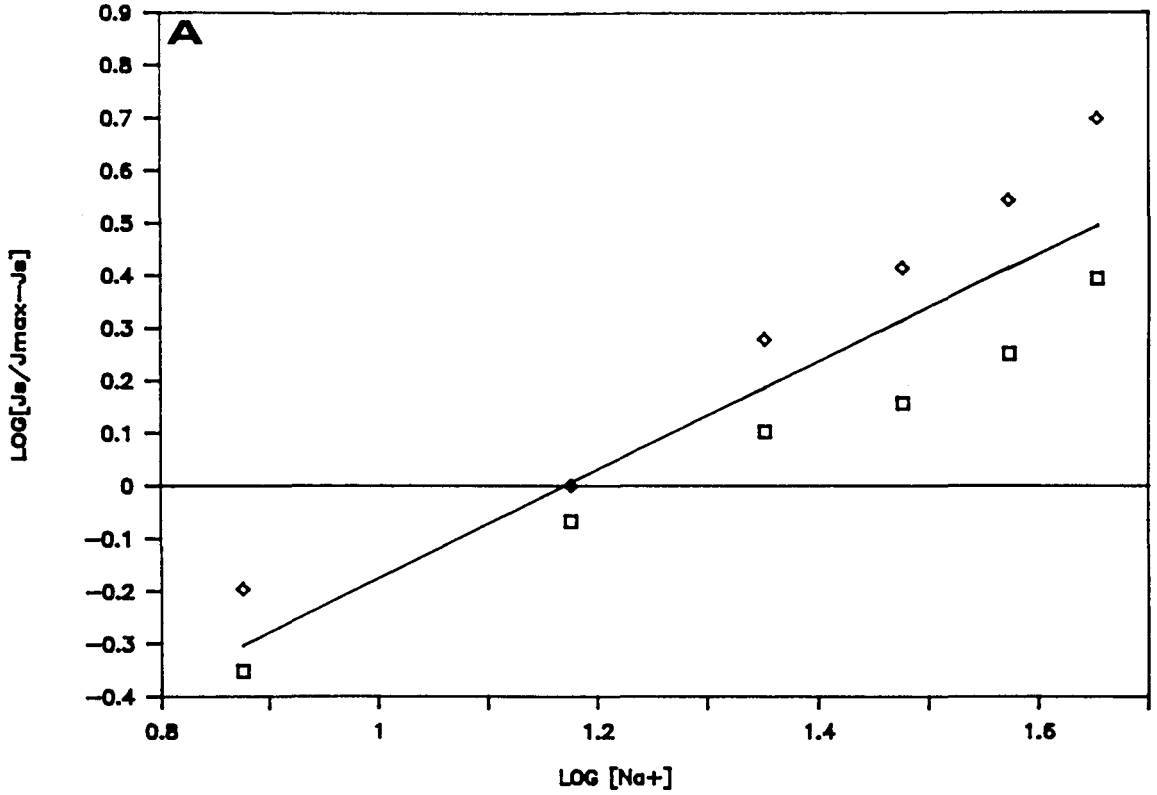


Animals acclimated to high salinity

 fluoresc.  stand dev.

Figure 10: Coupling ratios for Libinia dubia (A) and Menippe mercenaria (B). Ratios were determined using Hill plots.

L. dubia: coupling ratio 1.02



M. mercenaria: coupling ratio 1.73

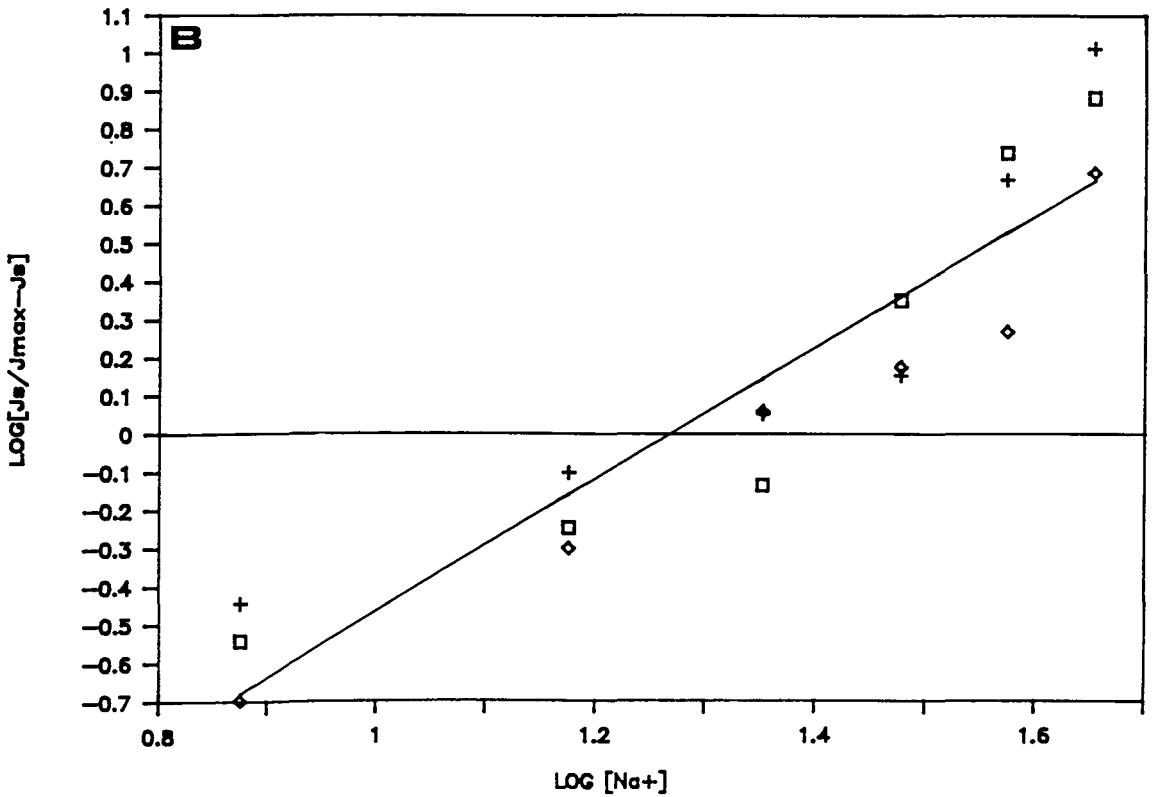
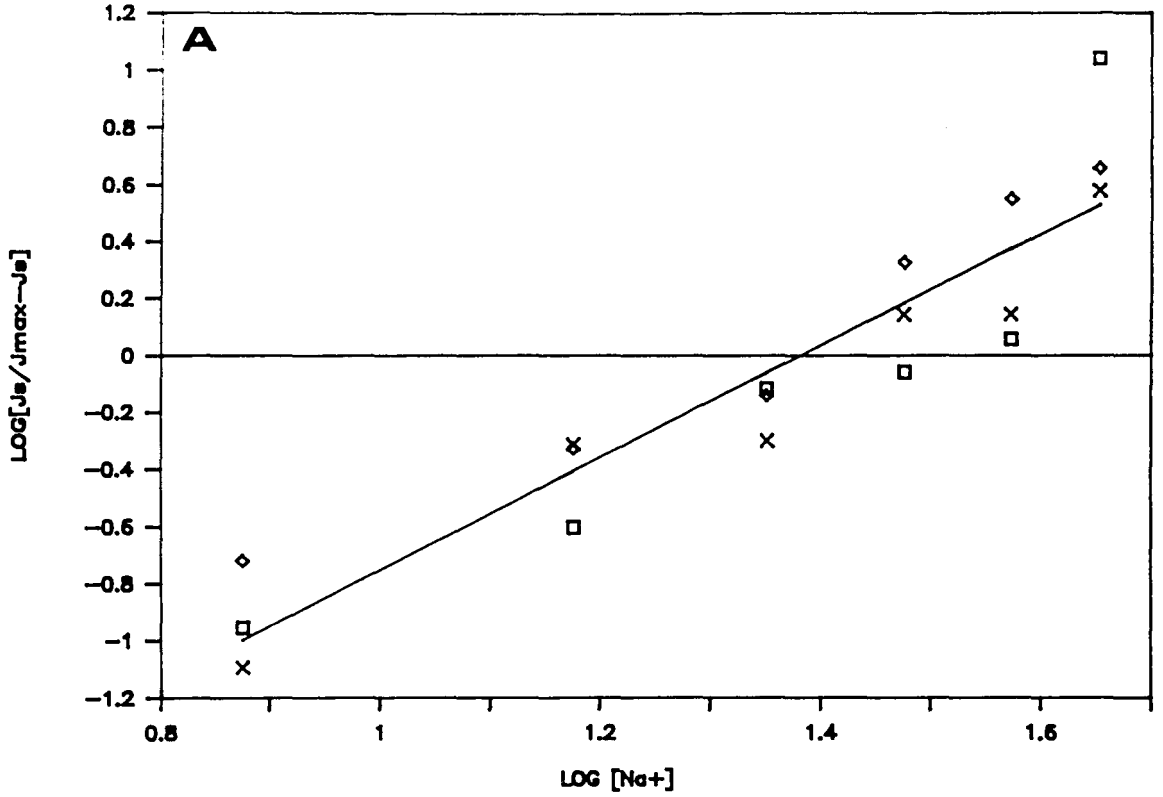


Figure 11: Coupling ratios for Callinectes sapidus males (A) and Callinectes sapidus females (B). Ratios were determined using Hill plots.

C. sapidus(m): coupling ratio 1.96



C. sapidus(f): coupling ratio 1.56

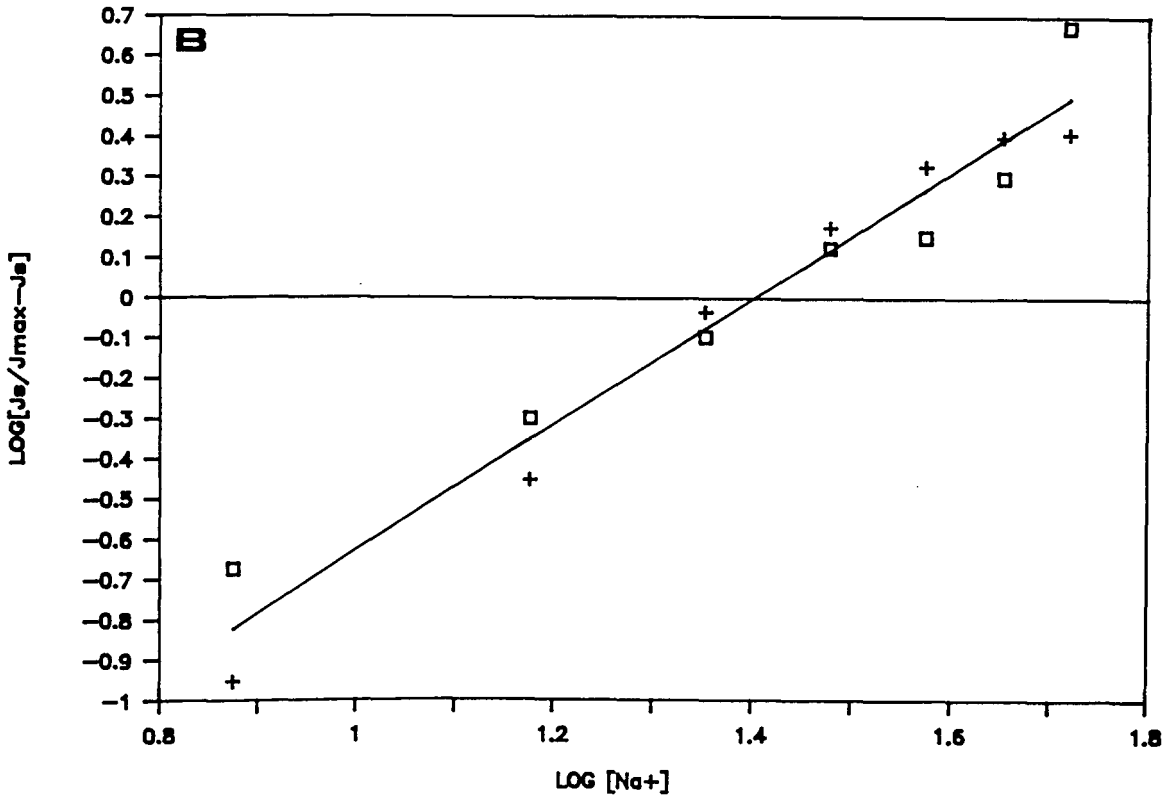


Figure 12: Michaelis-Menten plots for Libinia dubia (A) and Menippe mercenaria (B).

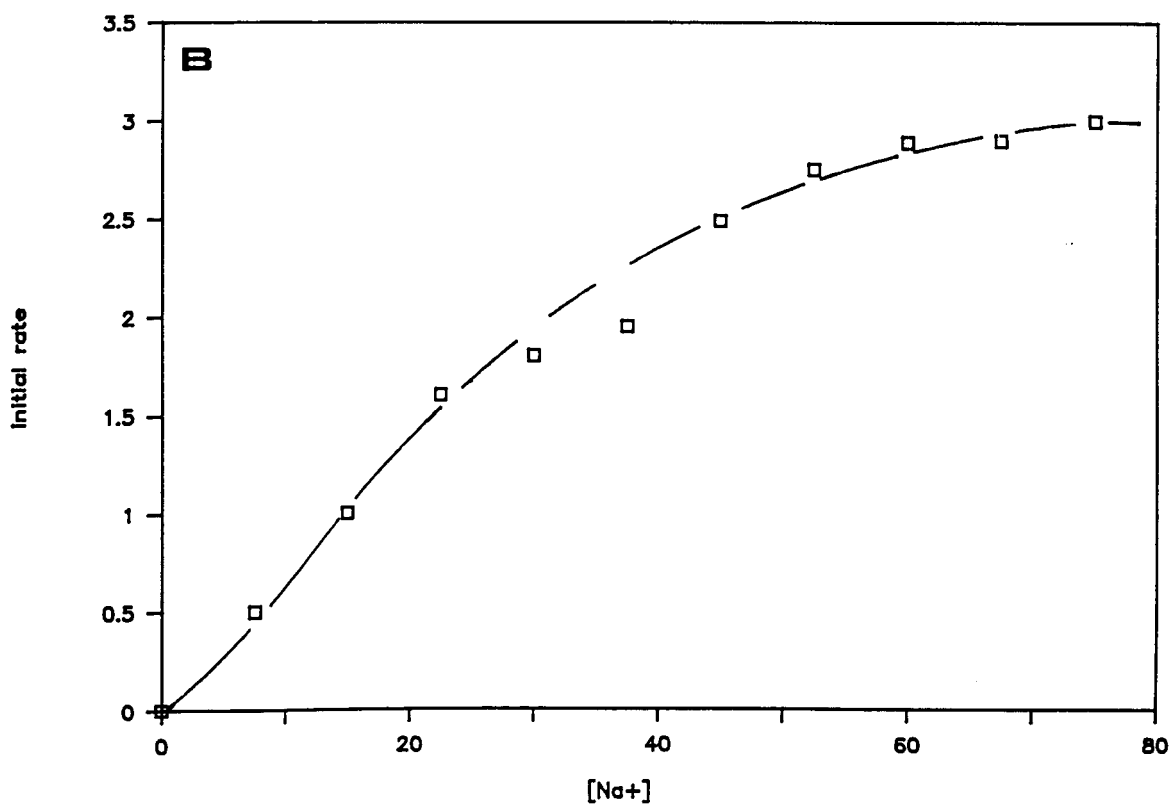
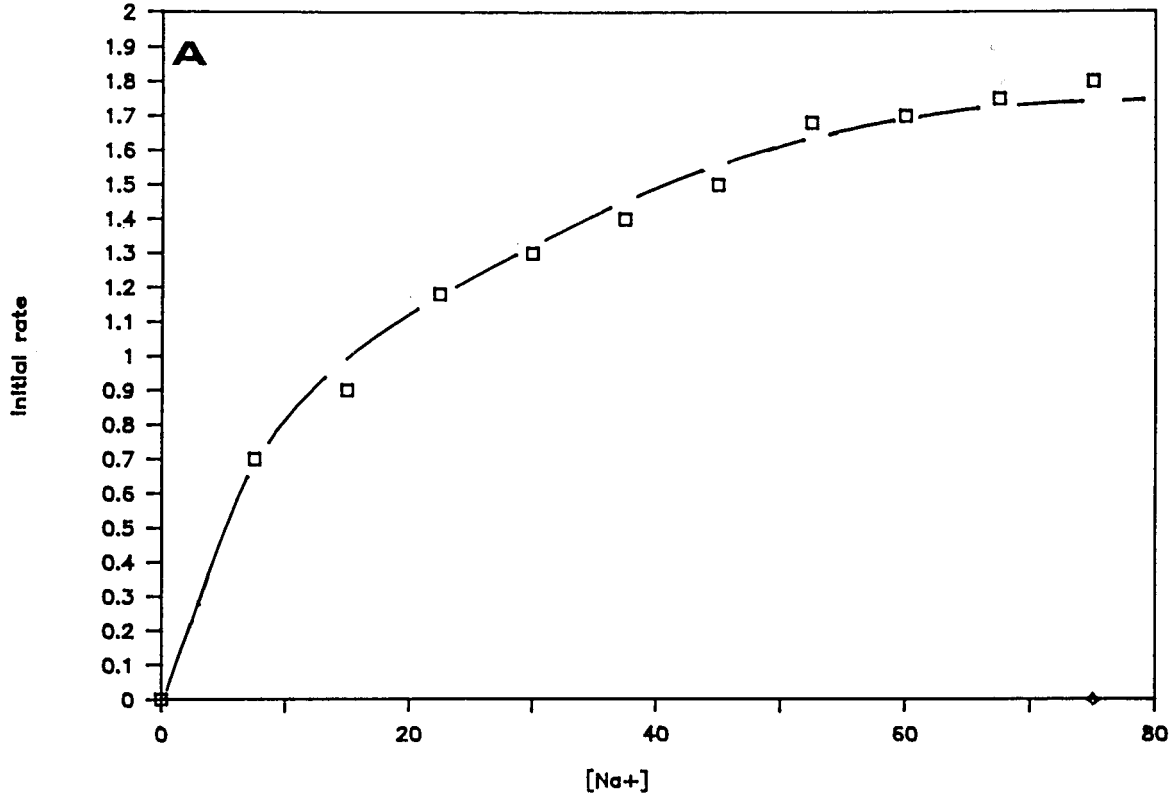
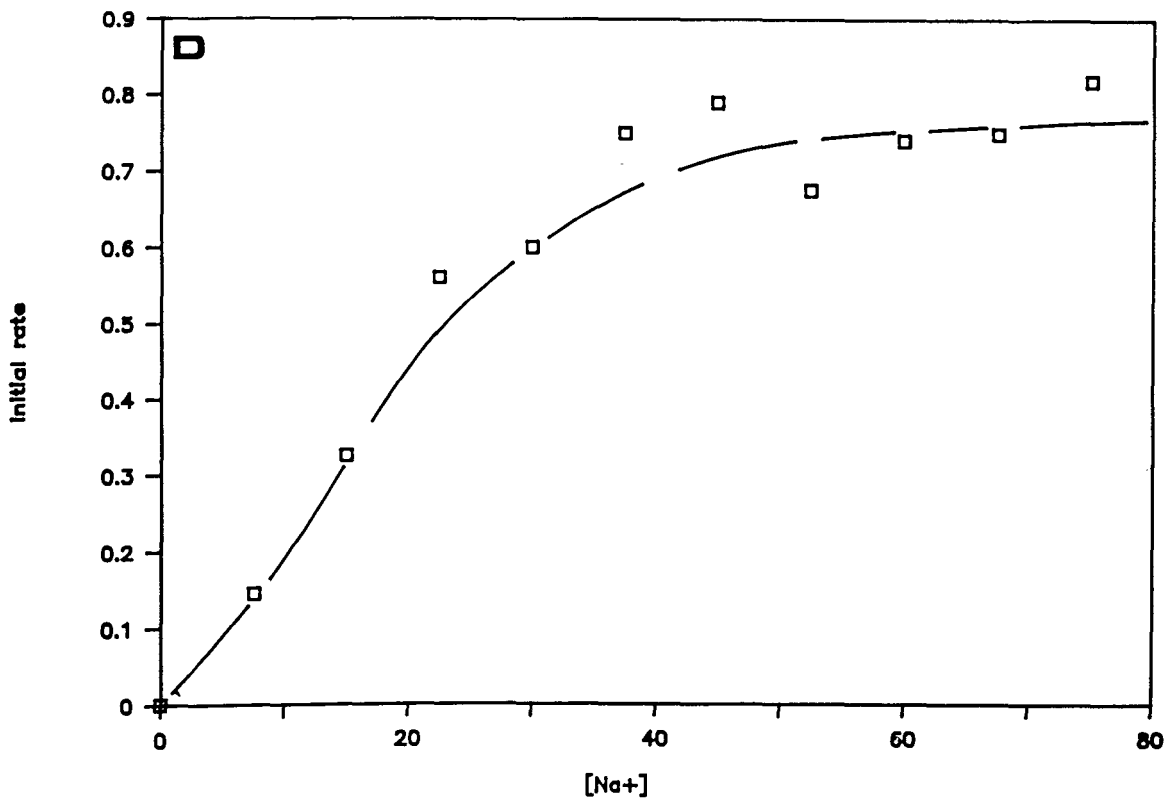
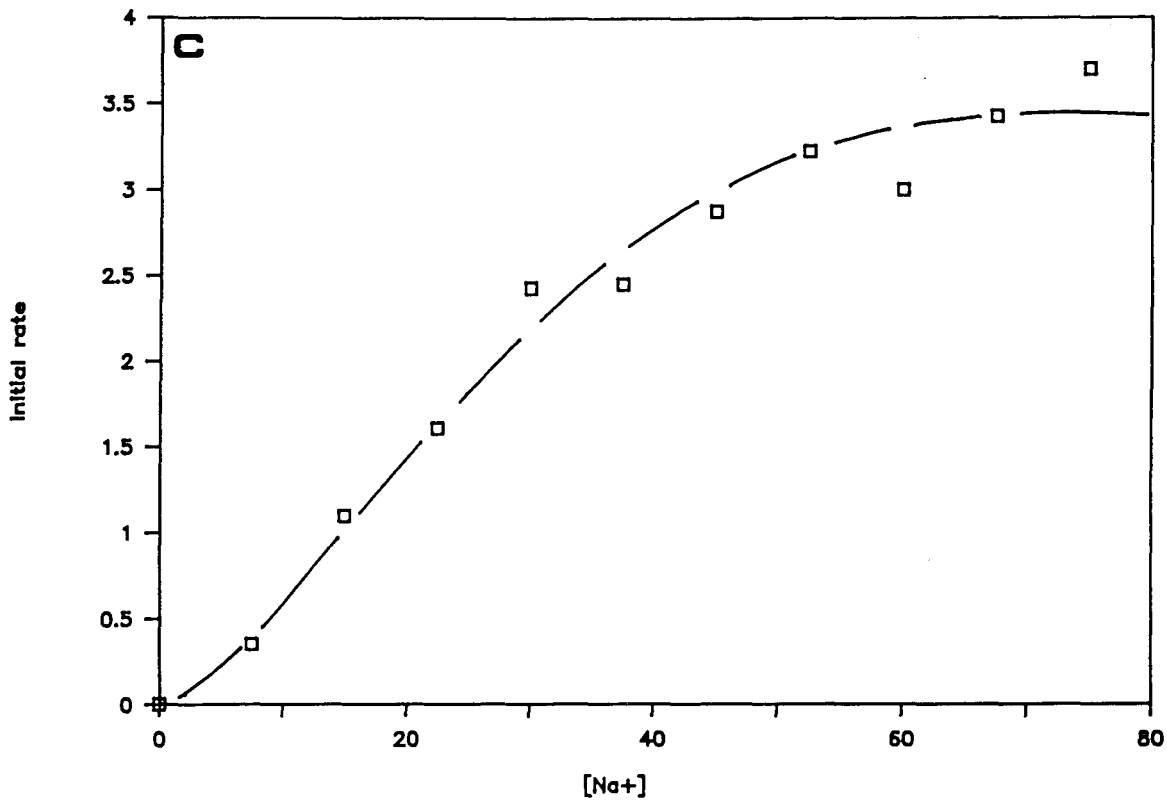


Figure 13: Michaelis-Menten plots for Callinectes sapidus males (C) and Callinectes sapidus females (D).



Vita

Nicholas J. Maiolo Jr. was born in Bridgeport, Connecticut in 1964. He attended grammar school in Stratford, Connecticut and graduated from Fairfield College Preparatory School in 1982. A Bachelor of Science degree was received in 1986 from University of Richmond, Va. He finished his Master's requirements at the University of Richmond, Va. in May, 1988. Future goals are to receive a Ph.D. and/or M.D. and ultimately pursue a career in research.