

OSMOTIC AND IONIC REGULATION IN SEVERAL WESTERN
ATLANTIC CALLIANASSIDAE (CRUSTACEA,
DECAPODA, THALASSINIDEA)¹

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Thalassinid mud shrimps of the genera *Callianassa* and *Upogebia* are frequently characterized as capable of ionic and volume regulation but incapable of osmotic regulation (Gross, 1957; Brown and Stein, 1960; Lockwood, 1962; Kinne, 1963). Studies by Zenkevich (1938), L. Thompson and Pritchard (1969), and Hill (1971), however, document osmoregulatory ability among upogebids. The assumed absence of this ability among callianassids is meanwhile supported by L. Thompson and Pritchard's (1969) studies of *Callianassa californiensis* and *C. filholi*. The recent report of strong ionic and osmotic regulation in *C. kraussi* from southern Africa (Forbes, 1974) constitutes the first evidence of such ability within the genus. However, other *Callianassa* species are also in some way adapted to low or varying salinities (Monod, 1927; Hedgpeth, 1950; Wass, 1955; Phillips, 1971; Rodrigues, 1971; LeLoeuff and Intes, 1974). Generalizations at the generic level must, therefore, await further physiological studies or perhaps be altogether abandoned until the systematic fate of the genus *Callianassa* Leach has been resolved; revisions proposed by de Saint Laurent (1973), for example, would partition *Callianassa* into six genera.

The present study compares osmotic adaptations of three species of Callianassidae from Louisiana and correlates these adaptations to local distributions. Specifically, salinity tolerance, osmotic regulation, and ionic regulation are reported. Despite the wide distribution of the species concerned, their trophic significance (Frankenberg, Coles, and Johannes, 1967), their potential as bait fisheries (Hailstone and Stephenson, 1961; Bybee, 1969), and the value of mud shrimp burrows in interpreting ancient environments (Weimer and Hoyt, 1964; Dewindt, 1974), basic understanding of their salinity tolerances and regulatory capacities is lacking.

Species concerned in the present study are *Callianassa jamaicense* Schmitt, 1935, *C. major* Say, 1818, and *C. islagrande* Schmitt, 1935, all of which fall within the subgenus *Callichirus* Stimpson, 1866. In a study of western African thalassinids, LeLoeuff and Intes (1974) note that *Callichirus* is frequently euryhaline and typically restricted to littoral waters in tropical latitudes. Habitats of *Callianassa* on the Louisiana coast are poorly documented, except in observations made on several coastal islands by Willis (1942); he notes predominance of *C. islagrande* on front beaches, interspersions of *C. islagrande* and *C. major* on ends of islands,

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and predominance of *C. jamaicense* on back sides of islands and in backbeach pools. Distributions are largely attributed to sediment characteristics as in a later study of *C. islagrande* and *C. jamaicense* on the Mississippi coast (Phillips, 1971).

North Atlantic coastal habitats of *C. major* are described by Lunz (1937), Pohl (1946), Weimer and Hoyt (1964), and Frankenberg *et al.* (1967); limited colonization of estuary mouths is noted, and *C. major* is usually reported from higher-salinity open beaches. Rodrigues (1971) suggests some tolerance to variations in salinity by *C. major* in Brazil but reports *C. jamaicense* to survive at the mouth of the Rio Caravelas. Hedgpeth (1950) notes *C. jamaicense* to inhabit estuarine mud flats on the Texas coast. Wass (1955) reports *C. jamaicense* from estuaries in northwestern Florida but lists *C. islagrande* only from the higher-salinity intertidal zone of Gulf beaches.

MATERIALS AND METHODS

Studies were conducted from January, 1972, to December, 1974. Initially, distributional records were supplemented by collecting callianassids from all accessible localities. Collecting techniques included shoveling and sieving, coring with a "yabby pump" (Hailstone and Stephenson, 1961), and using a portable water jet to obtain specimens, much as described by Bybee (1969). Except for some *C. islagrande* taken by shovel and sieve, animals for experimental studies were collected by the water jet method as it was the most productive and least injurious to animals.

All specimens of *C. jamaicense* used in experimental studies were collected from the perimeter of a tidally influenced pond near the Louisiana Wildlife and Fisheries Commission Marine Laboratory on Grand Terre Island. To prevent injury to animals, each was placed into a perforated, plastic vial. An insulated ice chest containing water from the collecting site was used to transport animals to the laboratory.

Animals were maintained unfed in individual, perforated vials throughout acclimation periods. Early in the study, free-swimming animals were held in sea water (SW) without isolation, and over 90% of 140 *C. jamaicense* perished within two days of collection. Aggressive encounters between individuals in a common container resulted in mutilation and consequent bleeding which accounted for high mortality.

Within two days of collection, after water in the ice chest had equilibrated to room temperature ($25 \pm 1^\circ \text{C}$), the animals and vials were transferred to artificial SW equivalent ($\pm 1\text{‰}$ salinity) to that from the pond. Two to three days were then allowed for attrition of animals injured during collecting. Ovigerous females, injured animals, and animals showing postmolt characteristics detailed by L. Thompson and Pritchard (1969) were not used in experimental studies.

Acclimation solutions were prepared by dilution of artificial SW with deionized water. Salinities were approximated with a refractometer. Animals were acclimated stepwise in 5‰ increments or decrements per day in the dark at 25°C with continuous aeration. Animals were maintained at the final acclimation salinity for nine days before blood was sampled.

One group of *C. jamaicense* was acclimated to 20‰ for nine days after which half were weighed and transferred directly to 3‰; the rest were weighed and transferred to 37‰. Animals were rinsed with deionized water and thoroughly blotted dry before being weighed to the nearest milligram. Five individuals were removed from each salinity extreme at timed intervals, rinsed, blotted and reweighed; blood was then sampled and the animals were lyophilized to constant weight. The same rinsing and blotting procedures were followed with all animals from which blood was sampled.

Blood was obtained by puncturing the arthrodistal membrane just posterior to the coxa of the fifth pereopod; 20 μ l were drawn for determination of osmotic concentration and another 20 μ l were immediately diluted for ion analyses; squeezing of animals was avoided. Osmotic concentrations (mOsmol/Kg H₂O) of whole blood and acclimation media were determined with a Hewlett-Packard vapor pressure osmometer. An Aminco chloride titrator was used for chloride analyses. Sodium was determined with a Coleman flame photometer and magnesium with a Perkin-Elmer atomic absorption spectrophotometer.

Individuals of *C. major* used for experimental studies were collected from Grand Isle and Grand Terre Island and those of *C. islagrande* were taken from Isles Dernieres and Chenier Caminada. Acclimation of these species to salinities below 15‰ was in some cases attempted in 2.5‰ steps. Collecting, acclimating, blood sampling, and analysis techniques were otherwise as described for *C. jamaicense*. Direct transfers into 3 and 37‰ media were not attempted with *C. major* or *C. islagrande*.

RESULTS

Distributions

As noted by Willis (1942) and Phillips (1971), distributions of the species studied are in part determined by substrate characteristics. *Callianassa jamaicense* is found most often in muddy substrates of back-beach ponds, estuarine flats, and tidal streams. Sandier substrates of beaches facing the open Gulf are the usual habitat of *C. major* and *C. islagrande*. However, lower salinities also typify most habitats of *C. jamaicense*; its distribution in Louisiana extends to well inside the 5‰ isohaline (Chabreck, 1972). Dense populations, with burrows exceeding 200/m², are found at 2 to 3‰ salinities near Johnson's Bayou and at 5 to 7‰ in the Lafourche Delta. On Grand Terre Island *C. jamaicense* occurs in salinities which vary seasonally from 6 to 28‰, and habitat includes bayward margins of Barataria Pass. Salinities at Barataria Pass commonly change by 10 to 15‰ over a period of a few hours (Hewatt, 1951).

By contrast, *C. major* and *C. islagrande* occur only in areas outside the 15‰ August isohalines of Chabreck (1972) and *C. islagrande* rarely occurs inside the 20‰ isohaline. *Callianassa islagrande* is the only callianassid found on Isles Dernieres front beaches which are bathed by high salinity coastal waters. Both *C. major* and *C. islagrande* are found on front beaches of Chenier Caminada and Grand Isle, but *C. major* predominates on the eastern portion of Grand Isle where salinities are less stable. On Grande Terre front beaches, which are inside the

20‰ isohaline (Chabreck, 1972), *C. major* is abundant and *C. islagrande* is uncommon.

During May, 1975, a mixed population of *C. major* and *C. islagrande* on Grand Isle was bathed by low salinity water (~ 7.0 ‰) for at least four days. Following the low salinities, numerous identifiable decomposing fragments of *C. islagrande* were found, but only *C. major* was found alive. Salinity of water issuing from *C. major* burrows ranged from 12 to 14‰. Of 40 *C. major* collected here half were held at a salinity of 7‰ and half were placed in artificial SW of 15‰ salinity. Those held at 7‰ were dead within two days, while most of those in 15‰ lived more than two weeks. Apparently, substrate interstitial water may adequately buffer *C. major* from some low overlying salinities, while *C. islagrande* succumbs under the same conditions. Populations of *C. islagrande* likely undergo mass mortalities where waters bathing beaches are subject to occasional extended periods of low salinity, as may be brought about by heavy rainfall, high rates of discharge from the Mississippi River, and the influence of winds and tides on water movement (Hewatt, 1951). The least vulnerable populations of *C. islagrande* are probably those on the front beaches of Isles Dernieres, Timbalier Island, and the Chandeleur Islands where salinity rarely falls to low levels.

Mortality, acclimation, and lower lethal limits

All experiments were completed within two to three weeks after animals were collected. Of the three species, *C. jamaicensis* proved most hardy in the laboratory during and beyond this period, provided animals were isolated in individual vials. Mortality of *C. major* and *C. islagrande* during the first two to three days after collection ranged from 7 to 10%, probably from injuries during collection; during two to three weeks thereafter attrition ranged from 2 to 4% per week. Mortality of *C. jamaicensis* seldom exceeded 2% during the first two to three days and

TABLE I

Survival during attempts to acclimate C. jamaicensis, C. major, and C. islagrande to low salinities.

Species	Salinity transfer (‰)			Number at Start	Number surviving at final salinity		
	From	To	Step/day		Day 1	Day 3	Day 9
<i>C. jamaicensis</i>	2.0	0.0	2.0	5	5	3	0
	15.0	2.0	5.0 and 3.0	10	10	9	9
	15.0	2.5	5.0 and 2.5	8	8	8	8
	20.0	3.0	17.0	50	50	50	50
	15.0	5.0	5.0	10	10	10	9
<i>C. major</i>	15.0	5.0	5.0	6	5	1	0
	15.0	7.5	5.0 and 2.5	12	9	4	0
	15.0	8.0	5.0 and 2.0	7	6	2	2
	15.0	10.0	2.5	20	20	15	14
<i>C. islagrande</i>	20.0	5.0	5.0	7	6	1*	1*
	20.0	10.0	5.0	7	5	2	1*

* Juvenile

TABLE II

Changes in wet weight during attempts to acclimate *C. major* and *C. islagrande* to 5 and 10‰ media. (Per cent difference from original wet weight is given as mean \pm standard error when more than one animal survived; numbers in parentheses for mortalities indicate numbers of animals surviving at the end of each day.)

Species	Salinity transfer (‰)		Number of animals	Difference (%) from original wet weight				
	From	To		1 day	2 days	3 days	4 days	9 days
Mortalities:								
<i>C. major</i>	25	10	2	9.7 \pm 0.8 (2)	15.3 \pm 4.6 (2)	20.9 (1)	(0)	—
	10	5	5	15.5 \pm 1.3 (5)	21.4 \pm 3.0 (5)	21.2 (1)	(0)	—
<i>C. islagrande</i>	15	10	4	14.4 \pm 1.4 (4)	17.7 \pm 1.8 (3)	23.5 \pm 4.5 (2)	31.2 (1)	(0)
	10	5	5	37.6 \pm 3.5 (5)	42.6 (1)	(0)	—	—
Survivors:								
<i>C. major</i>	15	10	5	14.7 \pm 1.5	10.9 \pm 1.7	9.3 \pm 1.2	4.8 \pm 1.5	3.2 \pm 0.7
<i>C. islagrande</i>	15	10	1*	13.3	9.0	2.7	0.4	0.4
	10	5	1*	16.9	12.3	11.9	5.6	3.2

* Juvenile

averaged $< 1\%$ per week under stable conditions for up to one month thereafter.

Of 180 *C. jamaicense* specimens collected in March and April, 1974, isolated in vials, and held unfed in static aerated aquaria at $25 \pm 1^\circ \text{C}$, $> 80\%$ were still alive in November. Under similar conditions, $< 40\%$ of the *C. major* and 17% of the *C. islagrande* specimens survived beyond two months.

Lower and upper lethal limits of salinity were apparently not reached when *C. jamaicense* was acclimated to salinities from 2 to 45‰; mortalities were no more pronounced at extremes of salinity than at midrange salinities. Three specimens, transferred to deionized water after acclimation to 2‰ and sampling of blood, survived in excess of five days (Table I). No mortalities occurred during nine days after direct transfer of 50 *C. jamaicense* specimens from 20 to 3‰, and only one animal died during nine days after direct transfer of another 50 from 20 to 37‰.

The lower lethal salinity for *C. major* was attained just below 10‰ in several acclimation attempts (Table I). Although specimens were acclimated to 10‰ on several occasions, mortalities during nine days at the final salinity exceeded 25%; most deaths occurred during the first three days after the step from 12.5 to 10‰. Mortalities for *C. major* during acclimation to salinities from 12.5 to 40‰ did not exceed 10%. In an attempt to acclimate five specimens to 45‰, all animals died between the seventh and eighth days after transfer from 40‰.

Few specimens of *C. islagrande* were available for experimental studies and tolerance data are preliminary. Mortality was less than 10% during acclimation to salinities from 20 to 45‰. Below 20‰, acclimation was much less successful. Although one *C. islagrande* juvenile survived nine days at 5‰, and another survived nine days at 10‰, all attempts to acclimate adults to salinities $\leq 10\%$ resulted in 100% mortalities within five days (Table I).

Weight changes were monitored during acclimation of some *C. islagrande* and *C. major* specimens to low salinities. Mortalities were preceded by substantial increases in wet weights, which suggests inability to regulate volume (Table II). Moribund animals under low-salinity stress had turgid abdomens

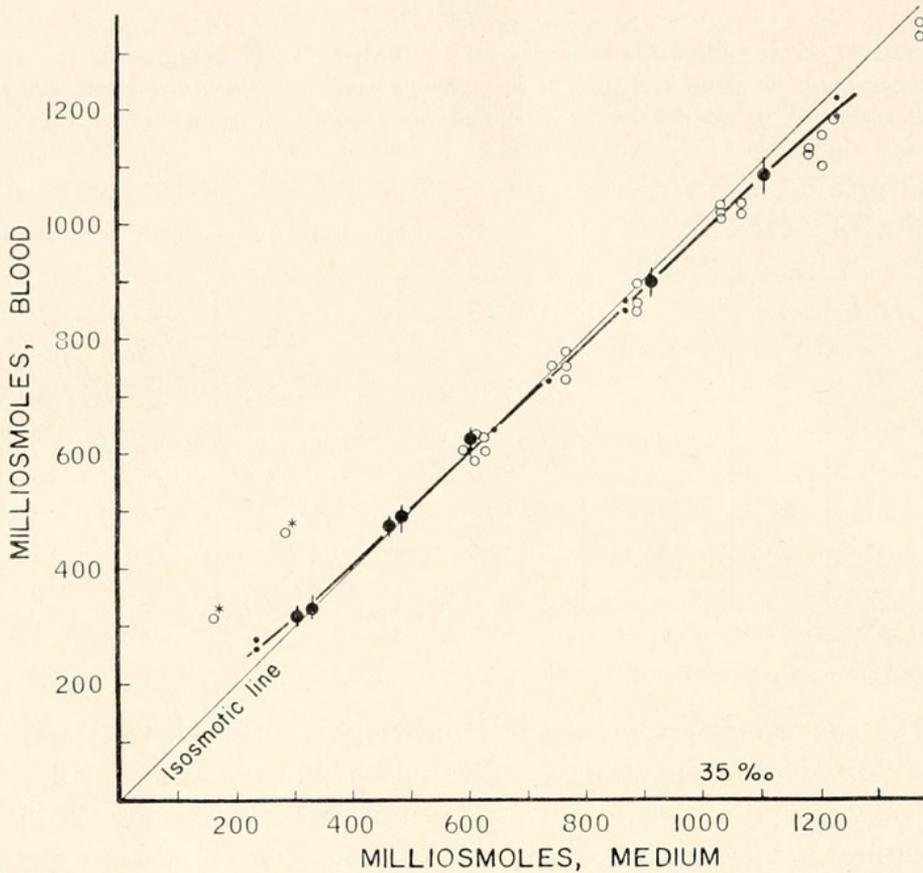


FIGURE 1. Blood osmotic concentration in acclimated *C. major* (solid circles) and *C. islagrande* (open circles) as a function of media osmotic concentrations. Each large solid circle is mean of 6 to 10 determinations; vertical lines indicate range; open circles and small solid circles are individual determinations. Heavy line is fitted to points for *C. major*. Asterisk denotes juveniles.

and branchiostegites which, by restricting movement and ventilation, probably caused respiratory stress. Those which survived low-salinity acclimation increased in weight initially, but began to regulate volume within three days; by the fourth day to ninth day of acclimation, wet weights returned to near original values.

Osmotic and ionic regulation

Blood of *C. major* and *C. islagrande* is nearly isosmotic to media over the entire salinity range in which these animals survive (Fig. 1). Slightly hyperosmotic values obtained for *C. major* at 8‰ salinity represent a low percentage of animals which survived at that extreme. Likewise, hyperosmotic values for *C. islagrande* at low salinities are from two juveniles which survived while nine adults died at these salinities.

Blood of *C. jamaicense* is hyperosmotically regulated at salinities $\leq 20‰$ and shows little depression of osmolality at the lowest salinity extreme of 2‰ (Fig. 2). Slightly higher levels of hyperosmicity are maintained by January animals collected from a field salinity of 11‰ and temperature of 8° C than by August animals collected from 23‰ and 33° C. Blood is isosmotic to most media salinities $\geq 25‰$ and very slightly hyposmotic at the upper-salinity extreme of 45‰.

Blood chloride is hypoionic in *C. major* and adult *C. islagrande* over the entire range of acclimation salinities, though less so at lower salinities (Fig. 3). Blood chloride was hyperionic in the two juveniles of *C. islagrande* which survived acclimation to 5 and 10‰ salinities. In *C. jamaicense* blood chloride is hypoionically regulated at salinities ≥ 20 ‰, isoionic at 15‰, and hyperionic at salinities < 15 ‰ (Fig. 3).

At salinities ≤ 20 ‰ blood sodium in *C. major* is isoionic to acclimation media (Fig. 4). Hypoionic regulation of sodium is exhibited at higher salinities, but not to levels as low as chloride. As with osmolality and chloride, sodium is hyper-regulated in the *C. islagrande* juveniles surviving low-salinity acclimation (Fig. 4). In *C. islagrande* adults acclimated to salinities from 25 to 45‰ blood sodium is hypoionically regulated to levels approximating those for *C. major*. Sodium levels in acclimated *C. jamaicense* are slightly hypoionic to media at salinities ≥ 25 ‰, near isoionic at 20‰, and markedly hyperionic at lower salinities from 15 to 2‰ (Fig. 5).

Blood magnesium in acclimated *C. major* is to some degree hyper-regulated at salinities ≤ 30 ‰ (Fig. 6); blood concentrations are maintained at about 6.0 mM/liter higher than media concentrations in salinities from 8 to 20‰. Hyper-regulation of magnesium is diminished at 30‰ and concentrations fall to slightly hypoionic levels at 37‰. In acclimated *C. jamaicense*, magnesium is slightly hyper-regulated

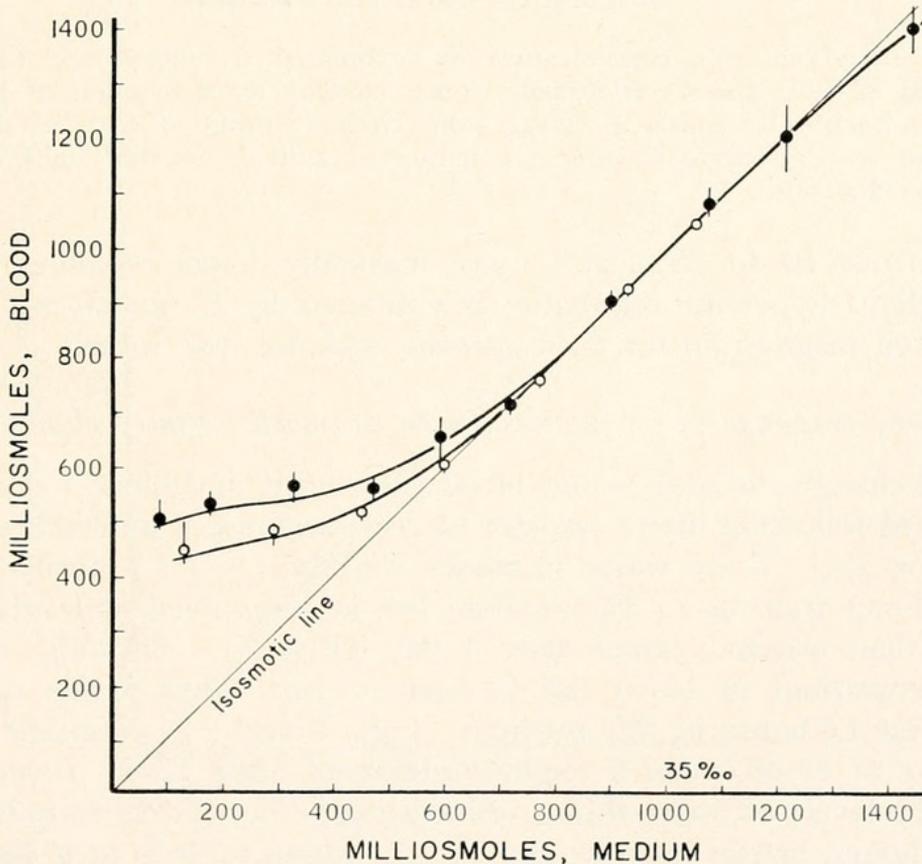


FIGURE 2. Blood osmotic concentration in *C. jamaicense* as a function of media osmotic concentrations after acclimation of summer (open circles) and winter (solid circles) animals at a media temperature of 25° C. Each open circle is mean of 5 to 6 determinations, and each solid circle is mean of 8 to 10 determinations; vertical lines indicate range.

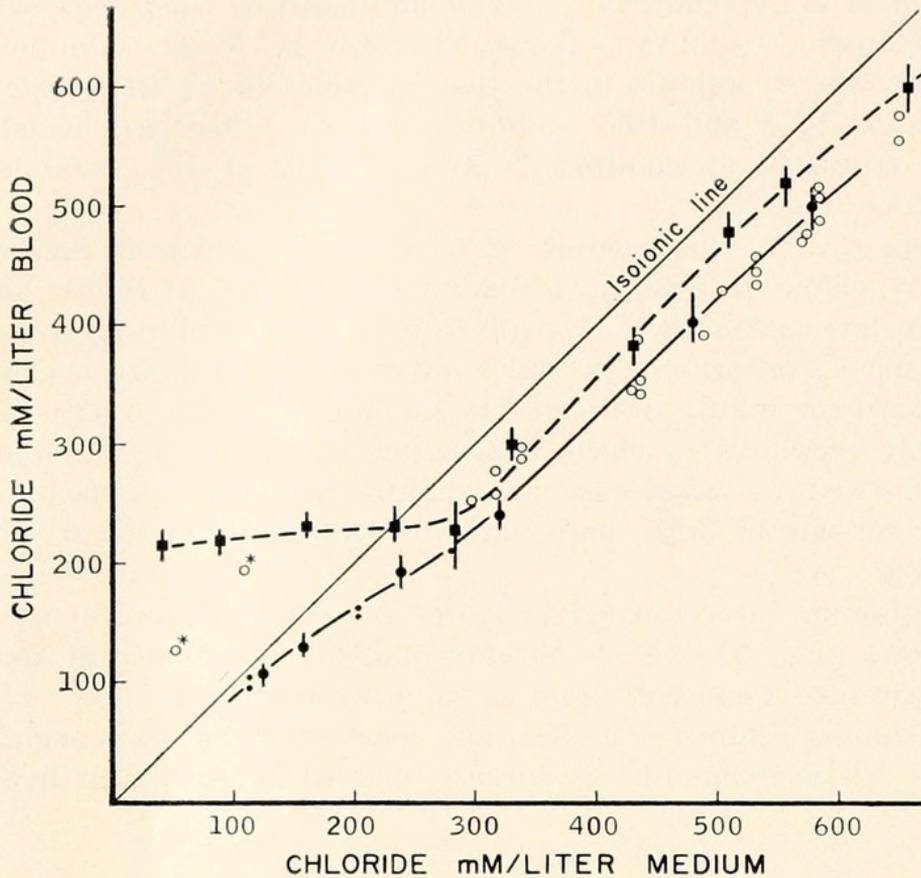


FIGURE 3. Blood chloride concentration in acclimated *C. jamaicense* (solid squares), *C. major* (solid circles) and *C. islagrande* (open circles) as a function of media chloride concentrations. Each solid square or large solid circle is mean of 6 to 10 determinations; each small solid or open circle is value for individual animal; vertical lines indicate range. Asterisk denotes juveniles.

at salinities from 10 to 25‰ and more markedly hyper-regulated below 10‰ (Fig. 6). Slight hypoionic regulation is exhibited by *C. jamaicense* at salinities ≥ 35 ‰. Blood magnesium for *C. islagrande* was not determined.

Osmoregulatory response of C. jamaicense to dramatic salinity changes

Temporal changes in body water, blood osmolality, and blood ion concentration were monitored following direct transfer of 20‰-acclimated animals to salinities of either 37‰ or 3‰. Body water increases slightly ($\sim 1\%$) during the first 12 hours after direct transfer to 3‰ medium but is maintained at levels equal to or slightly less than original values after 1 day (Fig. 7). Osmotic, chloride, and sodium concentrations of blood fall to near or just below stable concentrations during the first 12 hours in 3‰ medium (Figs. 8 and 9). Osmotic and sodium concentrations of blood show a slight undershoot after 12–24 hours, but at no time fall to the levels of the medium. Blood magnesium levels drop little over the first 6 to 12 hours, briefly recover, and then continue to drop at a decreasing rate over the entire nine day period (Fig. 10). Near stable levels of blood magnesium are achieved after four days, and concentrations are maintained above that of the 3‰ medium.

When *C. jamaicense* is directly transferred to 37‰ medium, body water de-

creases by $\sim 3\%$ over the first six hours and remains below original levels until the second day (Fig. 7). Osmotic, chloride, and sodium concentrations of blood increase markedly during the first day and continue to increase, at a decreasing rate, through day 9 (Figs. 8 and 9); near stable levels are attained by day 4. The levels of blood osmolality and sodium on day 9 approximate those of the 37‰ medium. Blood chloride remains slightly hypoionic to chloride concentrations of the medium through day 9. Blood magnesium increases slowly until the fourth day when it stabilizes at a level just below that of the medium (Fig. 10).

DISCUSSION

Investigations by Teal (1958), Snelling (1959), Kinne (1963), and Barnes (1967) are among those which correlate osmoregulatory capacities of decapod crustaceans to their differential penetration of estuaries. Distributions of callianassids on the Louisiana coast also correlate with their osmoregulatory capacities and tolerance of dilute media. This is not to say that habitat preference is solely or even primarily determined by salinities. For example, despite its survival in varying salinities, *Emerita talpoida* is localized on wave-washed beaches by its feeding specialization (Burse and Bonner, 1977). Devine (1966), Phillips (1971), and McLachlin and Grindley (1974) note the importance of substrate stability and composition in limiting distributions of burrowing thalassinids. How-

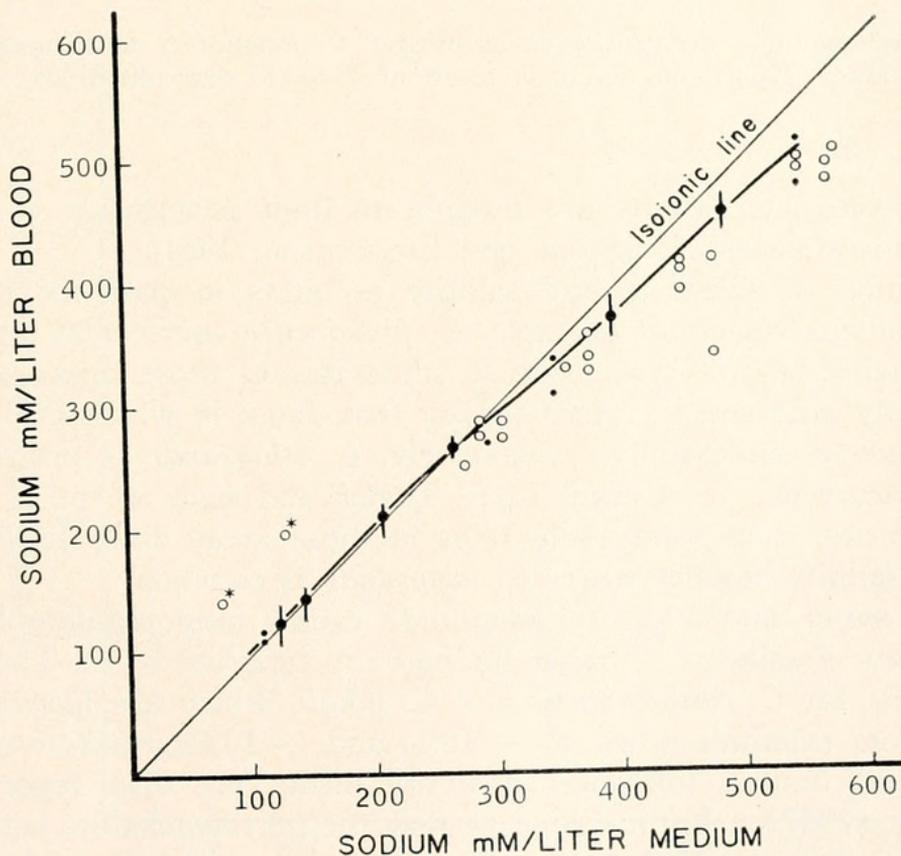


FIGURE 4. Blood sodium concentration in acclimated *C. major* (solid circles) and *C. islagrande* (open circles) as a function of media sodium concentrations. Each large solid circle is mean of 6 to 9 determinations; each small solid or open circle is value for individual animal; vertical lines indicate range. Heavy line is fitted to points for *C. major*. Asterisk denotes juveniles.

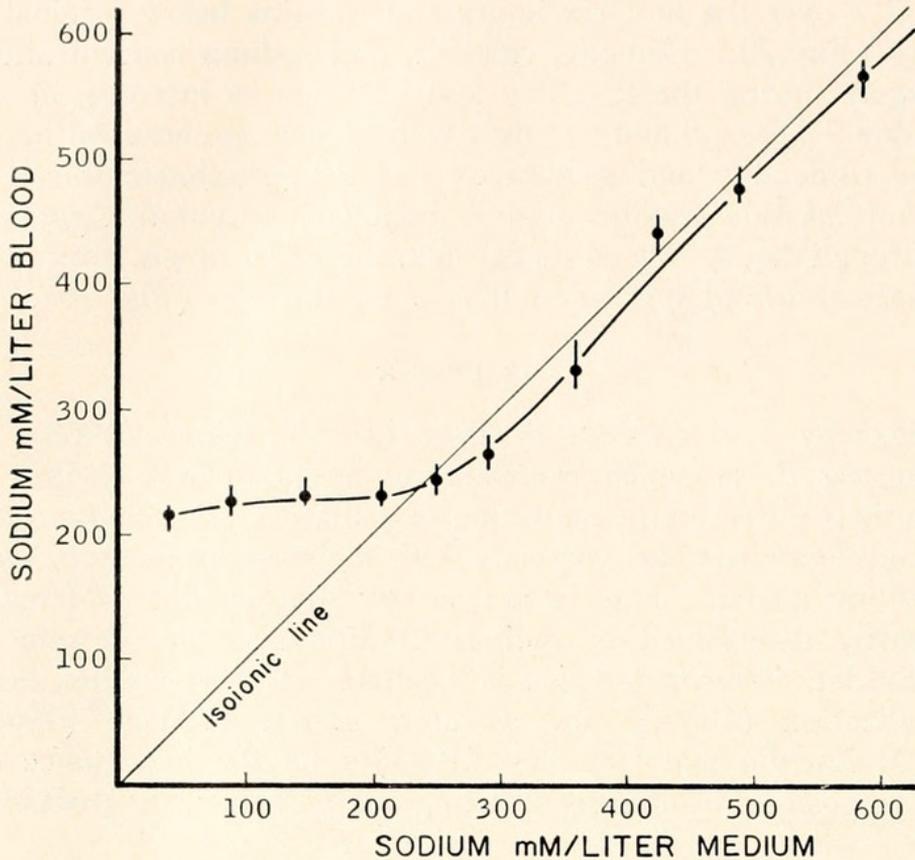


FIGURE 5. Blood sodium concentration in acclimated *C. jamaicense* as a function of media sodium concentrations. Each solid circle is mean of 7 to 10 determinations; vertical lines indicate range.

ever, both substrate and salinity are thought to limit penetration of *Callinassa australiensis* into estuaries (Hailstone and Stephenson, 1961).

The interaction of substrate and salinity accounts in part for distributions of Louisiana Callianassidae; for example, *C. jamaicense* survives at high salinities but is seldom taken above 25‰, because substrates in those areas of the coast are predominantly sand and therefore coarser than those in which Phillips (1971) reports it to burrow successfully. Conversely, *C. islagrande* is probably limited to transitory occurrence on Grand Terre Island and ends of other islands by fluctuating salinities, since sandy substrates in those areas differ little from substrates of high salinity beaches where *C. islagrande* is common.

Callinassa major and adult *C. islagrande* cannot osmoregulate but tolerate limited reductions of salinity. Similar findings are reported by L. Thompson and Pritchard (1969) for *C. californiensis* and *C. filholi*, which are likewise poikilosmotic but tolerate salinities down to ~10‰ and ~13‰, respectively. Osmoconformation and limited tolerance of dilute media are also reported for *C. affinis* by Gross (1957). It thus appears that the polystenohaline categorization, which was prematurely applied in general to *Callinassa* and *Upogebia* by earlier workers (Lockwood, 1962; Kinne, 1963), may be retained for at least five species of Callianassidae and probably for others which occupy similar habitats. However, some of these species are less stenohaline than others; the ability of *Callinassa major* to tolerate acclimation to 10‰ salinities while *C. islagrande* usually dies at

this salinity in part explains more frequent occurrence of *C. major* inside the 20‰ isohaline and its predominance on ends of coastal islands where salinities occasionally fluctuate. Preliminary evidence of low-salinity tolerance and slight hyperosmotic regulation in juveniles of *C. islagrande* suggests an ontogenic loss of tolerance and regulatory ability, although the two juveniles studied furnish an insufficient sample for firm conclusions. Juveniles of the hermit crab, *Pagurus bernhardus*, regulate volume in lower salinities than adults, and Davenport (1972a) suggests that the aperture of the nephropores in relation to body size limits this capacity in adults.

Tolerance of dilute media by *Callianassa major* and *C. islagrande* may prove of only short-term benefit for survival of populations. Hill (1971) notes that while *Upogebia africana* can tolerate a salinity of 1.7‰, it can only survive through a molt in a salinity ≥ 3.4 ‰. Although *C. major* and adult *C. islagrande* do not appear to osmoregulate (Fig. 1), their tolerance of dilute media may in part relate to accommodation of short-term increases in blood volume. The anterior portion of the abdomen is soft, and its elasticity may minimize mechanical effects of turgor. Davenport (1972b) suggests such an adaptation in *Pagurus bernhardus* and shows that with increased blood volume in low salinity, a larger proportion of the blood shifts from the thorax to the abdomen.

The degree of hypoionicity in blood chloride of *Callianassa major* and adult

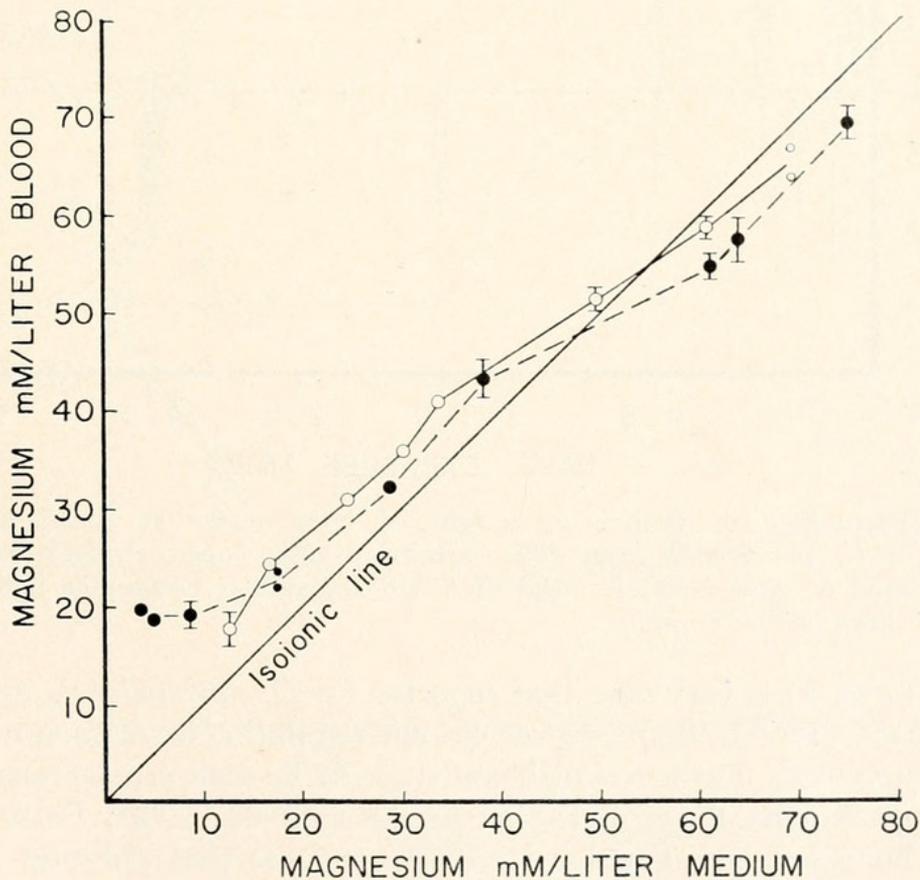


FIGURE 6. Blood magnesium concentration in acclimated *C. jamaicense* (solid circles) and *C. major* (open circles) as a function of media magnesium concentrations. Each large solid or open circle is mean of 6 to 10 determinations; vertical lines indicate standard errors where they exceed ± 1.0 ; small solid or open circles are individual determinations.

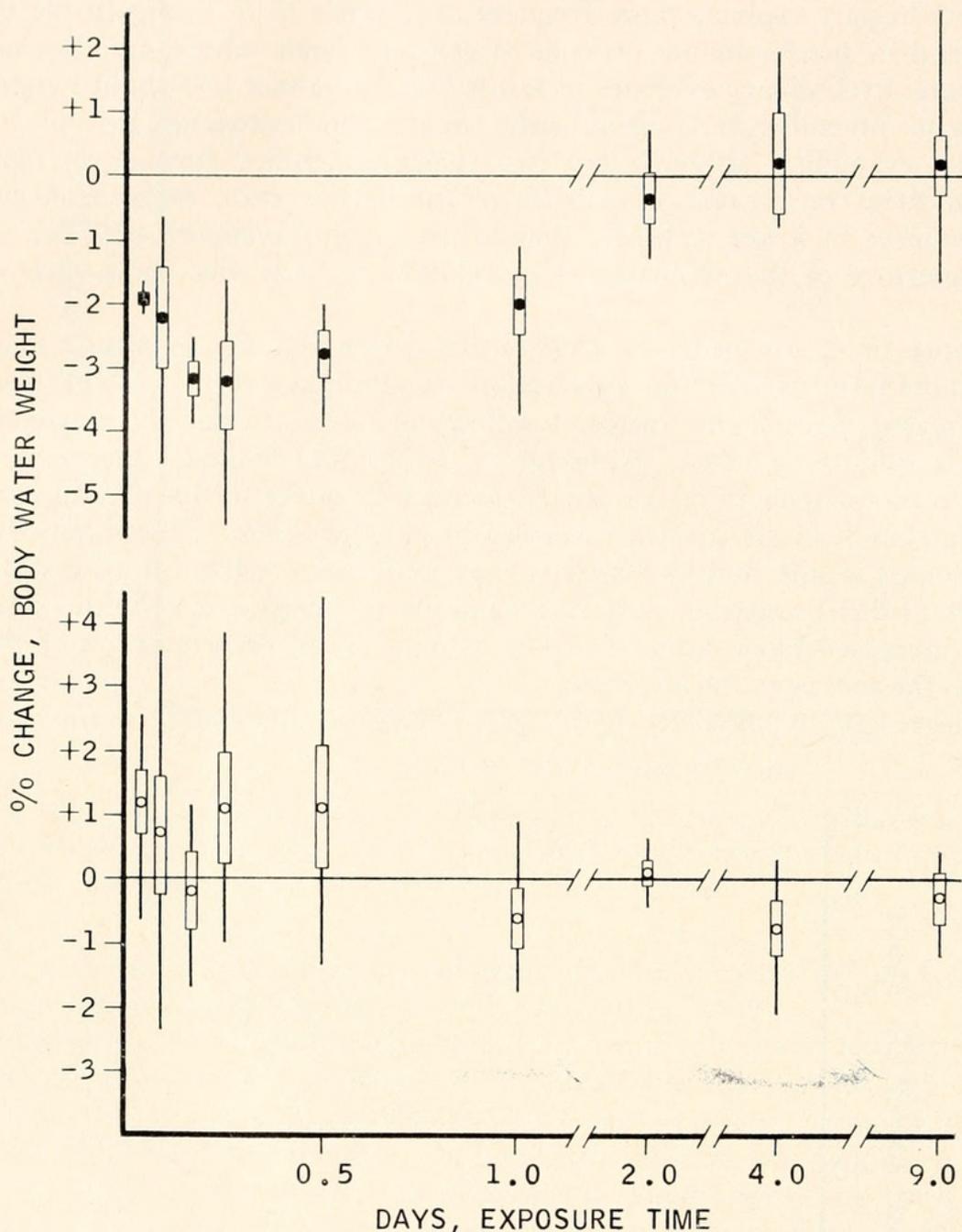


FIGURE 7. Percentage of change in weight of body water at timed intervals after direct transfer of *C. jamaicense* from 20‰ salinity to 3‰ (open circles) or 37‰ (solid circles). Each solid or open circle is mean of 5 determinations; rectangles indicate standard errors; vertical lines indicate range.

C. islagrande (Fig. 3) is very near that reported for *C. californiensis* by L. Thompson and Pritchard (1969). Some degree of ionic regulation is common to osmotically conforming crustaceans, but levels of blood chloride in such crustaceans are usually reported to approximate those of the media (Robertson, 1960; Potts and Parry, 1964). L. Thompson and Pritchard (1969) suggest that chloride hypoionicity may be attributable to a protein anionic component of blood in *C. californiensis*. However, as noted by Dall (1974), blood chloride is virtually equivalent to blood sodium at any given salinity despite the apparent difference when blood ion concentrations are plotted against media concentrations of the same ion. Hence,

where sodium and chloride in media are at normal SW ratios, sodium concentration being slightly less than that of chloride, equilibrium between the two ions is reflected in hypoionicity of chloride at any given medium concentration of chloride provided blood sodium is near or below sodium concentrations of the

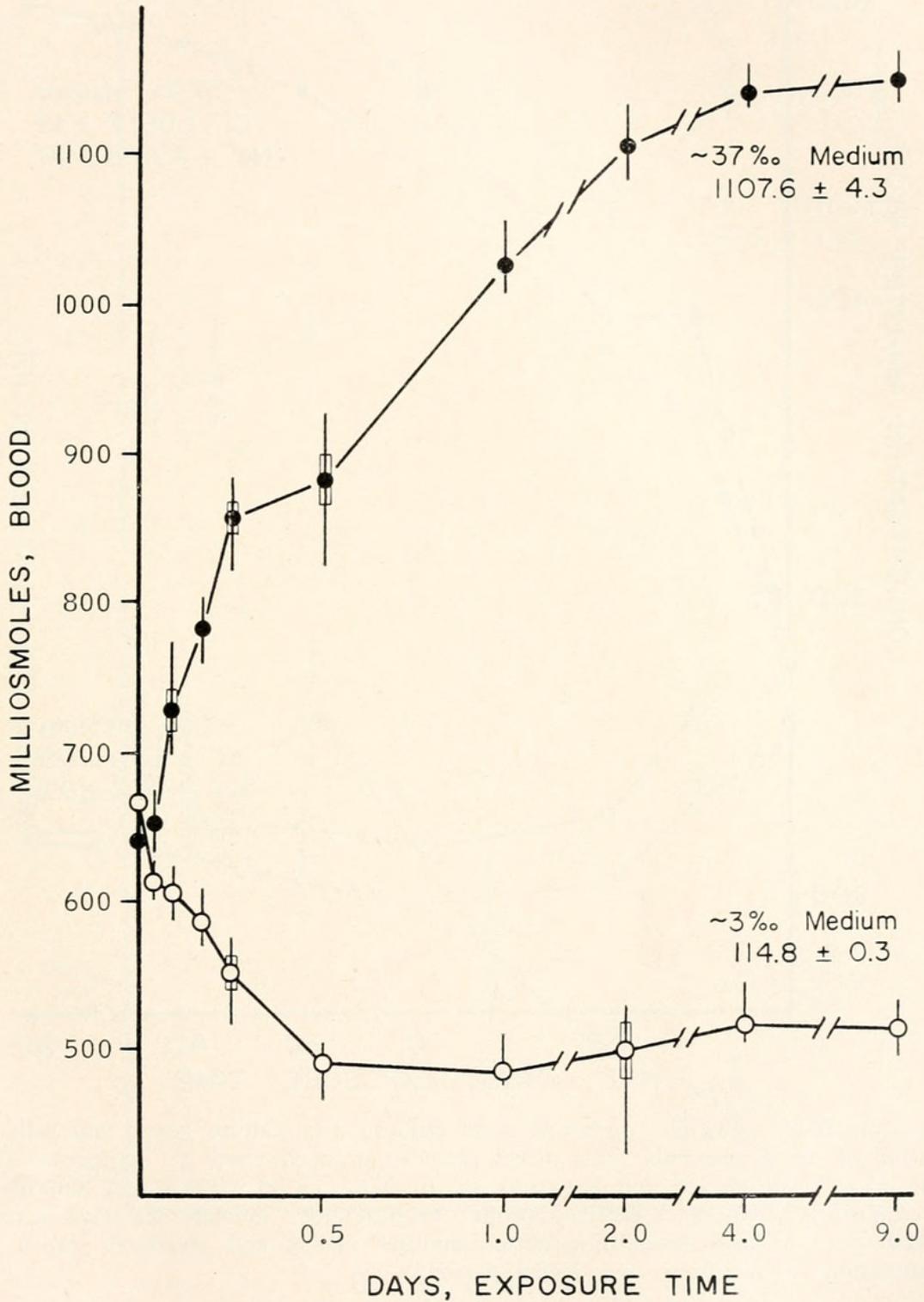


FIGURE 8. Blood osmotic concentration at timed intervals after direct transfer of acclimated *C. jamaicensis* from 20‰ salinity to 3‰ (open circles) or to 37‰ (solid circles). Each solid or open circle is mean of 5 determinations; vertical lines indicate range; rectangles indicate standard errors where they exceed ± 10 . Figures beneath salinities indicate means and standard errors of media osmotic concentrations over 9-day period.

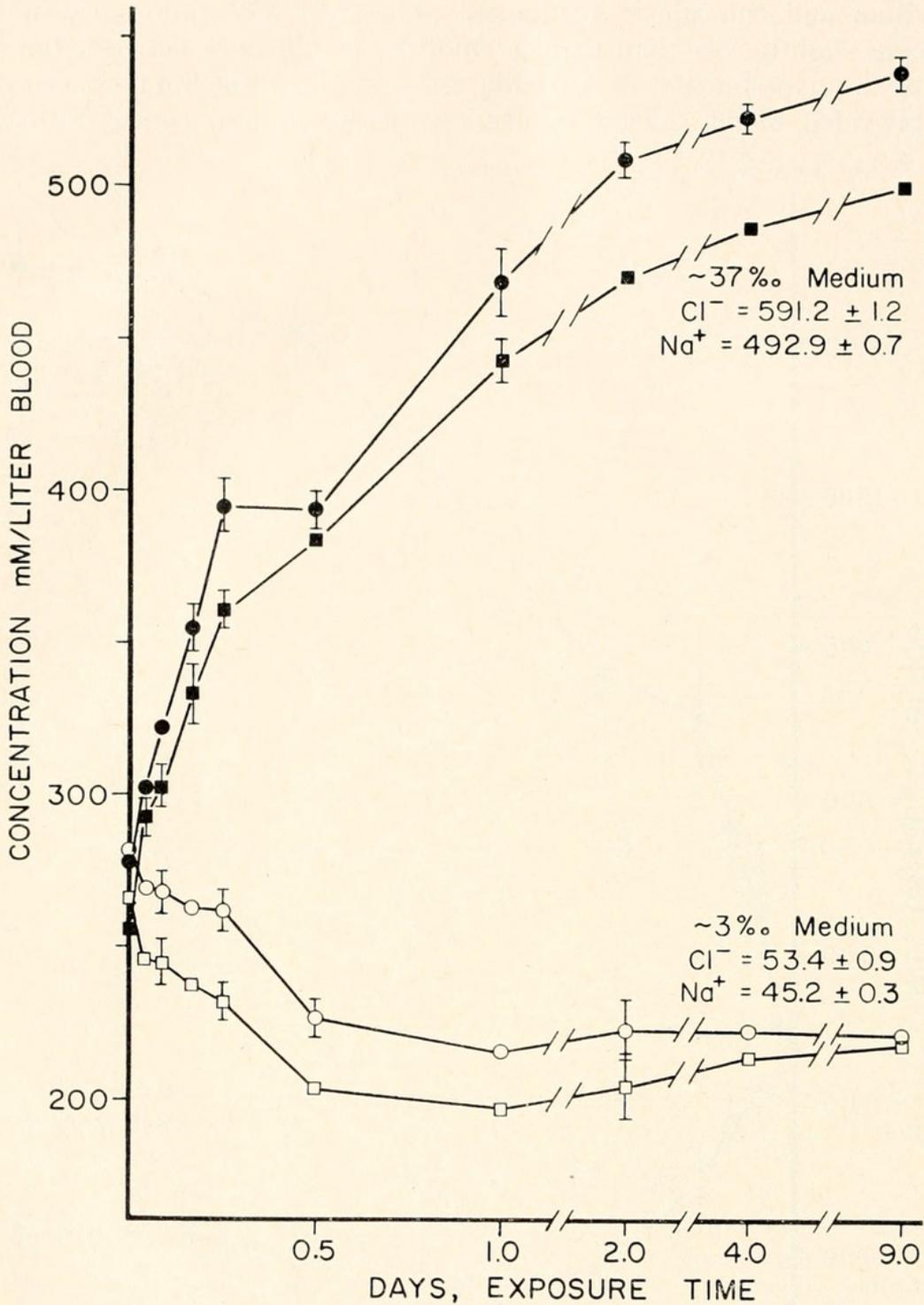


FIGURE 9. Blood chloride (open and solid circles) and sodium (open and solid squares) concentration at timed intervals after direct transfer of acclimated *C. jamaicensis* from 20‰ salinity to 3‰ (open circles and squares) or to 37‰ (solid circles and squares). Each circle or square is mean of 5 determinations; vertical lines indicate standard errors where they exceed ± 5 . Figures beneath salinities indicate means and standard errors of media ion concentrations in mm/liter over 9-day period.

medium. Blood chloride in *Callinassa* (Fig. 3) exceeds blood sodium concentrations (Figs. 4 and 5) at each acclimation salinity and the degree to which it does so increases with increasing salinity, probably in electrochemical response to increased concentrations of magnesium and other cations. Blood sodium in *C.*

major and adult *C. islagrande* is equivalent to media concentrations ≤ 300 mM/liter and, much as blood osmolality (Fig. 1), drops slightly below equilibrium at the upper extremes of salinity. Blood sodium and osmotic concentrations respond similarly in acclimations of *C. californiensis*, but both sodium and osmolality of

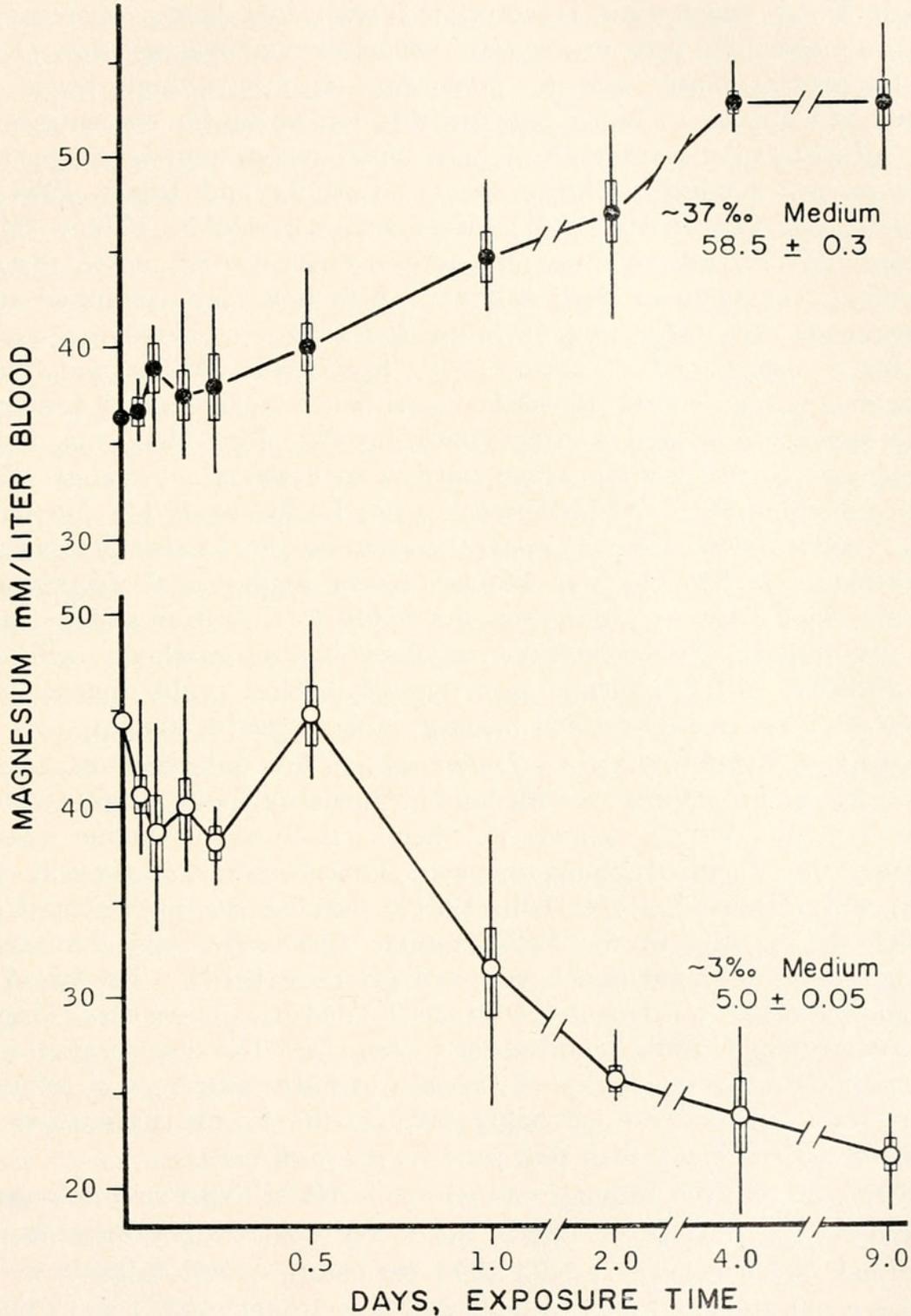


FIGURE 10. Blood magnesium concentration at timed intervals after direct transfer of acclimated *C. jamaicense* from 20‰ salinity to 3‰ (open circles) or to 37‰ (solid circles). Each solid or open circle is mean of 5 determinations; vertical lines indicate range; rectangles indicate standard errors. Figures beneath salinities indicate means and standard errors of media concentrations in mM/liter over 9-day period.

blood remain more nearly equivalent to media concentrations over the entire range of salinity (L. Thompson and Pritchard, 1969).

Most marine crustaceans strongly hyporegulate blood magnesium (Robertson, 1953). Exceptions to this rule include several brachyuran spider crabs, the primitive brachyuran, *Dromia vulgaris*, and the anomuran, *Lithodes maia*, in which relatively high blood magnesium is correlated with low levels of responsiveness attributed to magnesium interference with neuromuscular transmission (Robertson, 1960). *Callianassa major* and *C. jamaicense* also have high levels of blood magnesium and appear to hyper-regulate this ion at media concentrations < 50 mM/liter (Fig. 6). An advantage of high blood magnesium is suggested by its effects on oxygen binding in hemocyanins (Larimer and Riggs, 1964; Roxby, Miller, Blair, and Van Holde, 1974; Miller and Van Holde, 1974). Miller and Van Holde (1974) report a mean magnesium concentration of 48 mM/liter for *C. californiensis* at an unspecified salinity. Although high compared to that of most crustaceans, this value is well within the ranges of blood magnesium here reported for *C. major* and *C. jamaicense*. Specifically, Miller and Van Holde show that magnesium effects allosteric transitions in callianassid hemocyanin *in vitro*. By increasing oxygen binding (lowering P_{50}) high blood magnesium may be advantageous to thalassinids which burrow in hypoxic substrates. Such substrates are inhabited by *C. californiensis* on the Pacific coast (R. Thompson and Pritchard, 1969) and by the *Callianassa* species on the Louisiana coast (Felder, in preparation). Miller and Van Holde (1974) suggest that magnesium levels remain stable in *Callianassa*; this does not apply to *C. major* and *C. jamaicense* as blood magnesium, while somewhat regulated, varies markedly with changing salinity. Survival of these species in a hypoxic habitat could thus be influenced by interactions between salinity, ion balance, and oxygen availability.

Hyperosmotic regulation by *C. jamaicense* at low salinities, its tolerance of salinities $\leq 2\text{‰}$ and its ability to withstand abrupt changes in salinity with marked regulation of volume clearly support its categorization as euryhaline. Such capacities are well documented among upogebid Thalassinidea (Zenkevich, 1938; L. Thompson and Pritchard, 1969; Hill, 1971), but the South African *C. kraussi* is the only other species of the Callianassidae (*sensu de Saint Laurent, 1973*) in which hypersomotic regulation is reported (Forbes, 1974). The blood osmotic, sodium, and chloride concentrations in acclimated *C. jamaicense* (Figs. 2, 3, and 5) closely resemble those reported for *C. kraussi*. The deterioration of regulatory ability that Forbes reported in *C. kraussi* at lower extremes of salinity is not pronounced in *C. jamaicense*, probably because the lowest acclimation extreme used for *C. kraussi* is lower than that used for *C. jamaicense*.

The difference between summer and winter levels of hyperosmotic regulation in *C. jamaicense* (Fig. 2) likely reflects the lower field temperatures from which winter animals were collected; both field temperature and salinity were lower during winter collections. Lynch, Webb, and Van Engel (1973) and Charmantier (1975) list a number of studies documenting seasonal temperature effects upon blood osmotic and ionic concentrations in crustaceans. Higher blood osmotic and ionic concentrations occur in animals from colder water (Dehnel, 1962; Ballard and Abbott, 1969), even when, as in the present case, acclimations are

conducted at equivalent temperatures in the laboratory. Acclimation studies of *Callinectes sapidus* suggest that lower salinity could produce an effect opposite from that of low temperature, as blood osmotic concentration of acclimated crabs is lower when crabs are collected at low field salinity; however, in salinities $< 15\text{‰}$ blood osmotic concentrations of *Callinectes sapidus* depend little upon the direction from which the acclimation salinity is approached (Ballard and Abbott, 1969).

After direct transfers of *Callinassa jamaicense* from 20‰ , the animals placed into 3‰ regulate volume nearer original levels than do those placed into 37‰ , but in both cases volumes are near original levels after two days (Fig. 7). Limited data on weight changes of *C. major* and *C. islagrande* after less dramatic stepwise transfers to low salinities suggest much poorer volume control in those species (Table II). The means by which *C. jamaicense* controls volume and blood osmotic concentration is at present uncertain. Studies of urine in both hyperosmotically regulating (Forbes, 1974) and osmotically conforming (L. Thompson and Pritchard, 1969) Callianassidae show that, as in the great majority of euryhaline Crustacea (Potts and Parry, 1964), an isosmotic urine is produced by animals once acclimated to various salinities. However, urine volumes and osmolality are not reported during acclimation in either of these studies. Osmoregulatory functions of the antennal glands are suggested by increased urine volumes in the crab, *Carcinus maenas*, with decreased salinity (Binns, 1969) and by studies of the lobster, *Homarus americanus*, in which urine is near isosmotic to blood in animals fully acclimated to lowered salinity, but markedly hyposmotic during acclimation (Dall, 1970). Changes in permeability may also facilitate regulation of volume and blood osmolality, and such changes are documented in other euryhaline decapods subjected to dilute media (Capen, 1972; Spaargaren, 1975). Additionally, Heeg and Cannone (1966) describe an osmoregulatory diverticulum on the posterior mid-gut of grapsid crabs; a similar diverticulum is present in *Callinassa jamaicense*, *C. major*, and *C. islagrande*, although its function is unknown.

After direct transfer of *C. jamaicense* from 20‰ to 3‰ media, blood osmotic, chloride, and sodium concentrations are near new stable levels within 12 hours, but gradual changes in the sodium/chloride ratio continue to occur through day 9 (Figs. 8 and 9). Changes in blood osmotic and sodium concentrations of *C. jamaicense* are very nearly proportional over observed time increments after transfers to either 3‰ or 37‰ media. A similar close correlation between sodium and osmotic concentrations is reported in crustacean blood by other investigators (Colvocoresses, Lynch, and Webb, 1974) and such observations seem compatible with data indicating that the sodium transport system ultimately establishes the blood osmolality (Shaw, 1960). By day 9 after direct transfers, blood sodium/chloride ratios in *C. jamaicense* are higher at 3‰ than at 37‰ salinity. A similarly elevated sodium/chloride ratio is also observed after *C. kraussi* is acclimated to low salinity (Forbes, 1974).

Blood magnesium concentrations approach stable levels less rapidly than other ions after salinity transfers (Fig. 10). This may contribute to what Forbes (1974) describes as slower, smaller changes in blood osmotic concentrations after stabilization of blood sodium and chloride concentrations following salinity transfers of *C. kraussi*; Forbes (p. 310) speculates such changes could be asso-

ciated with "non-ionic osmotically active entities in the blood," but does not report divalent ion concentrations.

Evolution of hyperosmotic regulation in *C. kraussi* is attributed to the unique flood-influenced salinity gradient in southern African estuaries (Forbes, 1974); similar conditions occur in other areas including coastal estuaries of the Northern Gulf of Mexico (Hewatt, 1951; Barrett, Tarver, Latapie, Pollard, Mock, Adkins, Gaidry, White, and Mathis, 1971). Euryhalinity may be characteristic of a phyletic stock, rather than of an isolated species or genus and probably is a very conservative physiological adaptation once acquired (Hedgpeth, 1957); Ortmann (1902) furnishes examples of such phyletic stocks among crustaceans for the now freshwater Atyidae and the Palaemonidae which occur in marine, estuarine, and freshwater habitats. Since *Callianassa kraussi* and *C. jamaicense* share the conservative character of euryhalinity, further examination of their phylogenetic proximity may prove interesting. However, phylogenetic interpretations must be made with caution; osmoregulatory ability may be a conservative trait once acquired, but it could have been acquired independently following separation of ancestral stocks. Lockwood and Croghan (1957) suggest that only 700 years were required for development of a separate race of Baltic isopods which now possesses distinctly greater powers of osmotic and ionic regulation than its ancestral stocks.

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SUMMARY

Osmotic and ionic regulatory capacities of callianassid mud shrimps, *Callianassa jamaicense*, *C. major*, and *C. islagrande*, are correlated to their distributions on the Louisiana coast. *Callianassa jamaicense* burrows in muddy estuaries where salinity may commonly fall to $< 5\text{‰}$, but *C. major* and *C. islagrande* usually burrow in sandy beaches bathed by higher salinities. Lower lethal limits of salinity are $< 2\text{‰}$ for *C. jamaicense*, 7–8‰ for *C. major* and probably just below 15‰ for adult *C. islagrande*. After exposure to low salinity *C. jamaicense* exhibits better volume control than the other two species. Blood osmotic, sodium, and chloride concentrations in *C. jamaicense* are regulated near stable levels at acclimation salinities beneath $\sim 20\text{‰}$ but those of *C. major* and *C. islagrande* are not. Blood magnesium is slightly hyper-regulated by *C. jamaicense* at most acclimation salinities $< 25\text{‰}$ and more markedly hyper-regulated at salinities $< 10\text{‰}$; it is also slightly hyper-regulated by *C. major* at acclimation salinities $< 30\text{‰}$.

After direct transfer of *C. jamaicense* from 20‰ salinity to 3‰ salinity, blood osmotic, sodium, and chloride concentrations fall slightly but approach stable concentrations within 12 hours; blood magnesium concentration falls less rapidly. When *C. jamaicense* is transferred from 20 to 37‰, blood osmotic, sodium, and chloride concentrations increase markedly during the first day and continue to

slowly increase through day 9; blood magnesium increases to a near stable level by day 4.

Differences in osmoregulatory capacities, along with substrate preferences, appear to limit distributions of Callianassidae on the Louisiana coast. With one exception, previous studies suggest that osmoregulatory ability does not occur in this group. The present report of osmoregulatory ability in *C. jamaicense* documents a second exception.

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