Comp. Biochem. Physiol. Vol. 74A, No. 1, pp. 57 to 62, 1983
Printed in Great Britain.

0300-9629/83/010057-06$03.00/0

OSMOREGULATION AND RESPIRATORY METABOLISM IN BRAZILIAN *MACROBRACHIUM* (DECAPODA, PALAEMONIDAE)

GLORIA S. MOREIRA*§, JOHN C. MCNAMARA*, SANDRA E. SHUMWAY† and PLINIO S. MOREIRA‡

*Departamento de Fisiologia, Instituto de Biociências, Caixa Postal 11461, Universidade de São Paulo, 05421 São Paulo, Brasil; †Ecology and Evolution Department, Division of Biological Sciences, State University of New York at Stony Brook, Long Island, NY 11794, USA and ‡Departamento de Oceanografia Biológica, Instituto Oceanográfico, Caixa Postal 9075, Universidade de São Paulo, 05508 São Paulo, Brasil

(*Received* 26 *March* 1982)

Abstract—1. The effect of salinity (0, 7, 14, 21, 28 and 35‰) on osmoregulatory capability and metabolic rate in adult *Macrobrachium acanthurus*, *M. heterochirus*, *M. olfersii* and *M. potiuna*, was investigated at 20°C using a microcryoscope and Warburg respirometer.

2. All species are strong hyperosomotic regulators in freshwater or low salinities (0–14‰) while at high salinities (21–35‰), with the exception of *M. olfersii*, they are hypoconformers; *M. olfersii* exhibits a fair degree of hypo-osmotic regulatory capability.

3. Isosmotic points decrease in the sequence *M. heterochirus* > *M. acanthurus* > *M. olfersii* > *M. potiuna*.

4. For *M. acanthurus* and *M. olfersii*, the metabolism-salinity curves assume a dome shape with a high peak at 21‰ S, close to the isosmotic point. For *M. heterochirus* and *M. potiuna*, metabolic rates tended to decline with salinity increase.

5. These results are discussed in relation to the distribution patterns of the adult shrimps and to physiological modifications occurring during development.

INTRODUCTION

River shrimps belonging to the genus *Macrobrachium* are widely distributed throughout Brazilian territory (Holthuis, 1952). In north-eastern Brazil, commercial exploitation of natural populations of *M. amazonicum* reached 2000 tons in 1980 (Cordeiro & Correia, 1981). Large quantities of *M. acanthurus* and *M. carcinus* are also commonly encountered in local markets (McNamara & Moreira, personal observation). However, before extensive and economically successful cultivation of such decapods can be undertaken, profound investigation of the physiological requirements of the organisms concerned is necessary.

Macrobrachium acanthurus, *M. heterochirus*, *M. olfersii* and *M. potiuna* are very common in most of the rivers which discharge into the Atlantic Ocean along the coast of the State of São Paulo, Brazil. *Macrobrachium acanthurus* and *M. olfersii* occur from the river mouths up to several kilometers inland while *M. heterochirus*, less abundant than the other species, is usually found closer to the sea. Although these three species need brackish water for complete larval development, *M. potiuna* is a hololimnetic species having a reduced larval developmental phase (Müller, 1892) which shows no salinity requirement (McNamara, personal observation). Differences in physiological responses to salinity among these species and/or their developmental stages then might be expected.

Although several authors have studied the effects of salinity on haemolymph osmotic concentration and the metabolism–salinity relationship in many crustaceans (see Lockwood, 1976; Kirschner, 1979; Gilles & Jeuniaux, 1979; Gilles & Péqueux, 1981 for reviews), data for members of the genus *Macrobrachium* are relatively few. Most information available in this respect concerns the postlarvae, juveniles and adults of a single species, *M. rosenbergii*, indigenous to the Indo-Pacific region (e.g. Sandifer *et al.*, 1975; Nelson *et al.*, 1977; Singh, 1980; Stephenson & Knight, 1980; Armstrong *et al.*, 1981; Castille & Lawrence, 1981). Denne (1968) and Castille & Lawrence (1981) have studied osmotic and ionic regulation in adult *M. australiense* and *M. equidens* from Australia and *M. ohione* from Texas, respectively.

Moreira *et al.* (1980, 1982a,b) have verified the effect of salinity on the metabolic rates of the first zoeal stages of *M. acanthurus*, *M. amazonicum*, *M. carcinus*, *M. heterochirus*, *M. holthuisi* and *M. olfersii* from Brazil. As a continuation of this research programme, the effect of salinity on osmoregulatory capability and metabolic activity in adults of several Brazilian *Macrobrachium* species from different biotopes is examined in the current study.

MATERIAL AND METHODS

Specimens of *M. acanthurus*, *M. olfersii* and *M. potiuna* were collected by sieve from the marginal vegetation of the Vermelho Stream, about 6 km from its mouth at the Peruibe Beach (24°10′ 12″S; 46°50′00″W). *Macrobrachium*

§To whom correspondence should be addressed.

heterochirus was obtained during low tide from the supralittoral zone at the mouth of a small river discharging into the São Sebastião Channel at the Guaecá Beach (23°48′52″S; 45°24′26″W). These collection sites are located on the coast of the State of São Paulo, Brazil. Water temperatures taken during collections varied from 20 to 27°C; salinities were always 0‰.

Shrimps were transported to the laboratory and placed separately, according to species, sex and size, in groups of from 5 to 15, in aerated crystallising dishes (approx 25 cm dia) containing 2 l. of medium, of either 0, 7, 14, 21, 28 or 35‰ S. The dishes were placed in a constant temperature chamber at 20°C. All experimental procedures were initiated after a period of 24 hr in each test salinity. Dilutions were prepared from São Sebastião Channel sea-water (35‰ S) and Guaecá River water. Salinities were measured by refractometer.

A haemolymph sample of about 0.5 ml was taken directly from the pericardial region of each of five intermoult animals, from each salinity, with a small wedge of polyethylene tubing (No. 5, Hibiki, Tokyo). The fluid was drained into a centrifuge tube, covered with parafilm, ultra-centrifuged at 8000 rpm for 30 min and frozen at −20°C. After all samples were collected, measurements of freezing point depression were determined using a microcryoscope (Ramsay & Brown, 1955, modified by Salamão, 1980). Results are given as mOsm/kg water.

Measurements of oxygen consumption were made on adult female shrimps at 20°C, using a Warburg respirometer. For *M. acanthurus*, *M. heterochirus* and *M. olfersii*, respirometer flasks of about 140 ml were used; the animal and sufficient medium (0, 7, 14, 21 or 28‰ S) to make 40 ml were added to the vessel while 1.0 ml of 12% KOH and a strip of filter paper were placed in the flask side arm. Measurements were not made at 35‰ S as insufficient animals survived this salinity. For *M. potiuna*, 60 ml flasks were employed. The animal and sufficient medium to make 20 ml were added to the vessel; 0.3 ml of 12% KOH was added to the flask side arm. Flasks were shaken at a rate of 13 cycles/min. A minimum of 6 determinations was made for each salinity, each animal being used only once. Measurements were taken every 15 min over a 90 min period, the first two readings being discarded. Results are given as $\mu l O_2$ consumed per mg dry weight per hr.

To determine dry weights, the shrimps used in each respiration experiment were immediately killed by brief immersion in boiling water, dried overnight at 80°C, placed in a desiccator for 2–3 hr and weighed on a Mettler 6HT balance (0.1 mg sensitivity). Dry weights ($\bar{X} \pm$ SEM) in mg were: *M. acanthurus*, 503.0 ± 37.5 ($N = 27$); *M. heterochirus*, 768.0 ± 46.8 ($N = 31$); *M. olfersii*, 346.3 ± 15.5 ($N = 24$); *M. potiuna*, 197.1 ± 11.0 ($N = 30$).

Significant differences between means ($P \leqslant 0.05$) were calculated according to Zar (1974, p. 105).

RESULTS

Lethal salinity limits

While specific experiments to determine lethal salinity limits for the four species were not undertaken, tolerance of high salinity varied greatly among the species, none of which showed 100% survival after 24 hr in seawater. No mortality was recorded in either *M. acanthurus* or *M. heterochirus* until placed in full strength seawater. *Macrobrachium olfersii* exhibited some mortality in 28‰ S while the hololimnetic *M. potiuna* began showing mortality in 21‰ S.

Osmoregulation

The haemolymph osmotic concentrations (HOC) of the *Macrobrachium* species studied are shown in Fig. 1. All the species are strong hyper-osmotic regulators in freshwater or low salinities, maintaining HOC of between 425 and 524 mOsm in an external medium of 0–200 mOsm.

Macrobrachium acanthurus and *M. heterochirus* maintained their HOC constant in salinities of from 0 to 7‰. However, from 7 to 28‰, osmoregulatory capability is diminished, HOC increasing with that of the medium. The points at which haemolymph and medium are isosmotic occurred at 640 mOsm (22.4‰ S) for *M. acanthurus* and 647 mOsm (22.6‰ S) for *M. heterochirus*. In higher salinities, HOCs increase quickly but remain hypo-osmotic to the medium.

The osmotic concentration of *M. olfersii* haemolymph stays fairly constant (around 520 mOsm) in salinities of from 0 to 14‰. A sharp increase in HOC occurs between 14 and 21‰ S, the isosmotic point being reached at 21.7‰ S (620 mOsm). Haemolymph osmotic concentration remained constant from 21 to 28‰ S and strongly hypo-osmotic to the medium.

The osmotic concentration of *M. potiuna* haemolymph increased slightly from 0 to 21‰ S. The isosmotic point occurred at 19.3‰ S (552 mOsm), the lowest encountered of the species examined so far. From 21 to 28‰ S, osmoregulatory capability failed completely, increasing HOC parallelling that of the medium.

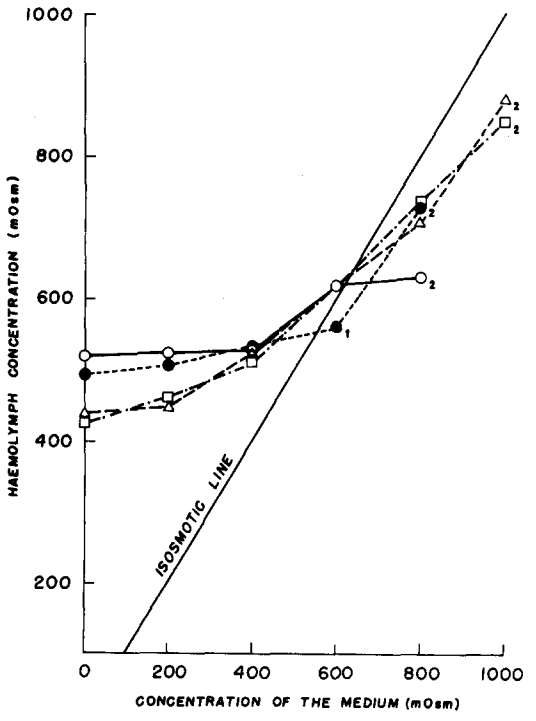

Fig. 1. Effect of salinity on the haemolymph osmotic concentrations of several Brazilian *Macrobrachium* species. Each point represents the mean of at least five determinations unless otherwise indicated. Standard errors were never more than ±5% of the means and are therefore not shown (△, *M. acanthurus* ; □, *M. heterochirus*; ○, *M. olfersii*; ●, *M. potiuna*).

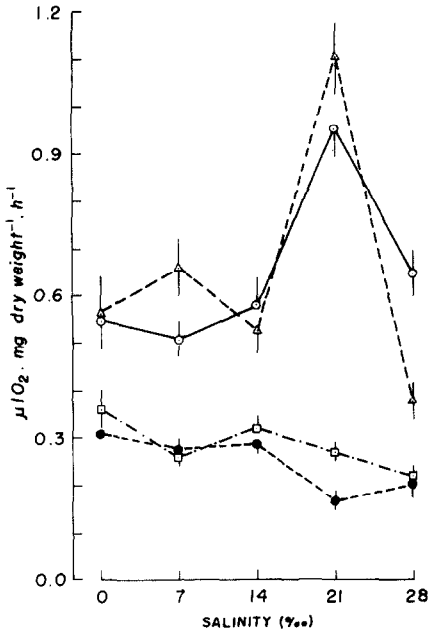

Fig. 2. Effect of salinity on the metabolic rates of several Brazilian *Macrobrachium* species. Each point represents the mean of at least six determinations. Vertical lines represent SEM (△, *M. acanthurus*; □, *M. heterochirus*; ○, *M. olfersii*; ●, *M. potiuna*).

Respiration

The effect of salinity on the rate of oxygen consumption by adult, female specimens of the *Macrobrachium* species studied is presented in Fig. 2.

The curves for *M. acanthurus* and *M. olfersii* are extremely similar in pattern. Both show a peak respiratory rate at 21‰ S but have relatively constant rates (no significant differences, $P > 0.05$) over the range 0–14‰ S. Metabolic rates varied from 0.51 to 0.96 $\mu l O_2$/mg dry wt per hr for *M. olfersii* and from 0.38 to 1.11 $\mu l O_2$/mg dry wt per hr for *M. acanthurus*.

Respiratory rates for *M. heterochirus* and *M. potiuna* were more constant, varying from 0.22 to 0.36 $\mu l O_2$/mg dry wt per hr for *M. heterochirus* and from 0.17 to 0.31 $\mu l O_2$/mg dry wt per hr for *M. potiuna*. In these species, metabolism showed a general tendency to decline with increase in salinity. However, no significant differences were found for the rates in salinities of from 7 to 21‰ for *M. heterochirus* and from 0 to 14‰ for *M. potiuna*.

DISCUSSION

Osmoregulation

Most of the catadromous *Macrobrachium* species studied so far exhibit osmoregulatory capabilities similar to those reported for other freshwater decapods, i.e. haemolymph is maintained hyperosmotic to the medium in low salinities but hypoconforms in salinities above the isosmotic point (e.g. *Macrobrachium rosenbergii*, Singh, 1980; *M. ohione*, Castille & Lawrence, 1981; *M. carcinus*, Moreira *et al.*, 1981; *M. acanthurus*, *M. heterochirus*, this study). Other members of the genus, e.g. *M. equidens* (Denne, 1968) and *M. olfersii* (this study) show hyper-hypo-osmotic regulatory capability, a characteristic generally considered to be associated with brackish water decapods (Beadle, 1943). Postlarval *M. rosenbergii* also exhibit considerable hypo-osmoregulatory capability in high salinities (Sandifer *et al.*, 1975), indicating that osmoregulatory patterns may differ with life cycle stages.

The hololimnetic species *M. potiuna* and *M. australiensis* (*cf.*, Fielder, 1970) also show the typical pattern observed for freshwater decapods. However, while the osmoregulatory curve for *M. potiuna* is similar to those presented by *M. acanthurus* and *M. heterochirus*, that for *M. australiensis* showed a sharp increase in HOC just before reaching isosmoticity (Denne, 1968). These results demonstrate that osmoregulatory capability may differ even among species whose reproductive strategies are similar showing a reduced number of larval developmental stages which are completely independent of brackish water.

The haemolymph osmotic concentrations in freshwater and isosmotic points for the *Macrobrachium* species studied so far are shown in Table 1. The widely differing values reported for *M. rosenbergii* may perhaps reflect differences between geographically separated populations and life cycle stages. Differences in experimental temperatures apparently have little effect on isosmotic point in the shrimp species studied so far (Dorgello, 1981). *Macrobrachium rosenbergii*, a predominantly freshwater species, has a low isosmotic point (485 mOsm) and does not survive in salinities above 28‰ (Singh, 1977). The brackish water species *M. equidens* has an upper lethal salinity limit of 40‰ and an isosmotic point of approx. 529 mOsm while *M. australiensis* has an upper lethal limit of 25‰ and an isosmotic point of about 475 mOsm (Denne, 1968). Isosmotic points for the Brazilian species are directly related to species biotopes as seen in previous studies on other species. The more strictly freshwater species (*M. carcinus* and *M. potiuna*) show the lowest isosmotic points while those species found in brackish and fresh waters (*M. olfersii*, *M. acanthurus* and *M. heterochirus*) have higher values.

Such data suggest differential degrees of adaptation to a freshwater existence among the species of the genus *Macrobrachium* confirming the hypotheses of earlier authors (Hedgpeth, 1949, 1957; Born, 1968; cf. Ortmann, 1902) that these decapods are still in the process of invading the freshwater environment.

Metabolism

Previous studies concerning the effect of salinity on the respiratory metabolism of *Macrobrachium* species have reported data for either postlarval or juvenile *M. rosenbergii* (Nelson *et al.*, 1977; Stephenson & Knight, 1980) or the first zoeal stages of several Brazilian species (Moreira *et al.*, 1980, 1982a,b). Since the adults of these species usually inhabit freshwater, a different metabolism–salinity relationship might be expected in the later phases of the life cycle.

Both postlarval (40–50 mg dry wt) and juvenile *M. rosenbergii* generally tend to increase metabolic rate at low salinities over the range 0–28‰ (Nelson *et al.*, 1977; Stephenson & Knight, 1980). Moreira *et al.* (1980, 1982a,b) have also observed an increase in metabolic rate associated with low salinities for the

Table 1. Comparison of haemolymph osmotic concentrations and isosmotic points of various *Macrobrachium* species

Species	Haemolymph osmotic concentration in freshwater (mOsm)	Isosmotic point (mOsm)	Temperature (°C)	Reference
M. acanthurus	440	640	20	This study
*M. australiense**	520	≃475	22 (?)	Denne (1968)
M. carcinus	461	492	28	Moreira *et al.* (1981)
*M. equidens**	†	529	22 (?)	Denne (1968)
M. heterochirus	425	647	20	This study
M. ohione	462	643	25	Castille & Lawrence (1981)
M. olfersii	520	620	20	This study
M. potiuna	493	552	20	This study
M. rosenbergii	479 (postlarva)*	†	28	Sandifer *et al.* (1975)
	473 (juvenile)*	≃515	28	Sandifer *et al.* (1975)
	450 (juvenile)	693	27	Armstrong *et al.* (1981)
	450 (juvenile)	†	25	Castille & Lawrence (1981)
	360 (adult)	485	†	Singh (1980)

* Recalculated from freezing point depression values.
† No data.

first zoeal stages of *M. acanthurus*, *M. amazonicum*, *M. holthuisi* and *M. olfersii*; the same trend was also observed in the present study for adult *M. heterochirus* and *M. potiuna*. Such a metabolism–salinity relationship has been commonly observed in euryhaline crustaceans, most of which exhibit strong osmoregulatory capabilities (e.g. *Palaemonetes varians*, Lofts, 1956; *Crangon vulgaris*, Hagerman, 1970). Gilles (1972), studying the effects of osmotic stress on the production of $^{14}CO_2$ by isolated *Callinectes sapidus* axons preloaded with various ^{14}C-labelled amino acids has verified that hypo-osmotic conditions induce increased $^{14}CO_2$ production concomitant with an increase in oxygen consumption. Hulbert *et al.* (1976) have presented evidence from studies with *Hemigrapsus nudus* gill preparations to suggest that increased oxygen consumption in low salinities was due in part to metabolic reorganisation gearing the cell to oxidative processes, specific increase in oxidative deamination of free amino acids and increased incorporation of free amino acids into osmotically inactive proteins. Although these results might explain *in vitro* variations in Q_{O_2} in different salinities, the interpretation of respiratory data obtained from whole animals is still controversial.

Some euryhaline crustaceans either maintain almost constant metabolic rates over a wide range of salinity (e.g. *Eriocheir sinensis*, Schwabe, 1933; *Artemia salina*, Gilchrist, 1956 and its nauplii, Kratowich, 1964; *M. carcinus* and *M. heterochirus* zoeae I, Moreira *et al.*, 1981) or decrease rates in both high and/or low salinities (e.g. *Corophium volutator*, McLusky, 1969; *Euphausia pacifica*, Gilfillan, 1972; *Euterpina acutifrons*, Moreira & Vernberg, 1978; *M. acanthurus* and *M. olfersii*, this study). If osmotic work were the sole cause of variation in M–S curves, lowest Q_{O_2} values should therefore be recorded in salinities isosmotic with the blood. Although such a result has been recorded for some crustaceans (e.g. *Metapenaeus monoceros*, Rao, 1958; *Crangon vulgaris*, Hagerman, 1970; *Eurytemora hirundoides*, Gyllenberg & Lundqvist, 1979; *Penaeus aztecus*, Bishop *et al.*, 1980), this is certainly not necessarily the case for *Macrobrachium* species. Sandifer *et al.* (1975) reported that the haemolymph isosmotic point of postlarval and juvenile *M. rosenbergii* was about 18‰ S. However, Stephenson & Knight (1980) have demonstrated the salinity independent nature of metabolic rate in *M. rosenbergii* postlarvae of up to 40 mg dry wt. Large postlarvae and juveniles increased metabolic rates in dilute salinities, the lowest Q_{O_2} value being recorded in 28‰ S (Nelson *et al.*, 1977). In the present study, *M. potiuna* alone had the lowest Q_{O_2} value close to the isosmotic point; Q_{O_2} values for *M. heterochirus* did not vary significantly between 7 and 28‰ S (haemolymph isosmotic point 22.6‰ S). In marked contrast, *M. acanthurus* and *M. olfersii* exhibited highest Q_{O_2} values in salinities very close to the isosmotic point. Although an explanation is not immediately evident for these metabolic peaks, such results are consistent with the data of McNamara & Moreira (1981) who showed that the duration of the intermoult cycle in adult, female *M. olfersii* is at a minimum in 21‰ S, an indirect reflection of increased rates of physiological processes. Singh (1980) has shown that growth rates are suboptimal in isosmotic salinities for *M. rosenbergii* but highest in low salinities.

Increased respiratory loss of carbon and reduced amount of energy available then could possibly be responsible for decreased growth rates in isosmotic salinities. Armstrong *et al.* (1981) have recently demonstrated apparent ammonia uptake from the medium in juvenile *M. rosenbergii* submitted to a hyperosmotic shock of 24‰ S, a salinity close to the isosmotic point. Such uptake could be used to increase intracellular ammonia concentrations prior to increased free amino acid synthesis. Reversal of the Na^+/NH_4^+ exchange pump following hyperosmotic shock might have some influence on the total metabolic rate.

Stephenson & Knight (1980) have suggested that during the developmental sequence, *Macrobrachium* species might show different M–S responses. With the exception of *M. potiuna*, M–S curves for the zoea I stage of the species treated in the present study have

been determined (Moreira *et al.*, 1982a). Larval and adult *M. heterochirus* both have constant metabolic rates in salinities of from 7 to 28‰ S, exhibiting differences only in the extreme salinities where larval Q_{O_2} values decrease in freshwater while adult values increase; larval values decline in 35‰ S, whereas adults do not survive this salinity. The M–S curves for adult *M. acanthurus* and *M. olfersii* are the inverse of those for their zoeae I. While the zoeae exhibited a U-shaped curve (lowest Q_{O_2} at isosmotic point?), adults showed only slight variations in Q_{O_2} values in salinities of from 0 to 14‰, after which the curve assumes a dome shape, the highest value occurring near the isosmotic point.

These differences, reflecting distinct physiological changes associated with different developmental phases, may be of ecological significance providing endogenous cues initiating migration to, and establishment of adults in entirely freshwater biotopes.

Acknowledgements—The authors are grateful to Dr Phan Van Ngan for the use of laboratory facilities and to Dr L. C. Salomão for the microcryoscope. This study was partially supported by the Organisation of American States (OAS).

REFERENCES

ARMSTRONG D. A., STRANGE J., CROWE J., KNIGHT A. W. & SIMMONS M. (1981) High salinity acclimation by the prawn *Macrobrachium rosenbergii*: uptake of exogenous ammonia and changes in endogenous nitrogen compounds. *Biol. Bull.* **160**, 349–365.

BEADLE L. C. (1943) Osmotic regulation and faunas of inland waters. *Biol. Rev.* **18**, 172–183.

BISHOP J. M., GOSSELINK J. G. & STONE J. H. (1980) Oxygen consumption and hemolymph osmolality of brown shrimp, *Penaeus aztecus*. *Fish. Bull.* **78**, 741–757.

BORN J. W. (1968) Osmoregulatory capacities of two caridean shrimps, *Syncaris pacifica* (Atyidae) and *Palaemon macrodactylus* (Palaemonidae). *Biol. Bull.* **134**, 235–244.

CASTILLE F. L. & LAWRENCE A. L. (1981) The effect of salinity on the osmotic, sodium, and chloride concentrations in the hemolymph of the freshwater shrimps, *Macrobrachium ohione* Smith and *Macrobrachium rosenbergii* De Man. *Comp. Biochem. Physiol.* **70A**, 47–52.

CORDEIRO E. A. & CORREIRA E. S. (1981) Produção em escala piloto de pós-larvas de camarões *Macrobrachium rosenbergii* (de Man) no Estado de Pernambuco. *Anais do II Congresso Brasileiro de Engenharia de Pesca, Recife, Brazil*, pp. 133–142.

DENNE L. B. (1968) Some aspects of osmotic and ionic regulation in the prawns *Macrobrachium australiensis* (Holthuis) and *M. equidens* (Dana). *Comp. Biochem. Physiol.* **26A**, 17–30.

DORGELLO J. (1981) Blood osmoregulation and temperature in crustaceans. *Hydrobiologia* **81**, 113–130.

FIELDER D. R. (1970) The larval development of *Macrobrachium australiensis* Holthuis, 1950 (Decapoda, Palaemonidae) reared in the laboratory. *Crustaceana* **18**, 60–74.

GILCHRIST B. M. (1956) The oxygen consumption of *Artemia salina* (L.) in different salinities. *Hydrobiologia* **8**, 54–65.

GILFILLAN E. (1972) Seasonal and latitudinal effects on the responses of *Euphausia pacifica* Hansen (Crustacea) to experimental changes of temperature and salinity. In *Biological Oceanography of the Northern North Pacific Ocean* (Edited by TAKENOUTI A. Y.) pp. 443–463. Idemitsu Shoten, Tokyo, Japan.

GILLES R. (1972) Amino-acid metabolism and isosmotic intracellular regulation in isolated surviving axons of *Callinectes sapidus*. *Life Sci.* **11**, 565–572.

GILLES R. & JEUNIAUX C. (1979) Osmoregulation and ecology in media of fluctuating salinity. In *Mechanisms of Osmoregulation in Animals* (Edited by GILLES R.) pp. 581–608. John Wiley, Chichester.

GILLES R. & PÉQUEUX A. (1981) Cell volume regulation in crustaceans: relationship between mechanisms for controlling the osmolality of extracellular and intracellular fluids. *J. exp. Zool.* **215**, 351–362.

GYLLENBERG G. & LUNDQVIST G. (1979) The effects of temperature and salinity on the oxygen consumption of *Eurytemora hirundoides* (Crustacea, Copepoda). *Ann. Zool. Fennici* **16**, 205–208.

HAGERMAN L. (1970) The oxygen consumption of *Crangon vulgaris* (Fabricus) (Crustacea, Natantia) in relation to salinity. *Ophelia* **7**, 283–292.

HEDGPETH, J. W. (1949) The North American species of *Macrobrachium*. *Texas J. Sci.* **1**, 28–38.

HEDGPETH J. W. (1957) Marine biogeography. In *Treatise on Marine Ecology and Palaeoecology* (Edited by HEDGPETH J. W.) *Geol. Soc. America, Memoir* **67**, 359–382.

HOLTHUIS L. B. (1952) A general revision of the Palaemonidae (Crustacea, Decapoda, Natantia) of the Americas. II. The subfamily Palaemoninae. *Allan Hancock Found. Publ., Occasional Paper* **12**, 1–396.

HULBERT W. C., SCHNEIDER D. E. & MOON T. W. (1976) Temperature and salinity adaptation in the purple shore crab *Hemigrapsus nudus*: an *in vitro* physiological study with excised gills. *Mar. Biol.* **36**, 217–222.

KIRSCHNER L. B. (1979) Control mechanisms in crustaceans and fishes. In *Mechanisms of Osmoregulation in Animals* (Edited by GILLES R.) pp. 157–222. John Wiley, Chichester.

KRATOWICH N. R. (1964) The effect of metabolic inhibitors on osmoregulation by nauplii of *Artemia salina* at various salinities. *Am. Zool.* **4**, 389–390.

LOCKWOOD A. P. M. (1976) Physiological adaptation to life in estuaries. In *Adaptation to Environment* (Edited by NEWELL R. C.) pp. 315–392. Butterworths, London.

LOFTS B. (1956) The effects of salinity changes on the respiratory rate of the prawn *Palaemonetes varians* (Leach). *J. exp. Biol.* **33**, 730–736.

MCLUSKY D. S. (1969) The oxygen consumption of *Corophium volutator* in relation to salinity. *Comp. Biochem. Physiol.* **29A**, 743–753.

MCNAMARA J. C. & MOREIRA G. S. (1981) Efeito da salinidade sobre a duração do ciclo de muda em *Macrobrachium olfersii* (Wiegmann). *14 Congresso Latinoamericano de Ciências Fisiologicas, São Paulo, Brazil, Resumos* p. 27.

MOREIRA G. S., MCNAMARA J. C. & MOREIRA P. S. (1982a) The effect of salinity on the metabolic rates of some plaaemonid shrimp larvae. *Aquaculture.* **28**, In press.

MOREIRA G. S., MCNAMARA J. C. & MOREIRA P. S. (1982b) The effect of salinity on the respiratory metabolism, survival and moulting of the first zoeal stage of *Macrobrachium amazonicum* Heller (Crustacea, Palaemonidae). *Bolm Fisiol. Animal, Univ. S. Paulo* **7**. In press.

MOREIRA G. S., MCNAMARA J. C., MOREIRA P. S. & SHUMWAY S. E. (1981) Osmorregulação em *Macrobrachium acanthurus* e *Macrobrachium carcinus* (Decapoda: Palaemonidae) *Ciênc. Cult. Suppl., São Paulo, Brazil* **33**, 626.

MOREIRA G. S., MCNAMARA J. C., MOREIRA P. S. & WEINRICH M. (1980) Temperature and salinity effects on the respiratory metabolism of the first zoeal stage of *Macrobrachium holthuisi* Genofre & Lobão (Decapoda: Palaemonidae). *J. exp. mar. Biol. Ecol.* **47**, 141–148.

MOREIRA G. S. & VERNBERG W. B. (1978) Synergistic effects of environmental variables on the metabolism of the copepod *Euterpina acutifrons* from two different areas off the coast of the State of São Paulo, Brazil. *Fish. Bull.* **76**, 449–455.

MÜLLER F. (1892) O camarão preto, *Palaemon potiuna*. *Arch. Mus. Nat. Rio de Janeiro* **8**, 179–206.

NELSON S. G., ARMSTRONG D. A., KNIGHT A. W. & LI H. W. (1977) The effects of temperature and salinity on the metabolic rate of juvenile *Macrobrachium rosenbergii* (Crustacea: Palaemonidae). *Comp. Biochem. Physiol.* **56A**, 533–537.

ORTMANN A. E. (1902) The geographical distribution of freshwater decapods and its bearing upon ancient geography. *Proc. Am. Phil. Soc.* 41, 267–400.

RAMSAY J. A. & BROWN R. H. (1955) Simplified apparatus and procedures for freezing point determinations upon small volumes of fluid. *J. Sci. Instrum.* **32**, 372–375.

RAO K. P. (1958) Oxygen consumption as a function of size and salinity in *Metapenaeus monoceros* Fab. from marine and brackish water environments. *J. exp. Biol.* **35**, 307–313.

SALOMÃO L. C. (1980) Determinação da concentração osmótica de micro-amostras de fluidos através do ponto de congelamento. *Bolm Fisiol. Animal, Univ. S. Paulo* **4**, 133–141.

SANDIFER P. A., HOPKINS J. S. & SMITH T. I. (1975) Observations on salinity tolerance and osmoregulation in laboratory-reared *Macrobrachium rosenbergii* postlarvae (Caridea: Crustacea). *Aquaculture* **6**, 103–114.

SCHWABE E. (1933) Über die Osmoregulation verschiedener Krebse (Malacostracen). *Z. vergl. Physiol.* **19**, 183–236.

SINGH T. (1977) Osmotic and ionic regulation in *Macrobrachium rosenbergii* (De Man), 1879 (Decapoda: Caridea). M.Sc. Thesis, University of Malaya.

SINGH T. (1980) The isosmotic concept in relation to aquaculture of the giant prawn, *Macrobrachium rosenbergii*. *Aquaculture* **20**, 251–256.

STEPHENSON M. J. & KNIGHT A. W. (1980) The effects of temperature and salinity on oxygen consumption of post-larvae of *Macrobrachium rosenbergii* (De Man) (Crustacea: Palaemonidae). *Comp. Biochem. Physiol.* **67A**, 699–703.

ZAR J. H. (1974) *Biostatistical Analysis.* Prentice-Hall, Englewood Cliffs, NJ.