TABLE 2 — GROWTH RATE OF *P. monodon* REARED IN AQUARIA

| Period months | Total length mm | Growth increment* mm |
|---|---|---|
| Initial | 10·5 | — |
| 1 | 21·5 | 11·0 |
| 2 | 42·0 | 20·5 |
| 3 | 56·8 | 14·8 |
| 4 | 92·5 | 35·7 |
| 5 | 103·0 | 10·5 |
| 6 | 118·0 | 15·0 |
| 7 | 135·0 | 17·0 |
| 8+3 (weeks) | 150·0 | 8·8 |

*Monthly average 16·0 mm.

salinity prevailing at that time of the year in the estuary. Heavy mortalities were often encountered. The few lower growth rates reported here were seen in the experiments initiated in winter.

The growth rate derived in the laboratory is considered to be lower than the natural growth rate. In nature, the postlarvae attain a size of about 160-170 mm in about 6 months (Subrahmanyam, M., unpublished data). The reduced growth rate in the laboratory could be the result of small size of the containers[6].

The prawn is believed to attain a size of about 250 mm in one year in prawn ponds of the Philippines[7] and 20-25 cm in prawn ponds of Indonesia[8]. Delmendo and Rabanal[5] conducted experiments on growth of *P. monodon* for an year in ponds in the Philippines. They observed an average growth rate of 17·9 mm/month from postlarva to a maximum size of 250 mm. The growth rate of 16·0 mm/month observed in the laboratory is slightly less presumably due to the difference in the method of study. In an experiment conducted by Nakano[9] the postlarvae of *P. monodon* (average size 13·9 mm) grew to 28·2 mm in 26 days. In yet another experiment conducted by the same author, juvenile prawn (33·0 mm) grew to 116·7 mm in 182 days when cultured with *Chanos chanos*. These growth rates (postlarvae 16·5 mm/month; juveniles 13·8 mm/month) are also lower than the growth rates reported from pond studies and the laboratory. The experiments, however, convey the possibility of culturing *P. monodon* with other compatible species. Isnadi[10] reared advanced postlarvae (20-30 mm) in a brackishwater pond in Indonesia for 9½ months and obtained a growth rate of 13·45 mm/month. Poernomo[11] reared small postlarvae (9·6 mm) in a glass tank (170 litres) for 37 days, the resultant growth rate being 17·35 mm/month. These results also confirm that the growth rate in ponds and small containers is lower than the growth rate in natural waters. More work is needed to understand the environmental requirements to promote the growth and survival rates with increasing size of the prawn.

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## Blood Amino Acid Levels in the Freshwater Crab, *Barytelphusa guerini* H. Milne Edwards, as a Function of Salinity Adaptation

S. A. T. VENKATACHARI
Department of Zoology, Yeshwant Mahavidyalaya
Nanded 431601
&
RAMAMANI KESHAVAN
Department of Zoology, Science College, Nanded 431601

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**Total free amino acids varied both qualitatively and quantitatively on adaptation to different salinities. Amino acid content increased in 25 and 100% sea water (SW) and the per cent increase was more in females in both media. While the increase in 100% SW was significant in both the sexes, the increase in 25% SW was significant only in females. Females showed a slight but insignificant increase in the intermediate salinities while a slight but significant decrease was found in the males in the same salinities. Qualitative variations depended upon the sex and salinity of the medium. More new amino acids appeared in 25 and 100% SW while the new ones appearing at intermediate salinities were few. It is suggested that the concentration of amino acids in the blood and its contribution to the blood osmotic pressure depend more on the osmotic gradient than merely the salinity of the external medium. Importance of the choice of sex in studies on osmoregulation is also proposed in view of the present findings.**

EVER since Jeuniaux *et al.*[1] suggested the possible role of amino acids in the isosmotic intracellular regulation of euryhaline species, considerable evidence is accumulating in support of this hypothesis[2-6]. A proportionate increase in the free amino acid pool size on adaptation to increasing salinities and its reversibility in low salinities are shown in the muscle tissue of the euryhaline crustaceans like *Carcinus maenas*[7-9], *Astacus astacus*[10], *Leander serratus* and *Leander squilla*[1], *Eriocheir sinensis*[11], and the nerve tissue of *Eriocheir sinensis*[12,13]. However, studies on the variation in the free amino acid content of blood on salinity adaptation have not received considerable attention. The only such study seems to be that of Rao *et al.*[14] who showed a qualitative increase in the free amino

acid content of the body fluid of the crab, *Sesarma plicatum*, with increase in salinity.

The freshwater crab, *Barytelphusa guerini*, is capable of tolerating a wide range of salinity and the biochemical mechanisms of salinity adaptation are being investigated. The qualitative and quantitative variations in the amino acid content of the blood of this crab on salinity adaptation are reported in this paper.

The crabs, collected locally, were adapted to the laboratory conditions for a couple of days. Then they were separately adapted to 25, 50, 75 and 100% sea water (SW) (8.125, 16.25, 24.375 and 32.5‰S. respectively) for 20 days. Different grades of sea water were prepared by appropriately diluting 100% SW (32.5‰, S.) with distilled water. Another batch of crabs maintained in tap water for the same period served as the controls. The animals were fed with frog muscles and bits of earthworm twice a week. They were not fed one day prior to experimentation.

At the end of the adaptation, blood was collected from the chelate leg of each crab with the help of a hypodermic syringe, rinsed with sodium oxalate solution and used individually for analysis. Quantitative analysis of the total free amino acids was carried out with the deproteinized blood using the method of Danielson as given by Oser[15]. One-dimensional paper chromatography was employed for the qualitative assay of the individual amino acids. Butanol-acetic acid-water mixture (4 : 1 : 1) was used as the mobile phase. At least 6 observations were made for each medium.

Males and females of the size range 35-40 g were used throughout theinvestigation and the experiments were carried out at room temperature (26-28°C).

*Amino acid content in normal animals*-Thirteen amino acids were identified in the blood of males and

**TABLE ~-VARIATION IN AMINO ACIDS OF THE BLOOD ON SALINITY ADAPTATION**

(Data are compiled from six observations in each medium and the most consistently occurring amino acids are represented in the table)

| Amino acid | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Sea water | | | | Control | Sea water | | | |
| | | 25% | 50% | 75% | TOE | | 25% | 50% | 75% | 100% |
| Valine | + | + | + | + | + | + | + | + | + | + |
| Leucine | + | + | + | + | ▪ | + | + | + | + | + |
| Norleucine | + | + | — | + | + | + | + | + | + | + |
| C2&? acid | + | — | + | + | | + | + | + | — | + |
| | + | + | + | + | r | + | + | + | — | + |
| Tyrosine | + | — | — | — | | + | + | + | + | + |
| Alanine | | + | + | — | | + | — | + | — | + |
| Hydroxyphenyl-alanine | + | + | + | + | | + | + | — | + | — |
| Histidine | + | + | — | — | — | + | + | — | | + |
| Threonine | | — | + | + | — | + | — | — | | |
| Lysine | : | + | + | + | + | — | + | | + | T |
| Methionine | | + | — | + | + | + | + | : | | ++ |
| Aspartic acid | | + | + | — | + | — | + | | -t | |
| Isoleucine | | + | — | — | + | + | + | : | | . |
| Cystein | | — | + | — | + | + | — | + | 3 | + |
| Tryptophan | 5 | — | — | + | — | — | — | — | | |
| Cystine | - | + | — | — | — | + | | — | | 7 |
| Arginine | | + | + | — | — | — | r | + | | + |
| Wenvlalanine | — | + | + | — | — | — | | — | T | |
| Glycihe | — | + | — | — | — | — | 7 | — | + | - |
| Strine | — | — | + | — | — | — | | — | | |
| Hydroxyproline | — | — | + | — | — | — | + | — | - | |
| Total | 13 | 15 | 14 | 10 | 11 | 14 | 16 | 13 | 11 | 16 |

**TABLE 2 — TOTAL FREE AMINO ACID CONTENT OF BLOOD OF MALE AND FEMALE CRABS ON ADAPTATION TO DIFFERENT SALINITIES**

(Values, expressed as mg aa $N_2$/100 ml blood, are averages of 6 observations ± SD)

| | *Control* | 25% SW | 50% SW | 75% SW | 100% SW |
|---|---|---|---|---|---|
| **MALES** | | | | | |
| Av.± SD | 5·252 ± 0·167 | 5.307 ± 0.1689 | 4·392 ± 0·1005 | 4·588 ± 0.1024 | 5·803 ± 0·1574 |
| *t* | | 0-518 | 9·87 | 7·58 | 5-37 |
| *P** | | >0·001 | <0·001 | <0·001 | < 0.001 |
| **FEMALES** | | | | | |
| Av.± SD | 4·673 ± 0.162 | 5·179 ± 0·088 | 4·769 ± 0·172 | 4·89 ± 0·173 | 5·35 ± 0-210 |
| *t* | | 5.913 | 0.9088 | 2·135 | 6·215 |
| *P** | | <0·001 | >0·001 | >0·001 | < 0.001 |

*$P$ = <0·001 (significant) and $P$ = >0·001 (not significant)

14 in females; ten amino acids were common in both the sexes. Lysine, aspartic acid and tryptophan were found only in males, while isoleucine, methionine, cystein and cystine were found in females (Table 1). Quantitatively the content was significantly greater in males (Table 2).

*Amino acid variation on adaptation to* 100% *sea water* — The total number of amino acids detected in males was 11 as against 13 of the normal. Of these, 8 were same as in normal, while 5 disappeared (proline, dihydroxyphenylalanine, histidine, threonine and tryptophan) and 3 new amino acids appeared (methionine, isoleucine and cystein). In females, the total number of amino acids increased from 14 to 16 (Table 1) of which 11 were same as in normal, 3 disappeared (dihydroxyphenylalanine, threonine and cystein) and 5 appeared (lysine, aspartic acid, tryptophan, arginine and phenylalanine).

The blood amino acid content increased significantly in both the sexes (Table 2), but the per cent increase was slightly greater in females (Table 3).

*Amino acid variation at intermediate salinities* — Number of amino acids in males increased from 13 to 15 in 25% SW and 14 in 50% SW but decreased to 10 in 75% SW. In the case of females the number increased from 14 to 16 in 25% SW but decreased to 13 in 50% SW and 11 in 75% SW. Also the composition of the amino acid pool had been different in different media in both the sexes, as in 100% SW (Table 1).

Quantitatively, the amino acids increased in 25% SW but this increase was significant only in the females. Even in 50 and 75% SW the females showed a slight increase in the blood amino acid content but it was less and insignificant compared to that in 25% SW. On the contrary, males did not show any increase; instead a significant decrease was found (Tables 2 and 3).

The amino acid content was high in males in 25 and 100% SW as in normal, while it was lesser than that of females in 50 and 75% SW. The number of amino acids appearing in the blood was more in 25 and 100% SW than in 50 and 75% SW though they varied with sex.

Qualitative and quantitative increases in the amino acid content with increasing salinities were shown in the muscle and nerve of several crustaceans and their role in the isosmotic intracellular regulation has been confirmed[1-6], but variations in the blood amino acid level on such alterations in salinity have not received considerable attention. Although the blood amino acid level in crustaceans is lesser than that in their tissues[11,16-18] it is still greater than that in the vertebrate blood[2,19] and the blood amino acids of crustaceans contribute to a certain extent to its osmotic pressure[19]. As such changes in the blood amino acid levels are likely to occur on adaptation to different salinities. Rao *et al.*[14] demonstrated that the number of amino acids in the body fluid of the crab, *Sesarma plicatum*, increases on adaptation to increasing salinities. In the present investigation about 10 to 12% increase in the blood amino acid content was detected on adaptation to 100% SW. Such increase was reported earlier in the muscle and nerve of several crustaceans. However, the present results are not in agreement with those of Rao *et al.*[14] in that there is no qualitative increase in the amino acids with increasing salinities. Moreover, the composition of amino acid pool changed on adaptation to different salinities, especially in 100% SW. It may be suggested that an increase in the amino acid content of the blood would increase the blood osmotic pressure to a new regulated level and prevent dehydration as in the tissues and also check the entry of more chloride ions.

TABLE 3 — PER CENT VARIATION IN BLOOD AMINO ACID CONTENT ON ADAPTATION TO DIFFERENT SALINITIES

(Data compiled from the average of 6 observations)

| Sex | 25% SW | 50% SW | 75% SW | 100% SW |
|---|---|---|---|---|
| Males | +1·13 | −16·2 | −12·4 | +10·37 |
| Females | +10·8 | +2·1 | +2·55 | +12·8 |

The amino acids mostly involved in isosmotic intracellular regulation are alanine, glycine, glutamic acid, proline[2,4,6], aspartic acid[2,6], and arginine[4,6]. Many of these amino acids are undergoing qualitative changes in the present case suggesting their involvement in the osmoregulation.

It is of interest to note that while the amino acid content increased in 100% SW a proportionate increase over normal was not detected in the intermediate grades of sea water. An increase was found in 25% SW but in 50 and 75% SW the increase was less and even a decrease was found in some cases. It seems that the amino acid content is maintained or decreased when the crabs were in intermediate salinities (50 and 75% SW) while it increased on adaptation to extreme salinities (25 and 100% SW). This suggests that the blood amino acid level might depend on the osmotic gradient rather than merely on the salinity of the medium. Since the crab blood is isosmotic to a salinity range of 50-75% SW (Venkatachari, S. A. T., unpublished data) the gradient is less and the amino acids are contributing least to the osmotic pressure. As 25 and 100% SW are hypo- and hyperosmotic to the crab blood respectively the amino acids increase and contribute more to the blood osmotic pressure. Under such conditions, the freshwater crabs are expected to have a higher amino acid content because of the steeper osmotic gradient, but they possess only a smaller content. It is possible that the blood amino acid level of freshwater crabs is low because of (i) its being the natural habitat and (ii) certain other homeostatic mechanisms. In fact, it is shown earlier that the protein levels in various tissues are maintained at the normal level when the crabs were adapted to 50 and 75% SW and decreased in 25 and 100% SW[20]. From the variations in the proteins and amino acids, it might be suggested that amino acid variation might be due to variations in the protein content.

Intersex variations in the normal blood amino acid level and differences in the qualitative and quantitative variations of it in the two sexes on adaptation to different salinities suggest that though both the sexes are capable of osmoregulation the way in which it is achieved might be different in the two. It is shown earlier that there are intersex variations in the biochemical set-up of these animals[21]. As such the choice of sex may be an

important factor in studies on the mechanisms of osmoregulation.

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