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Selective biochemical studies in a freshwater prawn, *Macrobrachium nobilli* (Crustecea: Palaemodinae)

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Abstract
Calcium and phosphorous contents of abdomen and cheliped muscles of juvenile, male and female *Macrobrchium nobilii* were determined from field collected samples. In all the three groups calcium concentration was higher in chelipeds while the phosphorous content was more in abdomen muscles than in the chelipeds. However between three groups the calcium content varied significantly both in the abdomen and cheliped muscles ($P<0.001$) while the phosphorous content differed ($P<0.05$) only in abdomen muscles.
Key words: Calcium, Phosphorous, *Macrobrchium nobilii*

Introduction

A knowledge on the proximate composition (Ash, moisture, macro and micro nutrients and energy) of a animal chosen for aqualculture is essential for not only selecting an ideal stock but also to formulate an ideal diet (Davis *et al.* 1992, Davis and Gatlin 1996). Available literature on the micro and macro nutrient composition of various and even commercially important decapods indicate their inter and intra-species variations and even within species with developmental stages (Boyd and Teichert-Coddington 1995). However there is a paucity of information on mineral content of freshwater crustaceans and its requirement for normal growth. Crustaceans obtain the required minerals for growth from the environment either by ionic exchange across the gill membrane or from the ingested water through gut absorption (Chuang 1995). A dietary sources of some minerals is necessary to ensure normal growth since the periodic exudation of heavily mineralized exoskeleton leads to loss of some minerals despite their reabsorption in premolt period (Greenaway 1985).

Materials and method

Macrobrchium nobilii (Henderson and Mattai 1910) were collected from river Cauvery, near Tiruchirapalli and transported to the laboratory with enough aeration in large plastic container and used for the study immediately. Tissue samples were obtained from the muscle of abdomen and chelipeds in juvenile, male and female. A known quantity of sample was dried at 70^0C in a hot air oven for 24 hrs and reweigh to quantify the moisture content of the sample (Passoneau and Williams 1953). To find out the ash content, the dried samples were shed at 550-600^0C for 4 hrs in a muffle furnace. The left out inorganic constituents in the form of ash was then weighed (Huner *et al.* 1990). Calcium and phosphorous content of the tissue samples were estimated by drying the samples in a hot air oven at 70^0 C to a weight constancy and then digested (Van Loon 1985) in acid-washed test tubes with a mixture of concentrated nitric and perchloric acid. The samples were slowly boiled to dryness on a hot plate and allowed to cool to room temperature. The died samples were-re dissolved in concentrated HCL and de-ionized water to quantify the calcium and phosphorous content using the UV 1604 Shimadzu atomic spectrophotometer (Fiske and Subbarow 1925, Mendez *et al.* 1998). Variations in the moisture, ash, calcium and phosphorous contents among juvenile, male and female were analyzed through one way ANOVA. The variations in proximate composition between the abdomen and chelipeds muscle in each group was tested through Student's "t" test (Zar 1996).

Results and discussion

The moisture content of abdominal muscle of juvenile is 72.±121.31% which differs from that of cheliped (59.01± 1.85%) (Table 1). A similar trend is also found between the muscle of abdomen and cheliped of male ($p<0.01$, t 7.46) and female (t 7.99, $t_{0.01}$ 2.78, df = 4). To find out the variations between the moisture content of the samples obtained from the three groups, ANOVA was performed which indicates that there is a significant difference between the three groups also ($p<0.05$) (Table 2). But no such relationship is found in the moisture content of chelipeds (Table 2). Ash content (%) was more in male chelipeds (40.57±0.91) than in female (37.06±0.39) and juvenile (16.67± 0.55). The Ash content of abdomen muscle of three groups are 07.37±0.27 for juvenile, 10.29±0.12 for male and 8.64±0.28 for female which very significantly between the three groups ($p<0.05$) (Table 2), however no such difference ($p>0.05$) is observed in chelipeds of these groups. Between abdomen and cheliped the variation in ash content is statistically highly significantly within the groups ($p>0.001$, t 24.61 for juvenile, 33.06 for male and 32.38 for female, $t_{0.001}$ 8.64, df = 4). Higher amount of ash content in male cheliped is due to the heavier mineralization since they possess robust chela.

Table 1. Moisture, ash, calcium and phosphorous content (%) in the abdomen and chelipeds of juvenile, male and female *Macrobrachium nobilii*

Sample	Juvenile	Male	Female
	Moisture (%)		
Abdomen Muscle Chelipeds	72.12±1.32 59.01±1.85	78.17±1.07 61.18±2.01	79.39±1.18 58.72±1.87

		Ash (%)	
Abdomen Muscle Chelipeds	07.37±0.27 16.67±0.55	10.29±0.12 40.57±0.91	08.64±0.28 37.06±0.39
		Calcium (%)	
Abdomen Muscle Chelipeds	00.49±0.03 05.11±0.31	00.72±0.05 12.79±0.48	00.47±0.03 10.62±0.16
		Phosphorous (%)	
Abdomen Muscle Chelipeds	01.62±0.09 00.97±0.13	01.13±0.09 00.97±0.13	01.38±0.11 00.96±0.06

Table 2. ANOVA to find out the validity of relationship between the studied parameters in three chosen groups of *Macrobrachium nobilii*

Variation	SS	Df	MS	F
Moisture- abdomen muscles				
Total	79.12	14		
Between Groups	42.10	02	21.05	6.82*
Error	37.02	12	03.09	
Moisture-Chelipeds				
Total	80.46	14		
Between Groups	06.24	02	03.12	0.5^{NS}
Error	74.22	12	06.18	
Ash-Abdomen muscles				
Total	25.41	14		
Between Groups	21.91	02	10.96	37.5***
Error	03.51	12	00.29	
Ash-Chelipeds				
Total	715.7	14		
Between Groups	705.23	02	352.62	404.5***
Error	010.46	12	000.87	

*** Statistically highly significant ($P<0.001$) ** Statistically significant ($P<0.05$) NS- not significant ($P<0.05$)

Calcium content in the abdomen and cheliped muscle, is significantly differ between the groups (Table 3). The variation in the calcium content between abdomen and chelae also differ significantly within each group ($p<0.0011$, t 14.46 for juvenile, 25.17 for male and 61.44 for female, $t_{0.001}$ 8.61, df= 4). In juvenile *Macrobrchium nobilii* the percentage content of phosphorous is higher in abdominal muscle (1.62 ± 0.09) than male (1.13 ± 0.9) and female (1.38 ± 0.11). The phosphorous content of juvenile, male and female cheliped muscle did not vary significantly ($P>0.05$). However in the abdominal muscle there is a significant difference ($p<0.05$) in phosphorous content (Table 3). But within the groups there is a significant variation between abdomen and cheliped muscle observed only in juveniles ($p<0.05$).

Table 3. ANOVA to find out the validity of relationship between the studied parameters in three chosen groups of *Macrobrachium nobilii*

Variation	SS	Df	MS	F
Moisture- abdomen muscles				
Total	3.20	14		
Between Groups	2.16	02	1.08	12.51**
Error	1.03	12	0.09	
Moisture-Chelipeds				
Total	180.07	14		
Between Groups	172.73	02	86.37	141.25***
Error	007.34	12	00.61	
Ash-Abdomen muscles				
Total	6.99	14		
Between Groups	3.51	02	1.75	6.05*
Error	3.48	12	0.29	
Ash-Chelipeds				
Total	1.12	14		
Between Groups	4.81	02	2.41	0.03^{NS}
Error	1.12	12	0.09	

*** Statistically highly significant ($P<0.001$) ** Statistically Highly significant ($P<0.01$)
* Statistically significant ($P<0.05$) NS- not significant ($P<0.05$)

Crustaceans require high amount of minerals since there is a significant loss of there minerals during ecdysis (Huner *et al.* 1990) through the loss is partially compensated by minerals obtained from food. Hence it is recommended to include seven minerals (calcium, copper, magnesium, phosphorous, potassium, selenium and zinc) in crustacean diets (Davis and Gatlin 1996), of these calcium and phosphorous play a major role since they contribute per se to the structural components of hard tissues like exoskeleton. Apart from this, calcium is also essential for muscle function, proper nerve impulse transmission and act as a cofactor for enzymatic process (National Research Council 1993). Phosphorous is a component of a variety of organic phosphates, such as nucleotides, phospholipids, coenzymes, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Inorganic phosphates serves as buffers to maintain normal pH of intra and extra cellural fluids (Zubay 1983). Through various decapods obtain environmental calcium for their normal growth, dietary calcium also plays a supplementary role in mineralization of exoskeleton (Deshimaru *et al.* 1987, NRC 1993) and the interactions between calcium and phosphorous has also evaluated (Brown 1995).

Unlike calcium, phosphorous concentration is very low in natural sources and hence its proper incorporation in the crustacean diet plays a major role in their nutrition (Lall 1991, Mayeaux 1988). Lochnam *et al.* (1992) reported the vital requirement of supplemental phosphorous rather than other minerals for *Procambarus clarkii.* Usually incorporation of phosphorous at a concentration of 1-2% of the diet promotes optimum growth among various decapods; however, its proper utilization also depends upon the availability of calcium.

From the available literature, it is known that both calcium and phosphorous levels vary from species to species. For instance calcium content is 0.35% in M. *dayanum* (Paul and Gupta 1995) and 26.6% in *Autacus astacus* (Welinder 1974). The phosphorous content is vary from 0.49% in *Metapearus* spp. (Dall 1965) to 2% in *A. astacus* (Huner and Lindqvist 1985). For *M. nobilii* the calcium and phosphorous levels fall within this range. The difference in the uptake of minerals in a species is adduced to the availability of these elements in environment, age and sex of the animal (Greenaway 1985).

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References

Boyd, C.E. and D. Teichert-Coddington, 1995. Dry matter, ash and chemical composition of pond cultured *Penaeus vannamei* and *P. stylirostris. J. World Aquacul. Soc.,* 26: 88.

Brown, P.B., 1995. A review of nutritional research with crayfish. *J Shellfish. Res.*, 14: 561-568.

Chang, E.S., 1995. Physiological and biochemical changes during the molt cycle in decapod crustaceans: an overview. *J. Exp. Mar. Biol. Ecol.*, 193: 1-14.

Davis. D.A. and D.M. Gatlin, 1996. Dietary mineral requirement of fish and marine crustaceans *Rev. Fish. Sci.*, 4: 75-99

Davis, D.A., A.L. Lawrence and D.M. Gatlin, 1992. Mineral requirements of *Penaeus vannamei*: a preliminary examination of the dietary essentiality of thirteen minerals. *J. World Aquacul.. Soc.*, 23: 8-14.

Deshimaru, O., K. kuroki, S. Sakamoto and Y.Yone, 1978. Absorption of labeled ^{45} Ca by prawn from sea water. *Bull. Jpn. Soc. Sci. Fish.*, 44: 975-977.

Fiske, C.H. and Y. Subbarow, 1925. The colorimetric determination of phosphorous. *J. Biol. Chem.*, 66: 375-400.

Greenaway, P., 1985. Calcium balance and moulting in the crustacea. *Biol. Rev.*, 60: 425-454.

Henderson, J.R. and G. Matthai, 1990. On certain species of Palaemon from South India. Rec. *Indian. Mus.*, 5: 277-306.

Huner. J.V., H. Kononen and O.V. Lindqvist, 1990. Variation in body composition and exoskeleton mineralization as a function of the molt and reproductive cycles of the noble crayfish, *Astacus astacus* L. (Decapoda, Astacidae), from a pond in central Finland, Com. *Biochem. Physiol.*, 96: 225-240.

Lall, S.P., 1991. Digestibility, metabolism and excretion of dietary phosphorus. *In*: Nutritional strategies and aquaculture waste (eds. C.B. Cowey and C.Y. Cho). University of Guelph, Ontario. pp. 77-90.

Lochmann, R., W.R. McClain and D.M. Gatline, 1992. Evaluation of practical feed formulation and dietary supplements for red swamp crayfish. *J. World Aquacul. Soc.*, 23: 217-227.

Mayeaux, M.H., 1988. Nutrient requirement studies with the red swamp crayfish, *Procambarus clarkii*: phosphorus and lysine. MS thesis, Texas A & M University, College Station, Texas.

Mendez L., B. Acosta and I. Recotta, 1988. Mineral concentrations in muscle and hepatopancreas of newly caught wild and hatchery-exchausted spawners of Pacific white shrimp, *Penaeus vannamei*. *J. Appl. Aquacul.*, 8: 17-26

Passoneau, J.V. and C.M. Willimas, 1953. The molting of Cecropia. *J. Exp. Biol.*, 30: 545-560.

National Research Council (NRC), 1993. Nutrient Requirements of fish. National Academy Press, Washington D.C.

Van Loon, J.C., 1995. Selected methods of trace metal analysis: biological and environmental samples. Wiley-Interscience, Toronto, Canada. pp 1-357.

Welinder, B.S., 1974. The crustacean cuticle-I. Studies on the composition of the cuticle. *Comp. Biochem. Physiol.*, 47: 779-787.

Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall, Upper Saddle River, New Jersey, USA.

Zubay, G.L., 1983. Biochemistry. Addision-Wesley, Reading, UK.

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