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Studies on the Analysis of Proximal Changes During Molting Process in the Penaeid Prawn, *Penaeus monodon*

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Abstract: The molt cycle is of immense physiological importance in the crustaceans. The present study reveals the changes in the proximate chemical composition, fresh mass, water content, ash content, organic constituents, lipid and protein contents of penaeid prawn, *Penaeus monodon* during the molting process of this species. This study was initiated to investigate changes that may occur in mass and tissue composition during different molting stages. Molting stages was determined by microscopic examination. The results obtained in the present investigation through light on the significance of water, protein, inorganic and organic components and concluded that altered concentrations of these parameters were observed in different molting stages of the prawn *Penaeus monodon*.

Key words: Body composition · Changes · Molting · Prawn · *Penaeus monodon*

INTRODUCTION

The physiology, behaviour and reproduction of crustaceans is intrinsically linked to the molting cycle. Molting is a phenomenon, which increases the weight of the organisms in crustacea. Drach and Tchernigovtzeff [1] evolved the criteria for identification of different molting stages in crustaceans. The methodology used for molt staging generally involve observations of the degree of hardness of the exoskeleton and microscopic examination of the transparent edge of the uropods or pleopods, where epidermal withdrawl and development of new setae can be observed [2]. Molting cycle in crustaceans is composed of different stages including postmolt, (A-B stages), Intermolt (C-Stage), Premolt (D-Stage) and Ecdysis (E-Stage). Each and every stage has got its specific histological evidence to identify the specific stage. The penaeid prawns are considered to one the most important group of cultivable organisms which has attracted the several entrenuprenuers for going for culture of these species because of their high growth rates, adoptability and tolerance to varied environments and popular food materials in different parts of the world. The juvenile tropical penaeid prawns *Penaeus monodon*, *P. indicus*, *Metapenaeus monoceros* are most abundant littoral penaeids found in the inshore and estuarine environments. But mature prawns are commonly present in deeper offshore areas adjacent to the juvenile habitats. Despite in relative abundance, constituting over 75% of all prawn catches, the *Penaeus monodon* has received scant mention. With the exception of cursory investigations of its general biology, little data is available on molting physiology. Since the molt cycle is of immense physiological importance, the present study was initiated to investigate changes that may occur in mass and tissue composition during different molting stages. When comparing the changes in the biochemical composition of animals relative to various physiological phenomena, expression of results is of critical importance. Generally, results of this nature have been expressed as changes in percentage composition [3]. In crustacea where mass but not length changes during Intermolt, it would be convenient to describe changes in composition in terms of an animal of a given length. This would enable not only the changes in individual tissue components to be compared relative to each other, but would also allow changes in total mass to be determined during for

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example, a simple molt cycle. Use of a "Standard animal" to demonstrate absolute changes in body composition follows that of Caulton and Bussell [4], who illustrated the value of the technique. Although there is a wide variety of biological data on these prawns, the biochemical data relative to the molting biology of the penaeid prawn, *Penaeus monodon* are very scarce [5]. Knowledge of the biochemistry and metabolic changes during the molt cycle are essential for a complete understanding of crustacean molting process. Hence, present study was conducted to find out the changes in the proximate chemical composition, fresh mass, water content, ash content, organic constituents, lipid and protein contents of penaeid prawn, *Penaeus monodon* during the molting process.

MATERIALS AND METHODS

Collection and Preservation: Immature *Penaeus monodon* were collected from nearly estuaries. Mature animals were collected from Trawlers. The carapace length (distance between the Post-orbital notch and the posterior mid-dorsal margin of the carapace), fresh mass and molt cycle stage were recorded for each prawn immediately on capture.

Molt Stage: Molt stage determinations were made by microscopic examination of the dorsal surface of the exopodite of the uropod. Molt staging followed that of Read [6], modified from Scheer [7], Aiken [8] and Drach and Tchernigovtzeff [1]. Basically Post-molt was divided into A and B stages, inter-molt (C) and pre-molt was subdivided into five stages D_0, D_1, $D_{2,}$ D_3 and D_4 broadly, respectively.

Body Composition: To preclude any variation in tissue constituents imposed by the molt cycle, only on inter-molt (Stage C) prawns were chosen for analysis. The prawns were paper dried and weighed and the carapace length was recorded.

Most analysis was undertaken using intact individual prawns. Prawns were oven dried at 80° C for 48 hr, re-weighted and the loss of water recorded, as the water content. The dry carcass was then homogenized and stored in a deep freeze pending analysis. Protein was estimated using a standard Kjeldahl titrametric technique. Lipid determination followed a modified method of Bligh and Dyer [9]. Ash content was determined by combustion of 0.5 g homogenized aliquot for 4 hrs in a muffle furnace.

Calculations: The prawn with 18 mm carapace length (mean carapace length of immature prawns) was used to describe changes in composition during molting cycle. Regression equation was derived from the relationship between carapace length and various tissue constituents. These data incorporate all stages of molt cycle. The relative carapace length/tissue exponent (B) was item used to correct the tissue mass of any individual to that of the standard. The procedure followed can be illustrated by the following example. A prawn in stage C of the molt cycle with a carapace length of 26.97 mm has a measured protein content of 3.42 g. What is the predicted protein value (P_2) of a standard prawn of 17 mm carapace length. Using the Formula $P_2 = P_1/L^B \times L^B{}_{std}$ (Where P_1 is the measured protein mass of an individual of L mm carapace length and B the predetermined carapace length / tissue regression exponent. By substitution the values in the following equation:

$$P_2 = 3.42 / 26.97^{2.9753} \text{ X } 17.0^{2.9753} = 0.8688 \text{ g}$$

Thus the estimated protein value of the standard prawn is 0.8688 g. In this way each individual tissue constituent mass can be corrected to a prawn of standard size.

Statistical Analysis: The experiments were done in triplicate. Data are expressed as the means ± SD (standard deviation).

RESULTS AND DISCUSSION

Changes in Proximate Composition During Different Molting Stages: Figures 1, 2 and 3 illustrates the changes in fresh mass, water content and ash of juvenile prawn of standard carapace length (18 mm) during a single molt cycle. Figure 1 shows that fresh mass increases from 3.94 of to reach a maximum of 4.53 g at stage D_2. By means of comparison, measurements of mass increases in laboratory maintained juveniles during molt cycle can be described by a linear function of the form $M_2 = 0.0906 + 1.11269 M_1$ ($r = 0.9956$), where M_1 is the initial inter-molt mass in g and M_2 the inter-molt mass preceding ecdysis. From this equation a prawn of mass 3.94 g of would be expected to increase to 4.53 g, which agrees favorably with the increase shown for prawn shown in Figure 1. It was observed that, water content of prawns also increases (Fig. 2) similarly to that of fresh mass, which is not surprising since water percent accounts for approximately 75% of the fresh mass. Figure 3 shows

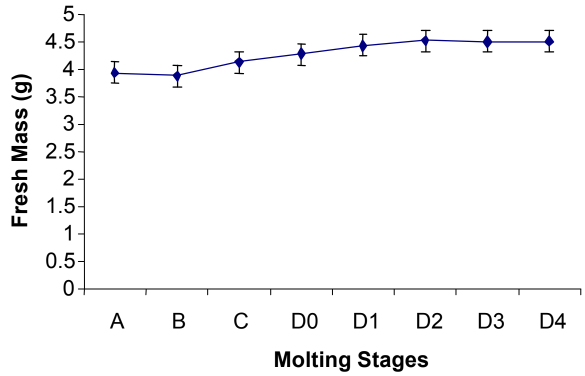

Fig. 1: Changes in freshmass (g) in different molting stages of prawn *Penaeus monodon*

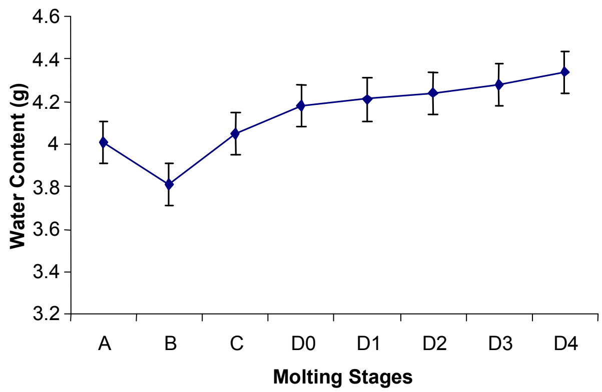

Fig. 2: Changes in water content (g) in different molting stages of prawn *Penaeus monodon*

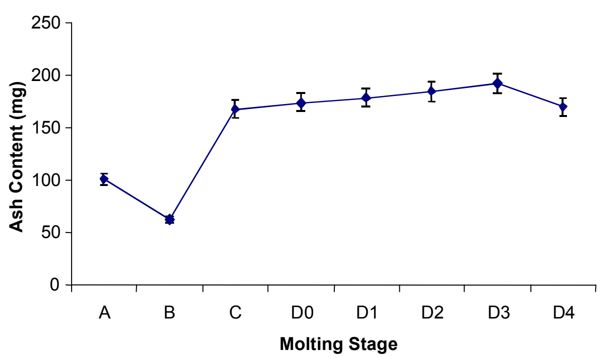

Fig. 3: Changes in ash content (mgs) in different molting states of prawn *Peneaus monodon*

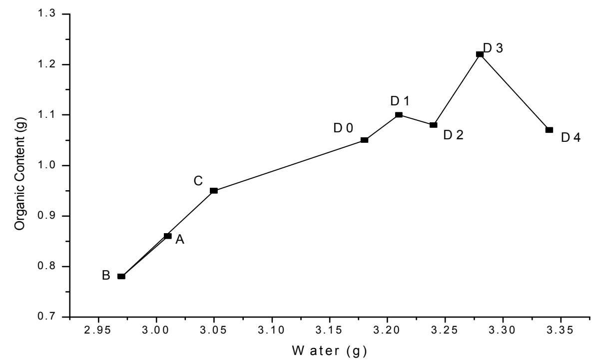

Fig. 4: Changes in the relationship between water (g) and organic constituents (g) during different molting stages of prawn *Peneaus monodon*

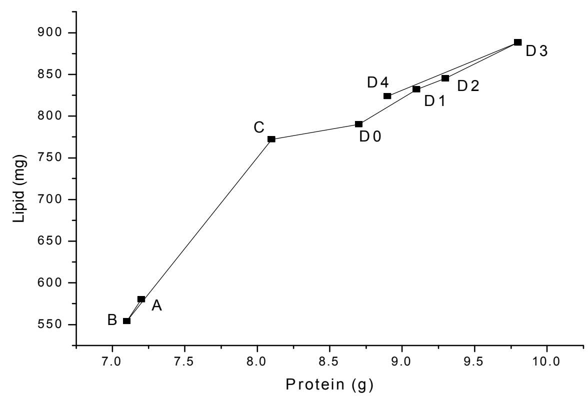

Fig. 5: Changes in the relationship between protein (g) and lipid (mgs) during different molting stages of prawn *Penaeus monodon*.

the ash content changes during molt cycle. To describe the relative changes between the major tissue components during the molt cycle trends are more apparent if the data are plotted as shown in Figures 4 and 5. The lipid content showed a decrease from stage A to B with a less marked decrease in protein content. This phase of catabolic energy utilization corresponds to a hardening of the new exoskeleton by the deposition of minerals (Fig. 3) during the period when the prawn is not feeding. But from stages B to C, there is a rapid synthesis of both lipid and protein occurs. This continues to stage D but with less emphasis being given to lipid synthesis during the phase compared with the previous phase. At stage D^4 the decline in both lipid and protein suggests a contribution to the catabolic energy required for the forthcoming molt. The results also supports that some organic compounds are also lost during this time (Fig. 5). The relationship between organic components i.e. lipid and protein and water can be followed in a similar manner (Fig. 4 and 5). During post ecdysal catabolism there is a small decrease in water content. The water content then increases during inter-molt but the most rapid increase occurs during remaining stages D. This trend continues to stage D_3, even though the organic and inorganic components appear to decrease. The energy content of the prawn varies according to the lipid protein ratio but averages 20 (20 kJ/g including ash or 24.00 kJ/g ash free). The energy content of exuviae were obtained from laboratory maintained prawns of known carapace length.

The relationship between exuvium energy and carapace length can be described by the equation $E=0.000025\ L^{3.46}$ ($r = 0.991$, $n = 14$), where E is the energy content of exuvium in KJ and L is lte carapace length in mm. From this equation it can be estimated that the energy content of the exuvium from a prawn of 18 mm carapace length would be 0.45 KJ demonstrating that some residual organic matter is lost at Ecdysis.

It is generally understood that the increase in size at ecdysis in crsutacea is due to water uptake [10, 11]. The volume increase due to this hydration governs the potential growth from one molt cycle to the next since "the tissues grow up to the volume established by this hydration" [2]. Little mention was made of the fate of this water after ecdysis but the impression given is that after the exoskeleton has hardened it is progressively lost as tissue is synthesized. Travis [13] clearly demonstrated that during molt cycle of *Panulirus orgies,* the ratio of water to fresh mass is greatest at ecdysis and during A stage and this proportion declines to early premolt before rising again at late pre-molt. A similar pattern is observed in *P. monodon* with a 76% water content at stage A steadily declining to 72% at D_1 and item increasing to 73 % at D_3. The water content appears to be minimum during post molt stages A and B. But the water content of the prawn decreases from stage A to B (Figure 2). Since it was not possible to determine if any animals were analyzed at ecdysis water uptake at this stage cannot be confirmed but it can be stated that a large proportion of any excess water that may be taken up is lost by stage A. At this stage is extremely short it would be expected that at least some of the prawns analyzed would show some indication of high water content. It is surprising that the water content of the stage D prawns does not show a greater variation to account for the water uptake that is understood to occur during this time.

From these results it appears that in the case of *Penaeus monodon* either the pre-ecdysial water is taken up and lost exceedingly rapidly or that the relatively small uptake between stages D_1 and D4 can be manipulated hydrostatically to create the necessary expansion that would be required to form an exoskeleton of the correct dimensions after ecdysis. Lipid and protein synthesis are not restricted to any particular period of the molt cycle but commences as soon as the exoskeleton hardens and the prawn begins feeding. The marked increase in lipid content from stage B to D_4 probably reflects an increase in heptopancreatic lipid ascribed to the commencement of feeding and an increase in lipid synthesis was reported in different crustaceans [14, 15]. Laboratory studies have indicated that *P. monodon* is capable of spawning at least three times during one complete molt cycle. During molt cycle in juvenile prawns there is a gradual increase in water content to reach a maximum at stage D_3 and D_4. Hannan and Evans [16] have demonstrated that *Penaeus duorarum* is capable of drinking water at the rate of 17.3 µl /g/hr in 100% seawater. If *P. monodon* has similar capabilities an animal of 38 mm carapace length could regain approximately 650 µl in 1 hr after spawning.

This study has shown that expression of the result on an absolute basis has enabled quantitative, compared with percentage differences during molt cycle. Of the changes in mass and chemical composition, the varying water content has proved the most revealing. It seems that not only does water play a vital role in ecdysis [11], but in the case of *P. monodon* during the molt cycle where a gradual increase in late pre-molt has been recorded. In light of these findings, where an accurate measurement of the increase in fresh mass is required, the choice of the stage of the molt cycle at which the weightings recorded should be given careful considerations. The results obtained in the present investigation through light on the significance of water, protein, inorganic and organic components in different molting stages of prawn *Penaeus monodon*.

REFERENCES

1. Drach, P. and C. Tchernigovtzeff, 1967. Sur la methode de determination des stades d'intermue et son application generale aux Crustads. Vie Milieu., 18: 596-609.
2. Yamaoka, L.H. and B.T. Scheer, 1970. Chemistry of growth and development, In: Chemical zoology, Eds, M. Flokin and B.T. Scheer, Pages 321-342V, A. Crustaceans. Academic Press, New York.
3. Johnson, J.T. and T.L. Hopkins, 1978. Biochemical components of the mysid shrimp Taphromysis, J. Exp. Mar. Biol. Ecol., 31: 1-9.
4. Caulton, M.S. and E. Bussell, 1977. The relationship between changes in condition and body composition of young *Tilapia rendalli*. J. Fish Biol., 38: 143-150.
5. Tuck, I.D., C.J. Chapman and R.J.A. Atkinson, 1997. Population biology of the Norway lobster, Nephrops norvegicus (L.) in the Firth of Clyde, Scotland-I: Growth and density. ICES, J. Mar. Sci., 54: 125-135.
6. Read, G.H.L., 1977. Aspects of lipid metabolism in *Penaeas indicus* Milne Edwards, M.S. Thesis, University of Natal, King George V Avenue, Durban, South Africa.

7. Scheer, B.T., 1960. Aspects of the intermolt cycle in natartians. Comp. Biochem. Physiol., 1: 3-18.
8. Aiken, D.E., 1973. Proecdysis, setal development and molt prediction in the American lobster (*Homarus americanus*). J. Fisheries Res. Board of Canada, 30: 1337-1344.
9. Bligh, E.G. and W.J. Dyer, 1959. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917
10. Passano, L.M., 1960. Molting and its control. *In:* T. H. Waterman, ed. The physiology of Crustacea Vol. 1: pp: 472-536. Academic Press, New York, New York.
11. O'Connor, J.D. and L.I. Gilbert, 1969. Alterations in lipid metabolism associated with premolt activity in a land crab and a freshwater crayfish. Comp. Biochem. Physiol., 29: 889-904.
12. Chan, S., S.M. Rankin and L.L. Keeley, 1988. Characterisation of the molt stages in *Penaeus vannamei*: setogenesis and hemolymph levels of total protein, ecdysteroids and glucose. Biological Bulletin, 175:185-192.
13. Travis, D.F., 1954. The molting cycle of the spiny lobster, *Panulirus argus* Latreille. I. Molting and growth in laboratory maintained individuals. Biol. Bull., 107: 433-450.
14. Patrois, J., H.J. Ceccaldi, T. Ando, A. Kanazawa and S. Teshina, 1978. Variation in the lipid synthesis from acetate during moulting of prawn, Bull. Jap. Soc. Sci. Fish., 44: 139-141.
15. Reaka, M.L., 1975. Molting in stomatopod crustaceans. J. Morphol., 146: 55-80.
16. Hannan, J.V. and D.H. Evans, 1973. Water permeability in some euryhaline decapods and *Limules polynya* sps. Comp. Biochem. Physiol., 44: 1199-1213.