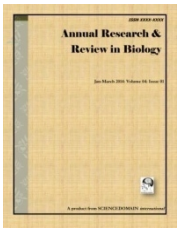

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Variation in the Biochemical Constituents during Different Moulting Stages in Green Tiger Shrimp, *Peneaus semisulcatus*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors BAP and AJA and MW designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author MSAK managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

A hallmark of crustacean physiology is the periodic shedding of their old exoskeleton achieved by moulting as an external manifestation of a discontinuous growth process. Crustacean metabolism, reproduction and behavior are all affected by the periodic shedding of the exoskeleton and the characteristics of moulting cycles. There are major gaps in our understanding of moulting patterns of commercially raised shrimp; hence, further investigations on the duration of each moult stage, in-moult cycle and the relationship between biochemical constituents and moult cycling are essentially needed. The present research work was aimed at describing characteristic features and biochemical changes that occur at various stages (A-D) during moulting cycle of the Indian green tiger shrimp *Penaeus semisulcatus*. Experimental animals (wet weight, 20 ± 2 g) were reared individually in aerated plastic aquaria under normal laboratory conditions (12L: 12D; 28.2ºC) and 50% of the water was replaced daily for one month. Animals were provided twice daily with commercial Magnum Scampi feed *ad libitum* and were observed regularly for moulting by setal development and biochemical analysis in conjunction with established parameters for morphological changes during the moulting cycle. Results

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showed that muscle protein content peaked during the post-moult stage A (51.23 ± 2.51 mg/g) and gradually declined through the inter-moult and pre-moult stages. Similarly in the hepatopancreas, distinct increase in the total protein content was observed during the post-moult stage B (15.78 ± 0.26 mg/g) and a steady decline was noticed thereafter ($p < 0.05$). Significantly higher levels of total sugars from muscle tissues were observed in late post-moult stage B (51.23 ± 2.65 mg/g) and minimum level of total sugars were observed in late pre-moult stage D_{2-3} both in muscle tissue (28.43 ± 2.98 mg/g) and in hepatopancreas (18.79 ±1.62 mg/g). A sharp fall in lipid content of muscle tissues was observed in inter-moult stage C (19.54 ± 1.45 mg/g) and a corresponding decline (48.21 ± 3.25 mg/g) was observed in the hepatopancreas. The present study documents and further expands our understanding of the physiological and biochemical changes occurring in *P. semisulcatus*during four different moulting stages and will provide useful criteria for identifying different stages in the life cycle of this commercially farmed shrimp.

Keywords: P. Semisulcatus; moulting; biochemical changes; physiology.

1. INTRODUCTION

One of the important aspects of crustacean physiology is the periodic shedding of old exoskeleton which is accomplished by moulting, an external manifestation of discontinuous growth process. The actual preparation for the subsequent moult, however, consists of numerous biochemical, physiological and morphological changes that temporally occupy much of the proceeding moult cycle [1].

The classical work of [1] made it possible to recognize three major moult stages such as inter-moult, pre-moult, and post-moult, which are further subdivided into several sub stages. The inter-moult is the interval between two successive moults, whereas the pre-moult and post-moult represent the preparatory as well as post-ecdysial stages, respectively. The schedule of moult staging on the basis of setal development has been described in many Penaeid species [2,3,4,5,6,7]. Preliminary studies on the moult staging of *Penaeus indicus* were carried out by [8,9,10] and for tiger shrimp *Penaeus monodon by* [11].

In this study, Indian green tiger shrimp *Penaeus semisulcatus* has been selected because of its growing importance for shrimp farming in the Indian sub-continent where its farming has been standardized. However, there is a great need to fill gaps in our knowledge on moulting patterns of this commercially important shrimp. The current basic problems of importance that need further investigation, include the duration of each moult stage within the moult cycle and the relationship between biochemical constituents and moult cycle. Metabolism, reproduction and behavior are affected both directly and indirectly by the periodic shedding of the exoskeleton [12]. The present study was restricted to study some characteristic features and biochemical changes in macromolecular components such as total proteins, total sugars and total lipids during various stages of moult cycle in the marine green tiger shrimp, *P. semisulcatus.*

2. MATERIALS AND METHODS

2.1 Collection of Shrimps

Adult fresh live shrimps, *P. semisulcatus,* were obtained from fishermen at fish landing center in Kasimedu, Chennai and stocked in large rectangular cement aquaria (90 × 60 × 60 cm; 175 L capacity) with adequate aeration. Experimental animals (wet weight, 20 ± 2 g) were reared individually in aerated plastic aquaria (16 L capacity) under normal laboratory conditions (12L: 12D; 28.2ºC) and 50% the water was exchanged every day for a month. Animals were fed, *ad libitum,* commercially available feed (Magnum Scampi feed; 31% crude protein, 4% crude fat, 7% crude fiber and 11% moisture) twice a day (08.00 h and 18.00 h) and were observed regularly for moulting.

2.2 Analysis of Moult Stages

Setal development of *P. semisulcatus* was observed at the posterior median part of the pleopods and uropods. Moult stages were determined based on morphological changes of the seta as described by [1] using light microscope (Labex, India). The pleopods and uropods were removed, mounted on a microscope slide in filtered sea water and stages of moulting were recorded with CCD micro-imaging systems (CCD_MODULE_BME_C_721, Korea).

2.3 Estimation of Total Protein, Sugars and Lipids

Total protein and sugars of muscle and hepatopancreas were quantified following the method of [13] and [14] respectively. Total lipid was quantified according to [15] and extraction of lipids from the samples was done as described by [16].

2.4 Statistical Analysis

Data obtained from biochemical analysis with reference to proteins, sugars and lipids at various stages of moulting were subjected to analysis of variance (ANOVA) and difference between different stages of moulting were determined by Duncun's test. Data are presented as mean ± SD. The values of $P<0.05$ were considered significant. Analyses were carried out using software SPSS v. 10.

3. RESULTS AND DISCUSSION

3.1 Description of Moulting Cycle

A systematic explanation of different moult stages of *P. semisulcatus* is given in Table 1. Moult stages were characterized by the morphological changes such as cuticle hardness, rigidity or changes in developing setae of pleopods and uropods.

On the basis of setogenesis, the moult cycle has been classified into 6 well defined stages viz. Post-moult (sub stages A & B), inter-moult (C) and pre-moult (sub stages D_0, D_1, D_{2-3}).

Table 1. Moulting cycle stages in *P. semisulcatus*

Moult stages	Diagnostic characters
Post-moult	Freshly moulted shrimps and extremely quiescent, cuticle soft
Stage A	and pliable, pleopod soft and transparent, Setae thin walled, granular protoplasmic matrix.
Stage B	Carapace hard, pleopod hard and rigid with the development of cuticular nodes.
Inter-moult	Exoskeleton remains hard, setal lumen becomes narrow; setal
Stage C	wall translucent; setal cone formation.
Pre-moult	Beginning of epidermal retraction (apolysis), protoplasmic
Stage D_0	invagination in the site of future setae resulting in scalloped epidermis
Stage D_1	Exoskeleton remains brittle, retracted zone between old cuticle and epidermis widens; the tip of new setae is either within the setal groove, or protrudes into the retracted zone. New setae clearly visible.
Stage D_{2-3}	Epidermal retraction continues; fully developed new setae appear in the matrix as tube-in-tube structure.

3.1.1 Stage A: early post-moult

The early post-moult (A) occurs just after ecdysis. The cuticle was thin and had a slotted appearance due to absorption of water by the animal through its soft exoskeleton. The setal lumen was continuous and not pinched off at the base of the setae (Fig. 1).

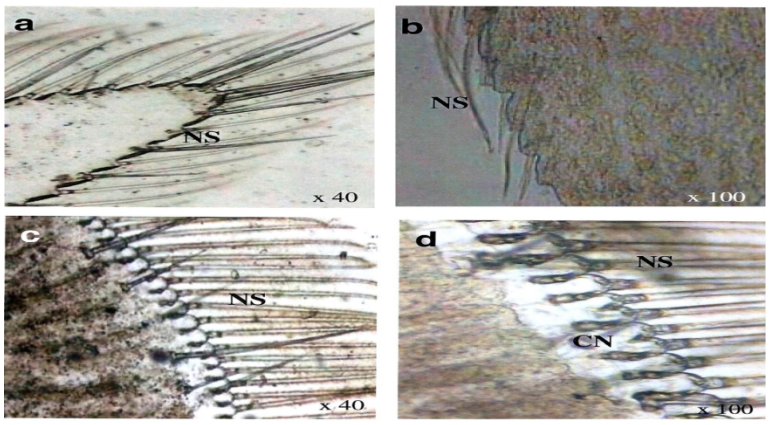

Fig. 1. Moulting stages of *Penaeus semisulcatus* - stage A (early post-moult)
a& b – Pleopodalsetogenesis,
c& d - Uropodalsetogenesis
CN - Cuticular nodes
NS - New setae

3.1.2 Stage B: late post-moult

The cuticle became thicker. The matrix was expanded throughout the setae, but remains vacuolated. The occurrence of chromatophores was noticed at this stage (Fig. 2).

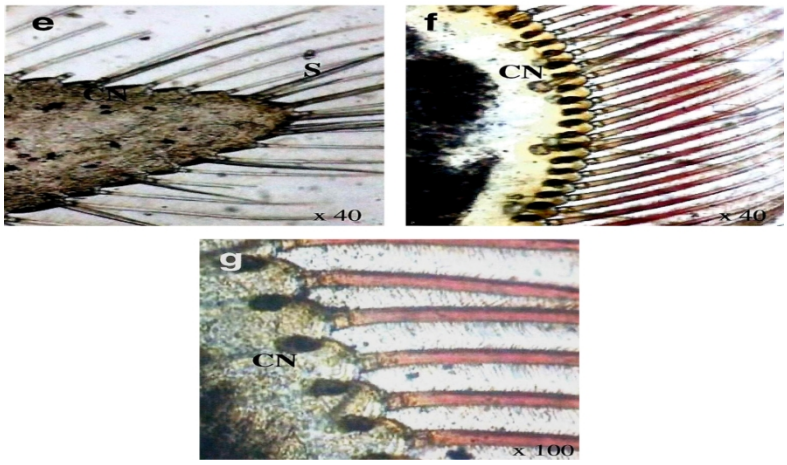

Fig. 2. Moulting stages of *Penaeus semisulcatus* - stage B (late post-moult)
e – Pleopodalsetogenesis
f& g - Uropodalsetogenesis
CN - Cuticular nodes
S - Setae

3.1.3 Stage C: inter-moult

Inter-moult was characterized by having a well formed cuticle. The setal lumen remains pinched off at the base and a gradual condensation of the setal lumen was observed followed by cone formation (Fig.3).

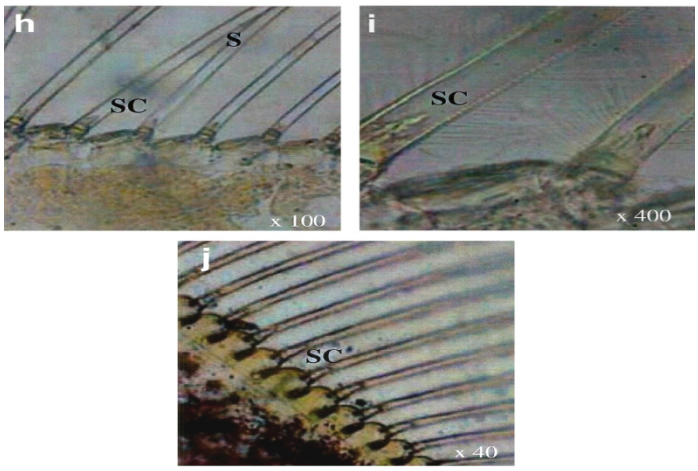

Fig. 3. Moulting stages of *Penaeus semisulcatus* - stage C (inter-moult)
H & i – Pleopodalsetogenesis
J - Uropodalsetogenesis
SC - Setal cone
S - Setae

3.1.4 Stage D: pre-moult

Setal development and pre-ecdysialcuticular changes can be observed in the pleopod and uropod from the earliest D_o stage through D_{2-3} stages. Degree of retraction of epidermal tissue determined different sub stages of pre-moult.

3.1.4.1 D_o stage: early pre-moult

Once the retraction begins, the shrimp is designated as stage D_o. The pre-moult stage is characterized by old cuticle resorption and new cuticle synthesis. The first morphologically distinguishable evidence of the pre-moult stage starts with apo-lysis, the retraction of the epidermis from the cuticle, and the creation of a moulting space for the formation of new cuticle. The setal cone in the setae becomes shorter (Fig. 4).

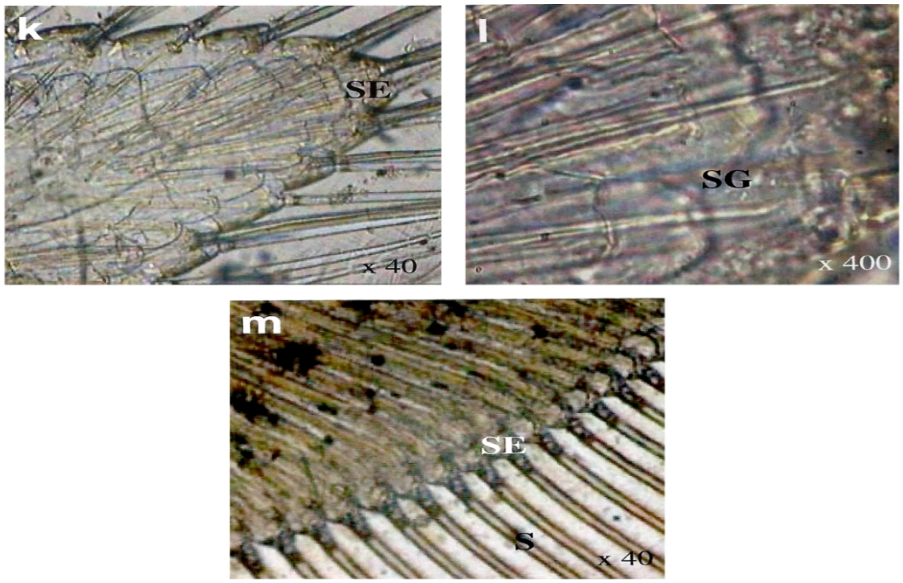

Fig. 4. Moulting stages of *Penaeus semisulcatus* - stage D_0 (early pre-moult)
k& l – Pleopodalsetogenesis
m - Uropodalsetogenesis
SE - scalloped epidermis
SG - setal groove
S - setae

3.1.4.2 D_1stage: early pre-moult

The anterior part of pleopod and uropod showed comparatively greater degree of epidermal retraction than the D_0 stage. Condensation of protoplasm was noticed in the region where new setae are formed. Later, the protoplasm invaginated at the site of future setae, giving rise to a scalloped appearance (Fig. 5).

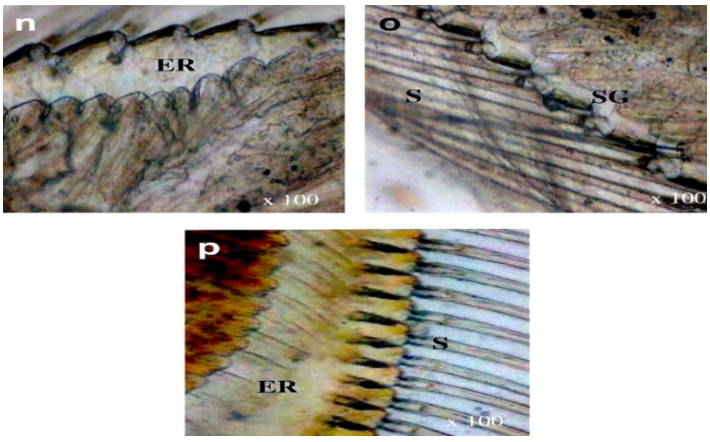

Fig. 5. Moulting stages of *Penaeus semisulcatus* - stage D_1 (early pre-moult)
n& o – Pleopodalsetogenesis
p - Uropodalsetogenesis
ER - epidermal retraction
SG - Setal groove
S - setae

3.1.4.3 D_{2-3}stage: late pre-moult

This stage precedes ecdysis and was characterized by maximal retraction of epidermis and is well developed along setae. New cuticle is clearly seen as a transparent layer that isfree of granules. The retraction of the epidermis in the anterior and lateral regions of pleopod and uropod are greater than in stage D_1. The split of delimitation broadens and the separation between inner and outer tubes of new seta becomes obvious. The new setules pinch off from the setal matrix at this stage. The cuticular lining of new setae and their tubes is thickened and becomes clearly visible as a 'tube in tube structure' giving a striated appearance (Fig. 6).

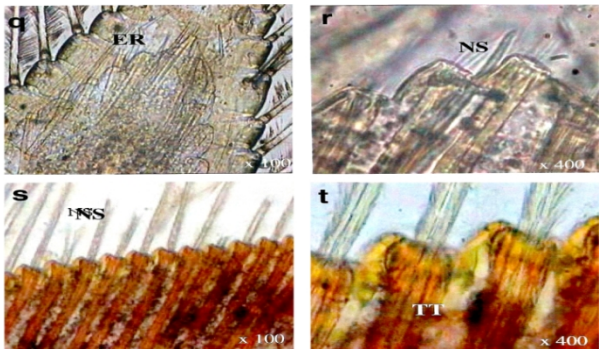

Fig. 6. Moulting stages of *Penaeus semisulcatus* - stage D_{2-3} (late pre-moult)
q& r – Pleopodalsetogenesis
r - Uropodalsetogenesis
ER - epidermal retraction
TT - tube in tube structure
NS - new setae

3.2 Biochemical Constituents

3.2.1 Total protein content of muscle tissue and hepatopancreas

Higher protein content was observed in muscle tissue, than in hepatopancreas during the different moult stages. Total protein content of muscle tissue was observed to be maximal during post-moult stages A (51.23 ± 2.51 mg/g) and B (48.57 ± 3.0 mg/g) and a steady decline was noticed during the inter-moult and pre-moult stages. Similar conditions were also observed in hepatopancreas with distinct increase in the total protein content during the post-moult stages A (15.23 ± 0.58 mg/g) and B (15.78 ± 0.26 mg/g) and steady decline was noticed thereafter. The variations in the total protein levels in muscle and hepatopancreas during moult stages were significantly different (*P* =0.05; Fig. 7).

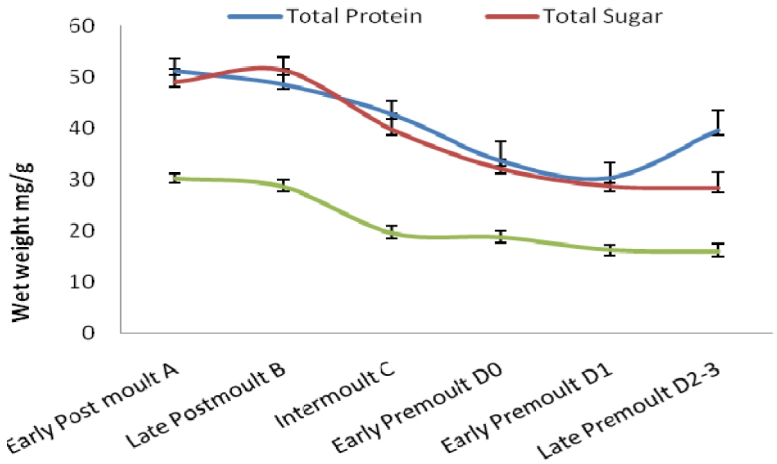

Fig. 7. Biochemical constituents in the muscle tissue of *P. semisulcatus* during different moulting stages. Moulting stages

3.2.2 Total sugar content of muscle tissue and hepatopancreas

The total sugar content was also observed to decrease during the early moulting stages. Significantly higher levels of total sugars were observed at late post-moult stage B in muscle tissue (51.23 ± 2.65 mg/g) and in hepatopancreas (38.16 ± 2.01 mg/g) $p < 0.05$. Gradual decline was observed in the preceding stages and minimum level of total sugars was observed in late pre-moult stage D_{2-3} in muscle tissue (28.43 ± 2.98 mg/g) and in hepatopancreas (18.79 ±1.62 mg/g). Variations observed between moult stages in total sugars levels within muscle and hepatopancreas were significantly different (*P* =.05; Fig. 8).

3.2.3 Total lipid content of muscle tissue and hepatopancreas

Significant variations were observed in the levels of total lipid during the various stages of moulting (*P* = 0.05). A sharp fall in lipid content was observed in inter-moult both in muscle tissue (19.54 ± 1.45 mg/g) and in hepatopancreas (48.21 ± 3.25 mg/g), when compared to maximal levels in post-moult A (30.21 ± 1.01 and 78.23 ± 2.65 mg/g) of muscle tissue and hepatopancreas respectively (Figs. 7 & 8).

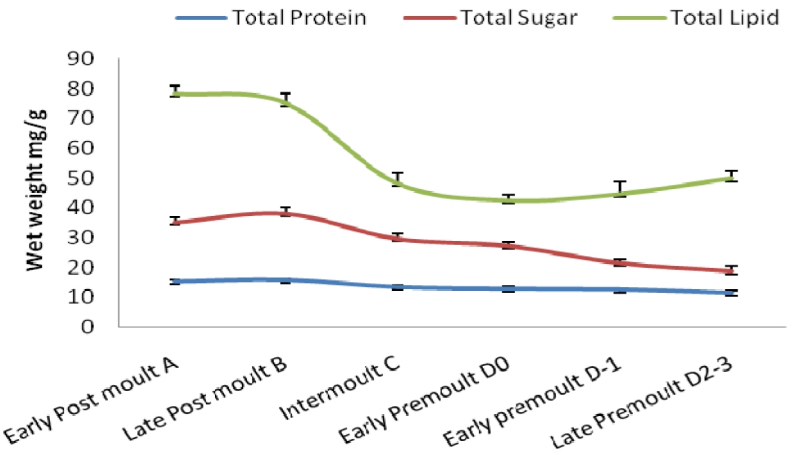

Fig. 8. Biochemical constituents in the hepatopencrease of *P. semisulcatus* during different moulting stages. Moulting stages

Statistical analysis clearly indicates that protein content in the muscle tissue did not differ significantly between early post-moult and late post-moult. However, the protein content significantly reduced up to early pre-moult D_1 stage. A similar trend was observed with total sugars and total lipids in the muscle tissue. Statistical analysis carried out with the data obtained for different biochemical constituents indicated that total protein did not show significant variation up to early pre-moult in the hepatopancreas. However, total sugars and total lipids decreased significantly from early post-moult A to early pre-moult D_1 stage. Subsequent increase in total sugars and total lipids in late pre-moult stage in hepatopancreas was probably due to formation or synthesis of new cuticle. Values with biochemical constituents studied in the hepatopancreas and muscle during different moulting stages show statistically significant difference (P = 0.05).

4. DISCUSSION

The moulting cycle in crustaceans is characterized by distinct morphological, physiological and biochemical events. The present study has identified and characterized several of these parameters for the green tiger shrimp, *P. semisulcatus*. For many years, setogenesis has been used as a criterion for describing moult stages in crustaceans [17]. Species variations in setal morphology and development results in differences among crustaceans in both staging criteria and in defining subdivisions of moult stages. The present study has attempted to establish identification criteria for the moult stages and sub stages in *P. semisulcatus*. These criteria include discernment in the pleopods of epidermis, setal lumen, internal cones and setal organs. Similar criteria have been used to determine stages for the Penaeidal shrimp, *P. duordarum* [2], *P. californensis* [19], *P. stylirostris* [20], *P. merguiensis* [21], *P. esculentus* [5] and *P. indicus* [10].

Moult staging may be accomplished using setogenesis in a variety of appendages. These appendages include the pleopods, as demonstrated in *A. leptodactylus* [22], *P. marginatus* [23] and *Chionoecetes opilio* [24] and the uropods in *Petrolisthes cinctipes* [25], *P. stylirostris*

[26] and *P. setiferus* [6]. Pleopods and uropods were used for the determination of moult stages because removal of other appendages results in trauma or death.

Complete retraction of the setal matrices observed in the present study has been reported in other penaeids during pre-moult, whereas in some individuals of *P. vannamei* retention of setal matrices was observed [7]. Deviations in setogenesis are even more pronounced in other decapods. In lobster, *Panulirus marginatus*, internal cones are lacking; thus, the distinction between stages B and C depends mainly on the thin and hollow appearance of the setal lumen in pre-moult [20]. These examples emphasize that moult staging must rely on a combination of setal characters. Furthermore, sub staging varies according to the investigators. In the present study, moulting cycle in *P. semisulcatus* was readily divided into stages, A, B, C, D_{0-3}. In the Cray fish, *Astacus leptodactylus* the moulting cycle was divided into A_{1-2}, B_{1-2}, C_{1-4} and D_{0-4} [20] and as A, B, C_{1-3} and D_{0-3} in *P. vannamei* [7]. These sub stages were not described in *P. semisulcatus* because these putative stages were of extremely short duration.

Available data for the protein levels in decapod crustaceans [23,24,9,25]) were mostly obtained from analysis of hemolymph collected during unspecified moulting stages. The range of values obtained for *P. semisulcatus*is were generally in agreement with these reports. Hepatoapanereas and muscle protein concentration in *P. semisulcatus* was maximal in the post-moult individuals; the lowest values were observed in pre-moult shrimps. Similar observations have been reported for *Panulirus argus* [26], *Orconectes limosus* [27], *Crangon vulgaris* [28] and *Penaeus monodon* [25]. The lower pre-moult protein levels in muscle and hepatopancreas are assumed to be due to re-adsorption from the chitin and epidermal protein complex of old exoskeleton and degradation of proteins for energy production [10,29]. Re-adsorption of the organic matter varies greatly among the species: 70% in *P. duorarum* [2] 23% in *Panulinus* [25] and 79% in *Carcinus* [12]. The increase in protein concentration in the post-moult stage results due to chitin formation for the new cuticle [25,12].

The titers of total sugars in hepatopancreas and muscle remained low during C and D stages and reached a maximal concentration in early and late post-moult A & B. Similar situation was reported for *Carcunus maenas* [30]. Although, titers of total sugars of *C. maenas* were more than twice the values reported for *P. semisulcatus*. In contrast to this pattern, [31] demonstrated that total sugars decreased shortly before ecdysis in three species of crabs. Since total sugar levels were highest after ecdysis, it is clear that the sugars have been mobilized from their storage organs viz., hepatopancreas and muscle, which are essential for either chitin synthesis for the new cuticle or as a source of energy during moulting [7].

The total lipid levels of hepatopancreas and muscle showed sharp decline from late post-moult B to inter-moult C and then gradually decreased up to late pre-moult stage D_{2-3}. In contrast, ovarian and haemolymph lipid levels have been found to increase during inter-moult and pre-moult period in other penaeid shrimp species [32]. These contradictory features witness the possible sequestration of yolk bodies and other lipids into the oocytes from the storage reserves, particularly within the hepatopancreas.

5. CONCLUSION

In conclusion, the developmental stage of setae in pleopods and uropods provides a rapid and accurate indication of moult stage in *P. semisulcatus*. It will be non-sacrificial to repeat measurements that are taken from the same animal to monitor the rate of development. The present study provides a detailed account on moulting stages such as epidermal retraction, setal formation and related structural changes in the cuticle. The present study also emphasizes variations in the biochemical constituents in muscle tissue and hepatopancreas during various moulting stages that will contributes to our understanding of moulting cycle physiology in *P. semisulcatus.*

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Drach P, Tchernigovtezff C. Sur la methode determination des stades d' intermueetsoh apllication general aux crustaces. Vie Milien. 1967;18A:595-610.
2. Schafer HJ. The determination of some stages of moulting cycle of *Penaeus duorarum* by microscope examination of the setae of the endopodities of the pleopods. FAO Fish. Rep. 1967;57:381-391.
3. Longmuir E. Setal development, moult staging an ecdysis in the banana prawn, *Penaeus merguiensis.* Mar. Biol. 1983;77:183-190.
4. Wassenberg TJ, Hill BJ. Moulting behaviour of the tiger prawn, *Penaeus esculentus.* Aust. J. Mar. Freshwater Res. 1984;35:561-571.
5. Smith DM, Dall W. Moult staging in the tiger prawn, *Penaeus esculentus.* In: Second Australian National Prawn Seminar, PC Rothlisberg, BJ Hill and DJ Staple (Eds.). NPS2, Cleveland, Australia. 1985;85-95.
6. Robertson L, Bray W, Leung-Trujillo J, Lawrence A. Practical moult staging of *Penaus setiferus* and *Penaeus stylirostris*. J. World Aqua. Soc. 1987;18:180-185.
7. Chan S, Rankin, SM, Kele LL. Characterization of the moult stages in the *Penaeus vannamei*: Setogenesis and hemolymph levels of total protein, ecdysteroids and glucose. Biol. Bull.1988;175:85-192.
8. Read GHL. Aspects of lipid metabolism in *Penaeus indicus*. Dissertation, University of Natal. 1977;1-162.
9. Diwan, AD, Usha T. Characterization of moult stages of *Penaeus indicus* based on developing uropod setae and some closely allied structures. Indian J. Fish. 1985;32:275-279.

10. Vijayan KK, Sunilkumar Mohamed K, Diwan AD. Studies on moult staging, moulting duration and moulting behaviour in Indian white shrimp, *Penaeus indicus.* J. Aqua Trop. 1997;12(1):53:64.
11. Diwan AD, Joseph, Shoji, Ayyappan S. Physiology of reproduction, breeding and culture of tiger shrimp *Penaeus monodon (Fabricius*). Narendra Publishing House, New Delhi. 2008;290-292.
12. Passono LM. Moulting and its control. In: "The Physiology of Crustacea", TH Waterman (ed.). Academic Press, Newyork. 1960;1:473-536.
13. Bradford MM. A rapid and sensitive method for the quatitation of microgram quantities of protein utilizing the principle of protein -dye binding. Anal. Biochem. 1976;72:248-254
14. Roe JR. The determination of sugar in blood and spinal fluid with anthrone reagent. J. Biol. Chem. 1955;20:335–345.
15. Branes, Blackstock. Estimation of lipids in marine animals and tissues; Detailed investigation of the sulpho phosphovanilun method for "total" lipids. Journal of Experimental Marine Biology and Ecology. 1973;12(1):103-118
16. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 1957;266:497–509.
17. Drach P. Mueet cycle d'internue chez les crustaces Decapods. Annis. Inst. Oceanogr. 1939;19:103–391.
18. Huner JV, Colvin LB. Observation on the moult cycles of two species of *juvenile stylirostris* (Decapoda: Crusstacea) Proc. Natl. Shellfish. Assoc. 1979;69:77- 84.
19. Van Herp F, Bellon-Humbert C. Setal development ad moult prediction in the larvae and adults of crayfish. *Astacus leptodactylus.* Aquaculture. 1978;14:289-301.
20. Lyle WG, MacDonald CD. Moult stage determination in the Hawaiian spiny lobster *Panulirus marginatus.* J. Crustacean. Biol. 1983;3:208-216.
21. Moriysau M, Mallet P. Moult stages of the snow crab *Chionoecetes opilio* by observation of morphogenesis of setae on the maxilla. J. Crustacean. Biol. 1986;6:709-718
22. Kurup NG. The intermoult cycle of an amomuram, *petrolisthes cinctipes Ramdall* (Decapoda). Biol. Bull. 1964;127:97-107.
23. Leone CA. Normal variation in the amount of protein in the sera of some decapod Crustacea. Science. 1953;118: 295-296.
24. Florkin M. Blood chemistry. In*:* The Physiology of Crustacea, T.H. Waterman (ed.). Academic Press, New York. 1960;1141-1159
25. Travis DF. The moulting cycle of the spiny lobster, *Panulirus argus* - III. Physiological changes which occur in the blood and urine during the normal moulting cycle. Biol. Bull. 1955;109:484-503.
26. Suneetha Y, Sreenivasula Reddy P, Naga Jyothi P, Srinivasulu Reddy M. Studies on the Analysis of Proximal Changes During Molting Process in the Penaeid Prawn, *Penaeus monodon*. World Journal of Zoology. 2009;4(4):286-290,
27. Andrews P. Seasonal variation of haemolymph composition in the crayfish *Orconectes limosus*. Z. vergl. Physiol. 1967;57:7-43.
28. Djangmah JS. The effects of feeding and starvation on copper in the blood and hepatopancreas and on blood proteins of *Crangon vulgaris (Fabricius*). Comp. Biochem. Physiol. 1970;32:709-731.

29. Bursey CR, Lane, CE. Ionic and protein concentration changes during the moult cycle of *Penaeus duorarum.* Comp. Biochem. Physiol. 1971;40(A):155-162.

30. Spindler-Barth M. Changes in the chemical composition of the common shore crab, *Carcinus maenas.* J. Comp. Physiol. 1976;105:197-205.

31. Telford M. Changes in blood sugar composition during the moult cycle of the lobster, *Homarus americanus*. Comp. Biochem. Physiol. 1968;26: 917-926.

32. Galindo C, Gaxiola G, Cuzon G, Chiappa-Carrara X. Physiological and biochemical variations during the molt cycle in juvenile *Litopenaeus vannamei* under laboratory conditions. Journal of Crustacean Biology. 2009;29:544–549.

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