STUDIES ON MOULT STAGING, MOULTING DURATION AND MOULTING BEHAVIOUR IN INDIAN WHITE SHRIMP *PENAEUS INDICUS* MILNE EDWARDS (DECAPODA: PENAEIDAE)

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ABSTRACT

Characterization and classification of complete moult cycle of *Penaeus indicus* have been worked out on the basis of setal development. Setogenic moult staging was found to be a rapid and simple technique. Since excision of appendage is not required, this technique is non-destructive and permits repetitive moult staging of an individual shrimp.

The average duration of one moult cycle with relative duration of each stage was determined in the adult *P. indicus* using setogenesis. Premoult occupied the major part of the moult cycle (70.9%), followed by intermoult (18.35%) and postmoult (10.45%). Significant relationship ($p < 0.05$) was observed between the size or age of the shrimp and the moult cycle duration. Observation on the moulting behaviour showed that most of the shrimp moulted during the late hours of the night, especially between midnight and 0400 h. *Penaeus indicus* is found to be very active prior to moulting, and the whole process of ecdysis was faster, lasting only for 30–50 sec.

Key words: *Penaeus indicus*, moult staging, setogenesis, moulting behaviour

Growth physiology and reproduction of penaeids are essentially linked to the moulting cycle. Hence a clear knowledge about the moult stage of the shrimp will be useful in the hatchery and farming operations of penaeid shrimps. After the classic work by Drach and Tchernigovtzeff (1967), the schedule of moult staging on the basis of setal development has been described for many species of penaeids (Schafer, 1967; Huner and Colvin, 1979; Longmuir, 1983; Wassenberg and Hill, 1984; Smith and Dall, 1984; Robertson *et al.*, 1987; Chan *et al.*, 1988; Kibra, 1993).

Indian white shrimp *P. indicus* has been selected for the present study considering the growing importance of this species in the shrimp farming fields of the

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Indian subcontinent. Preliminary works on the moult staging of *P. indicus* have been reported by Read (1977) and Diwan and Usha (1985). There are still gaps in our knowledge of moulting pattern of commercially important penaeids. Basic problems that need further investigations include the duration of each of the moult stages in the moult cycle, the relationship between body size and moulting duration, and mechanism of exuviation. In the present work a detailed study has been carried out with objectives to obtain data on (1) staging of moult cycle in greater detail using setogenesis, (2) assessment of stagewise moult cycle duration, (3) relationship between body size versus moult cycle duration, and (4) mechanism of exuviation.

MATERIALS AND METHODS

Live *P. indicus* were periodically obtained from the traditional shrimp farms of Vypeen Island, Cochin, India. The size of the shrimps used for the moult staging varied between 100 to 120 mm in total length (TL). Twenty shrimps were held individually in circular plastic cages (15 cm height, 21 cm diameter). The cages were floated in collapsible plastic pools of 3 foot diameter and 350 l capacity fitted with a biological filter (salinity 25×10^{-3}, pH 8 ± 0.2, and temperature $30 \pm 1°C$). Shrimps were fed *ad libitum* with fresh molluscan/crustacean meat. Daily observations on the setal development were made by observing the posterior median part of the inner uropod. Excision of uropod was not necessary and the shrimp could be held immobile and quiescent while wrapped in a wet paper towel. Moult stages were determined using morphological changes of the seta (Drach and Tchernigovtzeff, 1967) using a stereoscopic binocular microscope with transmitted light. Duration for each moult stage was calculated by examining the setogenesis daily. The total duration of time between two consecutive moults gave the duration of one moult cycle. From the day of first moult, prawns were kept under observation till the shrimp completed two moult cycles. For microphotography the endopodites of the uropods were removed, mounted in filtered sea water on microscopic slides, and photographed using an Orwo 125 ASA black and white film in Olympus PM Load Binocular compound microscope.

In order to study the moulting behaviour and mechanism of exuviation, shrimps of size 80–120 mm TL and moult stage D2-3 were selected and kept in separate glass troughs of 10 l capacity containing aerated sea water. The light was diffused by separating the troughs and light source by a cloth screen, since the shrimps were found disturbed in direct light during ecdysis. As and when the animals moulted, the mechanism of exuviation or shedding process of exoskeleton was closely followed by visual observation and duration was recorded.

To study the relationship between the body size versus moult cycle duration, observations on the moult cycle were conducted in three different size groups of *P. indicus*: 30–40 mm, 60–80 mm, and 80–120 mm (TL). Each group comprised 15 experimental animals. Animals were kept under observation till they completed two cycles of moult in the laboratory. Average moult cycle duration of each group was calculated and the results were statistically verified using Student's *t* test.

RESULTS

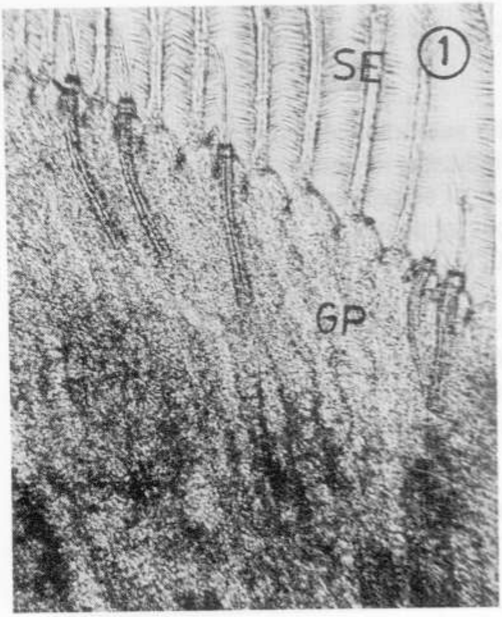
Description of moult cycle

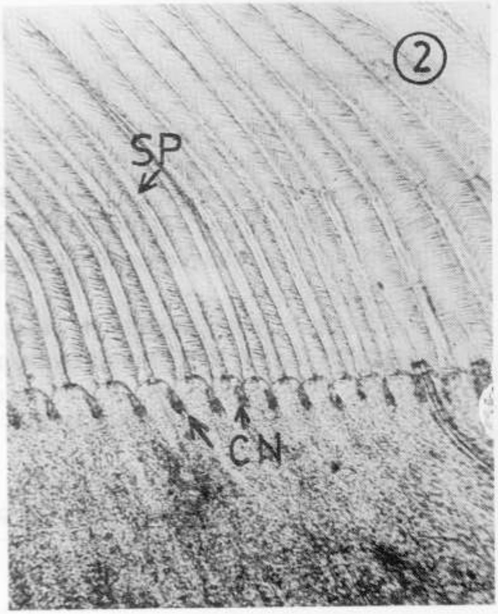
The morphological changes associated with setal development of uropod in the prawn *P. indicus* are found to be a good indicator for identification of the different stages of the moult cycle. On the basis of setogenesis the moult cycle has been classified into eight well-defined stages of postmoult (substages A and B), intermoult (C), and premoult (substages D0, D1′, D1″, D1‴ and D2-3 (Figs. 1–9, Table 1).

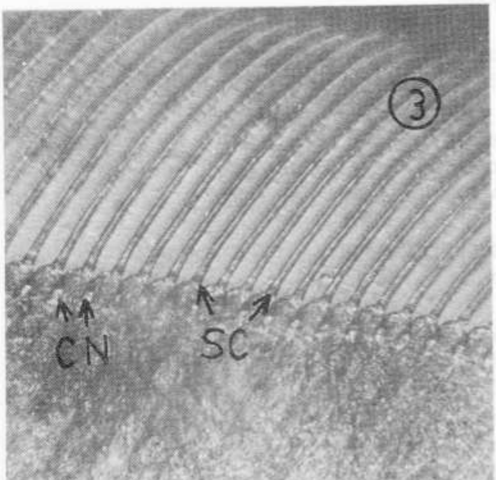
Stage A, (early postmoult, Fig. 1) represented a prolonged state of the actual moult and is the first stage immediately after the shrimp flicked clear of the old exuvia. The whole body and the exoskeleton were found very soft and slippery to touch. The granular protoplasmic matrix was continuous throughout the setae

Table 1. Criterion for the moult staging in *Penaeus indicus* on the basis of setogenesis

Moult stage	Characteristic features	Duration (h)	Average % duration	
A	Body soft and slippery. Rostrum flexible. Granular protoplasmic matrix (GP) continuous and fills the new setae (SE). Setal cones and culticular nodes absent. (Fig. 1)	3–7	2.1	10.45% Postmoult
B	Setal protoplasmic matrix (SP) shows retraction. Cuticular nodes (CN) are poorly developed. Setal cones absent. (Fig. 2)	16–22	8.35	
C	Rigid and hard exoskeleton with firm rostrum. Setal protoplasm retracts to form well-developed setalcones (SC). Well-developed cuticular nodes. (Figs. 3 and 4)	36–48	18.35	18.35% Intermoult
D0	Appearance of amber-coloured zone (AZ) at the tip of the uropod due to epidermal retraction (ER). (Fig. 5)	24–36	13.05	70.9% Premoult
D1'	Deepening of epidermal retraction and protoplasmic invagination in the site of future setae resulting in scalloped epidermis (SD). (Fig. 6)	48–72	26.0	
D1"	Protoplasm condenses in the region of the new setae. Setal invagination (SV) deepens, marking the appearance of new setal walls (SW) (Fig. 7).	24–48	15.2	
D1"'	New Setae appears in the uropod matrix with double wall. Setal invagination complete and setal shafts (SH) visible at the tip of the setae. Proximal part of the developing setae ill defined (PS) (Fig. 8).	20–36	11.9	
D2–3	Fully developed new setae appears in the matrix as "tube-in-tube" (TT) structures. Proximal part of the setae blunt (BD). Well-developed shafts and barbules (Fig. 9).	8–14	4.75	
E	Ecdysis/rejection of the old cuticle			







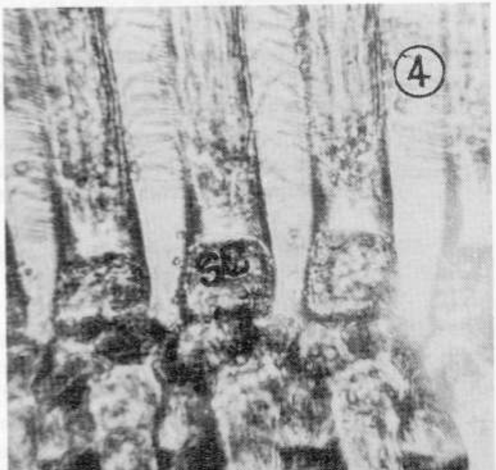


Fig. 1. Stage A, × 100
Fig. 2. Stage B, × 100
Fig. 3. Stage C, × 100
Fig. 4. Stage C, × 400

GP, granular protoplasmic matrix; CN, cuticular node, SC, setal cones; SE, setae; SP, setal protoplasmic matrix.

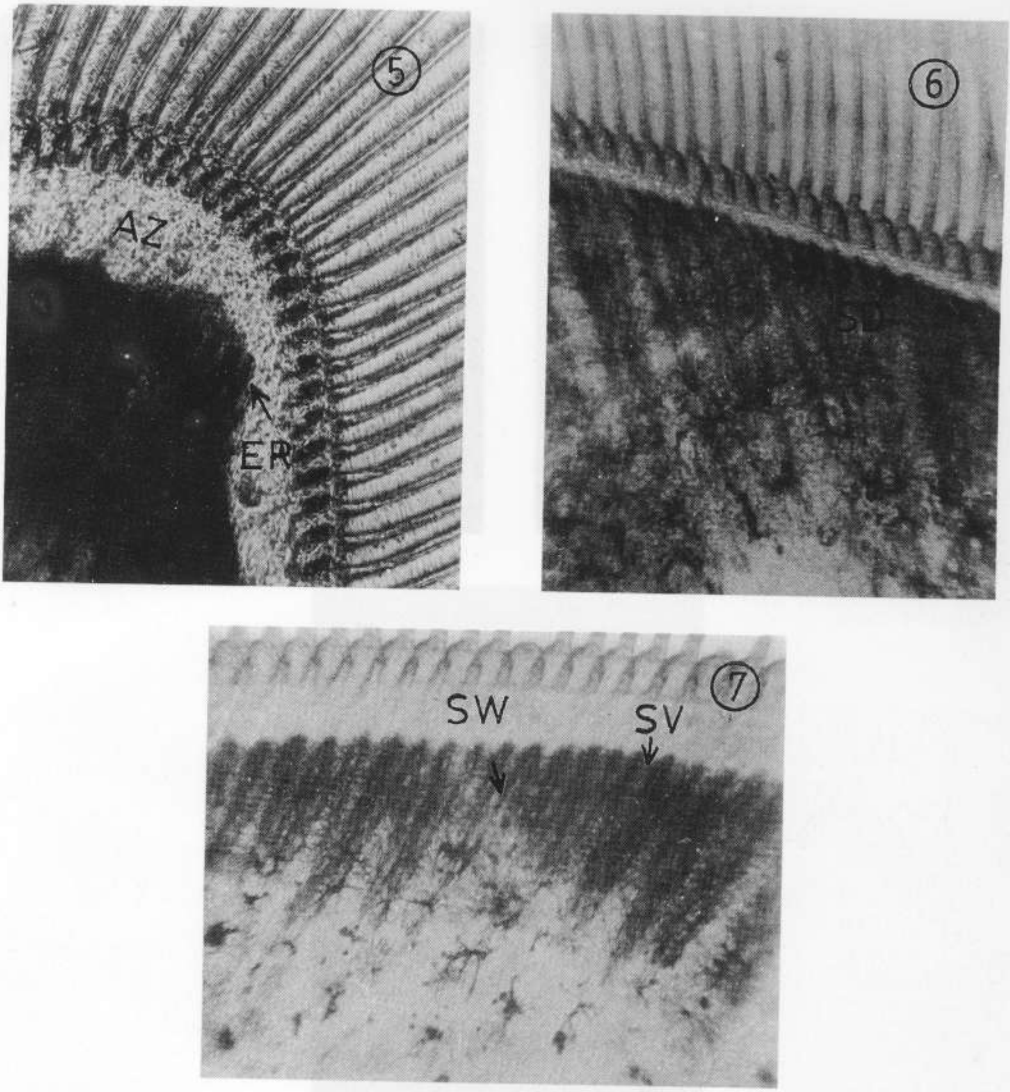
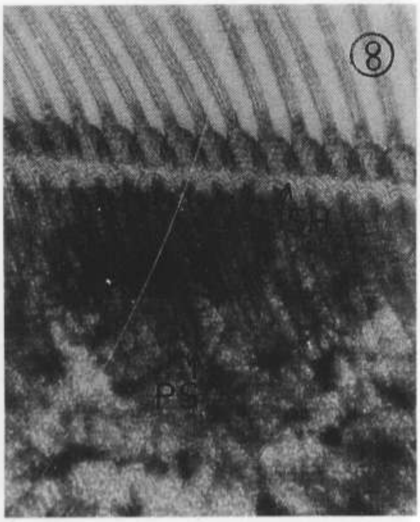
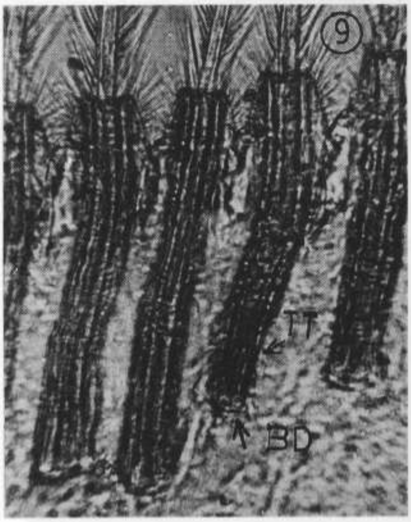


Fig. 5. Stage D0, × 100.
Fig. 6. Stage D1', × 100
Fig. 7. Stage D1", × 150
AZ, amber-coloured zone; ER, epidermal retraction; SD, scalloped epidermis; SV, setal invagination; SW, setal wall.

filling the new setal articulation and distal end of the setae. Stage A extended up to a period of 3–7 h.

Stage B (late postmoult, Fig. 2) was marked by the appearance of well-developed setal articulation and the beginning of cuticular node development. The setal protoplasmic matrix was found contracted within the setal lumen so as to fill only the proximal half of the setae. The exoskeleton, which at stage A showed a





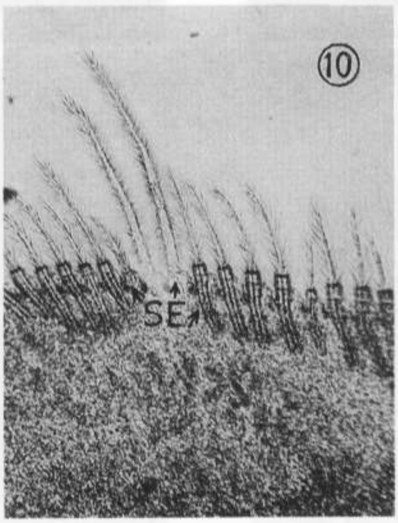


Fig. 8. Stage D1''', × 150
Fig. 9. Stage D2-3, × 600
Fig. 10. Stage E, × 100
BD, well-defined proximal part of the newly developed setae; PS, Ill-defined proximal part of the newly developing setae; SH, setal shafts; TT, tube-in-tube structure; SE, setal eversion.

parchment-like consistency, became relatively hard, but it was easily depressed. This stage lasted 16–22 h.

In stage C (intermoult, Figs. 3 and 4), the most significant observation was that of the fully developed cuticular nodes and setal cones. The distal part of the setae appeared clear and transparent because of the retraction of the setal protoplasmic matrix. Carapace and rostrum became firm and rigid. Stage C extended for a period of 36–48 h.

The onset of premoult period was marked by the separation of cuticle at the base of the setae due to the withdrawal of epidermis and subsequent development of new setae. Premoult is further divided into five substages—D0, D1, D1″, D1‴, and D2-3 on the basis of detailed observation on the morphology and extent of the neosetal development.

In stage D0 (early premoult, Fig. 5), an amber colour zone appears at the tip of the uropod, due to the retraction of epidermis from the cuticle between the bases of setae. The epidermal retraction was found to start from the tip and later on extended towards either side of the uropods. This stage lasted for 24–36 h.

In stage D1′ (early premoult, Fig. 6), retraction of the epidermis under the setae increased further. Condensation of protoplasm was noticed in the region of formation of new setae. Later, protoplasm invaginated at the site of future setae, giving a scalloped appearance. This stage lasted for 48–72 h.

In stage D1″ (late premoult, Fig. 7) relative to stage D1′, the setal invagination deepened and became more distinct. Development of new setal walls was observed, while setal shafts had not made their appearance. This stage lasted for 24–48 h.

In stage D1‴, (late premoult, Fig. 8), setal invagination reached its maximum, and the new setae were entirely visible in the matrix. The setal shafts of the developing setae were visible immediately above the epidermal surface. Fully developed setae with an ill-defined proximal end were characteristic of this stage. This stage lasted for 20–36 h.

Morphological observations have not revealed any notable differences between stages D2 and D3 (late pemoult, Fig. 9). Therefore, in the present species these two stages were combined and expressed as a single stage. Stage D2-3 varied from the previous stage of D1‴ in that the fully developed new setae had the appearance of a "tube in a tube" with well-defined, blunt proximal end. Development of the setae was completed with setal shafts bearing barbules. This is the final stage before ecdysis. D2-3 had a duration of 8–14 h.

The next process observed was ecdysis, which is termed stage E (Fig. 10). The prawn draws itself out from the old exoskeleton and everts the setae of the new exoskeleton. The process of exuviation in *P. indicus* takes less than 2 min.

Moult cycle duration

Observation on moult stage duration of *P. indicus* indicated the dominance of premoult period in the moult cycle, while the postmoult period was the shortest (Table 1). In the present study it was observed that the length of the premoult period was the longest, constituting 70.9% of the moult cycle. Postmoult and intermoult occupied the remaining part of the cycle, with 18.35% and 10.45% respectively.

Moult cycle duration for the three size groups of animals—30–40 mm, 60–80 mm and 80–120 mm—was studied so as to observe relation between the body size or age and duration of moult cycle. Results (Fig. 11) showed a significant ($p < 0.05$) variation between the size or the age of the animal and the moult cycle duration. The duration of the moult cycle was found to be greater with increase in the size of the animal. In juvenile prawns of size 30–40 mm, average moult cycle duration was 96 h with a premoult period of 76 h, whereas in young adults of size 60–80 mm average moult cycle duration recorded was 180 h with an average premoult period of 130 h. When the animals reached the adult size of 80–120 mm, the average moult cycle duration increased to 240 h with a premoult period of 165 h.

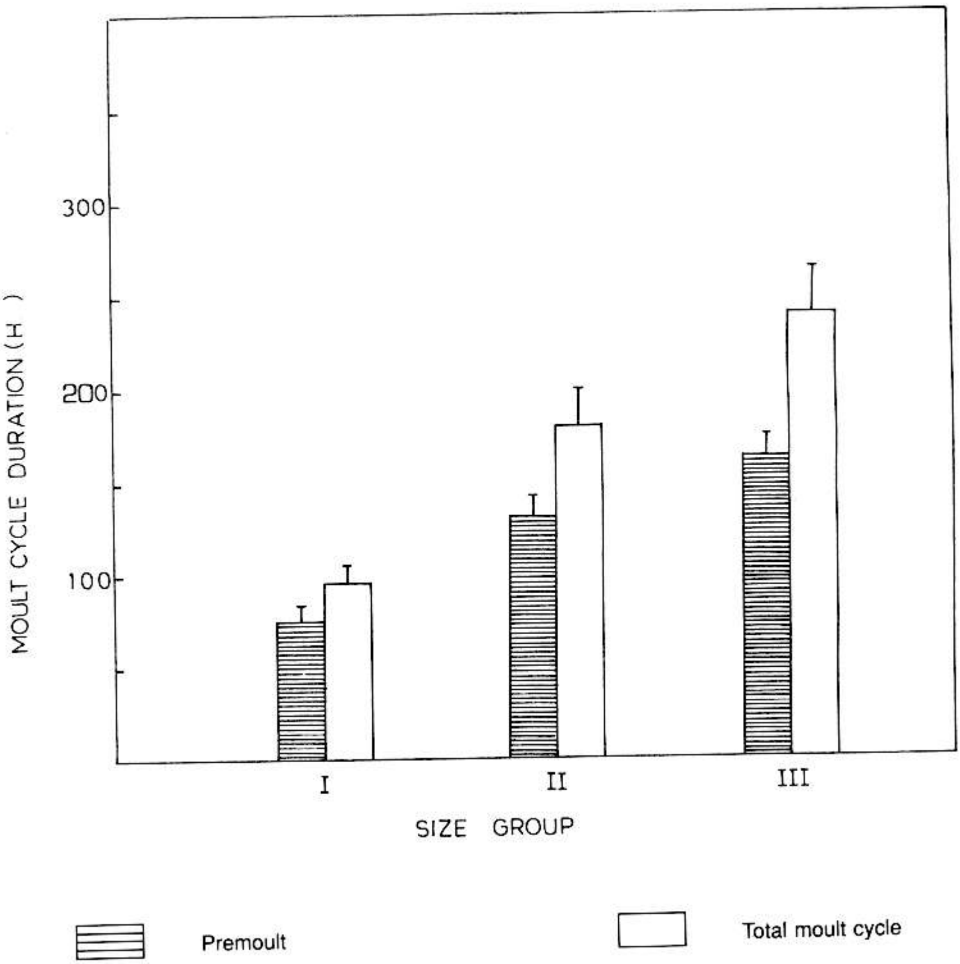


Fig. 11. Average moult cycle duration in different size groups of *P. indicus*. I, 30–40 mm; II, 60–80 mm; III, 80–120 mm.

Moulting behaviour

Of the 38 prawns observed in the laboratory 34 animals (94%) moulted during the night hours (24 animals moulted between 2400 and 0400 h, 8 animals moulted between 0400 and 0700 h, and 4 animals moulted between 2200 and 2400 h). Only two animals (6%) were observed to moult after 0700 h.

Prawns in the late premoult stage of D2-3 were generally found to be very active in the middle of the night. Prior to ecdysis, the prawns were observed to perform swimming, walking, jumping, flicking, rolling, and rotating movements. Rapid propulsion of the animal with the help of pereopods along with flexing of the body convexly at the cephalothorax or abdomen joint was common. Animals stretched the body vertically with the help of the arch centred on the third abdominal segment. This period of intense locomotion in the late hours of premoult probably helps the animal in the removal of the old exoskeleton from the newly formed one. It was observed that in the first phase of shedding, carapace was thrown out separately from the cephalothorax. The prawns then flicked violently, lifting the body out of the abdominal and ventral cephalothorax portion of the old exoskeleton. Following the moult, the prawns were found to lie on their side for an average of 5 to 30 min. before attaining the movements with the aid of pereopods. Prawns stopped feeding when they entered the last premoult and resumed feeding only in late postmoult stage B.

DISCUSSION

The method of determining moult stages in *P. indicus* based on developmental changes in the setae of the uropods enabled us to classify the moult cycle into eight well-defined stages (Table 1). This method is simple and quick so that repeated moult staging is possible in the experimental prawns. General characteristics of different moult stages in *P. indicus* were similar to those of other penaeids, with species-specific differences especially in premoult staging and moult cycle duration. The key features of the postmoult stages A and B are based on the changes in the distribution of granular protoplasmic matrix of the setae and formation of cuticular node due to the thickening of setal base. Other penaeid workers such as Longmuir (1983) and Smith and Dall (1984) have also used granular protoplasm of the setae to identify the postmoult stages of A and B. Granular protoplasmic matrix filling the new setae is believed to help in the evagination of setae at the time of ecdysis and support the setae during the stages of A and B (Smith and Dall, 1984). In the present study a fully developed internal cone and cuticular node marked the onset of stage C. However, further division of stage C based on microscopic examination of uropods was not possible.

The premoult stages vary the most among the assorted species of crustaceans studied and have been modified to the greatest extent. Species differences probably account, to a large extent, for the variation observed. Apolysis or withdrawal of epidermis from the setal base signals the onset of premoult stage D0 in *P. indicus*, similar to the observation of Jenkin and Hinton (1966) in other crustaceans. Virtually all investigation on crustacean setogenesis had taken the apolysis (D0) as the starting point of premoult development (Dall *et al.*, 1990). Subsequent classification of the premoult stages into substages on the basis of neosetal development is similar to those described for penaeids (Dall *et al.*, 1990). In the present work, stages D2 and D3 are combined and counted as a single last stage of the premoult as further division was not possible in the absence of noticeable differentiating characters. The fully developed setae in D2-3 stage in *P. indicus* appeared as a "tube-in-tube" structure, and this stage immediately follows stage E, during which the setal eversion and actual shedding of exoskeleton takes place.

Ecdysis in *P. indicus* is a rapid process that is not subdivided as reported by Longmuir (1983) in *Penaeus merguiensis* and Smith and Dall (1984) in *Penaeus esculentus*. During stage E of *P. indicus* all the setae are found to unfold under the cuticle like the straightening out of an inverted glove finger as reported by Van Herp and Bellon-Humbert (1978).

The present investigation showed the dominance of the premoult period in the moult cycle of *P. indicus* exhibiting a typical diecdysis moult cycle. This is in agreement with earlier studies in penaeid moult cycle (Dall *et al.*, 1990), except the study by Schafer (1967), who has reported a very long intermoult period and a very short premoult period for *P. duorarum*.

In the present study juvenile shrimps moulted faster with short moult cycle duration, while in the adults moult frequency was slow with a lengthy moult cycle (Table 2). This is in conformation with the general moulting pattern among the penaeids (Dall *et al.*, 1990).

Observation on the moulting behaviour showed that most of the shrimps (95%) moulted during the night. Wassenberg and Hill (1984), while working on *P. esculentus*, also observed that 63% of their experimental animals moulted during the night. The active movement pattern exhibited by *P. indicus* during the pre-ecdysal period resembled that observed by Longmuir (1983) in *P. merguiensis* and Wassenberg and Hill (1984) in *P. esculentus*. These authors have concluded that the pre-ecdysal movements serve to loosen the old exoskeleton, to enable easy shedding of exuvia. The process of ecdysis in *P. indicus* was faster, lasting 30–50 sec. In *P. merguiensis*, ecdysis lasted 40 sec. (Longmuir, 1983), whereas

Table 2. Moult cycle duration for different size groups of *Penaeus indicus*

Size group (mm)	No.	Average premoult duration with standard deviation (h)	Average moult cycle duration with standard deviation (h)
30–40	15	76 ± 14	96 ± 16
60–80	15	130 ± 20	180 ± 24
80–120	15	165 ± 24	240 ± 48

in *P. duorarum* Bursey and Lane (1971) reported that the time taken for ecdysis was 20–30 min. The disparity could be due to the difference in defining ecdysis. In the act of ecdysis, *P. indicus* throws out its carapace first, followed by the remaining exoskeleton of abdomen and other appendages as a single unit, similarly to the process described for *P. esculentus* by Wassenberg and Hill (1984).

The present study revealed the use of setogenesis as a practical tool in the moult staging of a penaeid shrimp. The moult staging scheme developed for *P. indicus* can be used in further research on this commercially important shrimp as well as in related penaeids.

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