

## Index of molt staging in the black tiger shrimp (*Penaeus monodon*)

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### Abstract

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Molting is a phenomenon of shedding the old cuticle and re-generating the new one found in crustaceans including shrimps and many other species of invertebrates. The molting cycle is a dynamic process, composed of pre-ecdysis (pre-molt, D stage), ecdysis (E stage), postecdysis (postmolt, A-B stages), and intermolting (C stage) stages. In healthy shrimp, molting cycles are repeated several times through shrimp life in order to increase body size and mass (growth). In this paper we gather the knowledges and important findings related to the molting cycle of crustaceans from the past until present, and highlight the physical evidence of cuticular tissue that we used for molt staging in the black tiger shrimp, *Penaeus monodon*. At the end of this paper we summarize the easily observed criteria used for molt staging in the black tiger shrimp.

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**Key words :** molting stages, *Penaeus monodon*, setae, setal cone

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## บทคัดย่อ

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 ดัชนีบ่งชี้ระยะการลอกคราบของกุ้งกุลาดำ (*Penaeus monodon*)  
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การลอกคราบคือกิจกรรมที่กุ้งสลัดเอาเปลือกเก่าที่ห่อหุ้มตัวออกและสร้างเปลือกใหม่ขึ้นมาแทนที่ การลอกคราบเป็นกระบวนการที่มีการเปลี่ยนแปลงของร่างกายเป็นวงจรอย่างต่อเนื่อง ซึ่งสามารถแบ่งย่อยเป็นระยะต่าง ๆ 4 ระยะ ได้แก่ ระยะก่อนการลอกคราบ ระยะลอกคราบ ระยะหลังการลอกคราบ และระยะระหว่างวงจรการลอกคราบ ทุก ๆ วงจรการลอกคราบของกุ้งที่อยู่ในสภาวะปกติ กุ้งจะมีขนาดเพิ่มขึ้นทุกครั้ง ดังนั้นจึงกล่าวได้ว่าการลอกคราบมีผลโดยตรงต่อการเจริญเติบโตของกุ้งกุลาดำ การศึกษาค้นคว้าได้ประมวลความรู้และงานวิจัยที่สำคัญที่เกี่ยวข้องกับการลอกคราบของสัตว์ในตระกูลปูและกุ้งตั้งแต่อดีตจนถึงปัจจุบัน โดยเน้นที่การอธิบายลักษณะทางกายภาพของเปลือกและเนื้อเยื่อผิวหนังที่เปลี่ยนแปลงตลอดวงจรการลอกคราบของกุ้งกุลาดำ และในตอนท้ายได้สรุปเกณฑ์สำคัญที่ใช้เป็นดัชนีบ่งชี้ระยะการลอกคราบของกุ้งกุลาดำ

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Molting, a phenomenon of shedding the old cuticle and re-generating the new one, occurs in many species of invertebrates, including crustaceans. It is an essential process for post-larval growth of crustaceans. In healthy animals, molting cycles are repeated several times in order to allow growth throughout crustacean life. Through the molting cycle, the animals show dramatic changes in many aspects, including body structure, biology and behavior. The regulatory mechanism of the molting process has long been studied in various species of crustaceans, mainly in crabs. The studies related to the molting cycle have so far paid attention to molt-inhibiting hormone from the X-organs located at the eye-stalks (Hubschman and Armstrong, 1972; Rao, 1973; Freeman and Costlow, 1979; Keller and Schmid, 1979; Bellon-Humbert and Van Herp, 1988; Sithigorngul *et al.*, 1999; Watson, 2001), and molt-stimulating ecdysteroid hormones secreted by the Y-organs (Carlisle, 1957; Hubschman and Armstrong, 1972; Charmantier and Trilles, 1973; 1979; Keller and Willig, 1976; Chaix *et al.*, 1976; Spaziani, 1999; Chang, 2001), although changes in the structures and biochemistry of hepatopancreas, haemolymph and integument related to molting cycle have also been reported in many

species (for example see Watanabe and Kono, 1997; Watanabe *et al.*, 2000; Sousa *et al.*, 2001; Fernandez *et al.*, 2001; Pratoomchart *et al.*, 2002a; b). These findings accumulate more knowledge about the regulatory mechanism of the molting. However, the direct evidence in the black tiger shrimps (*Penaeus monodon*), which is well known as an important agricultural export product of Thailand, has not been yet elucidated. The objective of this study is to gather background knowledge about molting stages and criteria used to determine molting stages in crustaceans, focusing especially on physical evidence used to determine molting stages in the black tiger shrimp. We hope that this paper will be useful for further researches which aim to promote shrimp growth, which is at the heart of shrimp farming.

#### Molting stages and criteria used to determine molting stages in crustaceans.

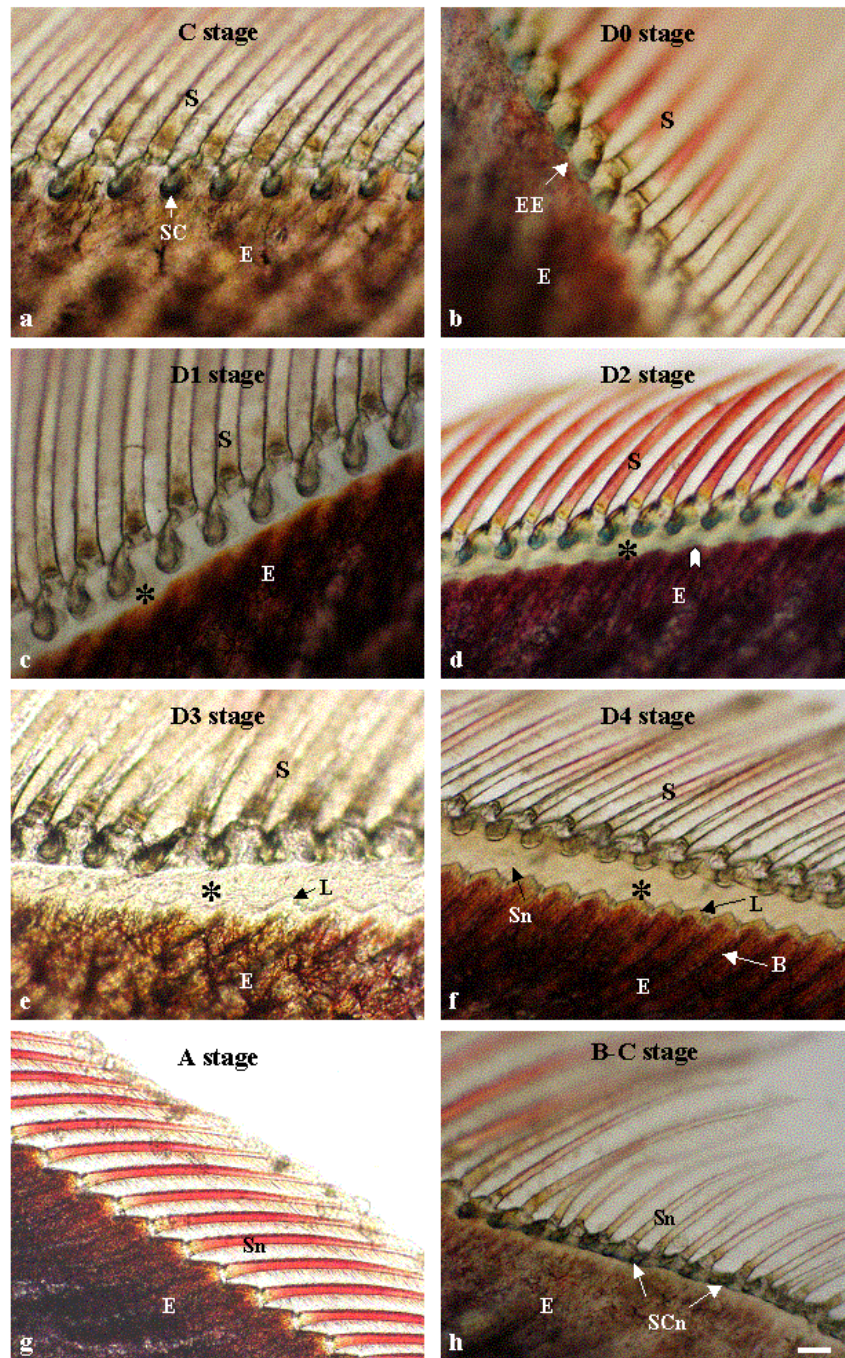
Each molting cycle of crustaceans is composed of different stages including preecdysis (pre-molt, D stage), ecdysis (E stage), postecdysis (postmolt, A-B stages), and intermolting (C stage) stages (Drach, 1939; Skinner, 1962). Various criteria are in use for staging the crustacean molt cycle. These include the degree of hardness of

the exoskeleton, changes in matrices of the setae (Drach, 1939), the growth of re-generating limb buds (Skinner, 1962; Stevenson *et al.*, 1968; Stevenson and Henry, 1971; Hopskin, 1982), the progressive development of gastroliths in the digestive organs (McWhinnie, 1962; Hopkins, 1977; Rao *et al.*, 1977), histological changes of organs and tissues (Travis, 1957; Skinner 1962; Stevenson, 1968; Stevenson *et al.*, 1968; Benhalima *et al.*, 1998), development of setae (Lyle and MacDonald, 1983; Moriyasu and Mallet, 1986), and a combination of the above methods (O' Halloran and O'Dor, 1988; Musgrove, 2000). The premolt stage is a period of biological preparation of organs and tissues all over the animal body for shedding its cuticle (exoskeleton). It is divided into four sub-stages; D1, D2, D3 and D4 depending on the degree of retraction of the epidermal tissue from the cuticle (Drach, 1939). After the D4 stage, the animals will spend a few seconds for shedding their old cuticle during ecdysis (E stage). The animals will subsequently enter postmolt stage where they will spend some time for building up and hardening the new cuticle. Postmolt is divided into an A and a B stage. The A stage follows immediately after the ecdysis. The main task of the A stage is the retraction of the contents of the new setae. The A stage is divided into A1 and A2 sub-stages. The B stage is the period for mineralisation of the cuticle, and is divided into the B1 and B2 sub-stages, the first is defined by the retraction of the contents of the setae to the region where cones will form inside, the latter is defined by the formation of setal cones. At the end of the B2 stage the formation of the setal cones and chemical change in the preecdysial layer of the newly-synthesized cuticle are completed (Drach, 1939). The animals subsequently enter the C stage, in which four sub-stages are distinguished; C1, C2, C3 and C4. Stages C1 and C2 are defined by an increase of integument rigidity, but there are no marked criteria to divide these. At stage C3 the integument reaches its final stage of rigidity, after which in stage C4 the synthesis of the membranous layer of the procuticle is completed. Thus C3-C4 stages are so-called intermolting stages, as the

formation of the new cuticle is finished, and the animals await the signals from ecdysteroid hormones from the Y-organs located at the mouth region to trigger re-entering the premolt of the next cycle (Carlisle, 1957; Hubschman and Armstrong, 1972; Charmantier and Trilles, 1973; 1979; Chaix *et al.*, 1976; Keller and Willig, 1976). While the animal body is not under limitation of the cuticle during the period from late premolt to postmolt, they are increasing up body size and mass, hence growing.

#### **Molt staging in the black tiger shrimp.**

Molt staging in penaeid shrimps was previously described by numbers of researchers based on various criteria (Schafer 1968; Longmuir 1983; Smith and Dall 1985; Yashiro, 1991). In this paper we reveal physical characteristics that we used to determine molting stages in the black tiger shrimp, *Penaeus monodon*, based on the criteria described by Drach, 1963 and a number of other researchers (for review see Skinner, 1985; Stevenson, 1985). From our direct observations, we found that in the intermolt stage (C stage, Figure a), setal cones (SC) were fully developed, and each was arranged in a neat line at the base of the setae. These two structures form a mature cuticle. The epidermal tissue (E), which is located under (interior to) the cuticle, was spreading throughout the area under the cuticle and between pairs of setal cones. When the premolt stage began, the process of continuous retraction of the epidermal tissue away from the cuticle started. The degree of retraction of the epidermal tissue determined the sub-stages of the premolt. Once the retraction begins, the shrimp is designated as stage D0. At the end of D0 stage, a clear straight margin of the epidermal tissue was observed at the base of the setal cones (Figure b). The D1 stage (Figure c) was defined by the presence of a narrow clear zone between the setal cones and the epidermal tissue. The clear zone implies the formation of a new cuticle. In the D2 stage (Figure d), the width of the clear zone between the setal cone and the epidermal tissue increased, and the edge of the epidermal tissue now had a wave-like pattern. This implies the continuous



Determination of molting stage. Uropods of the black tiger shrimps were examined and photographed under a light microscope (Olympus BX51) connected to a digital camera (Olympus DP11). The criteria used for molt staging followed Drach's staging. The images of physical characteristics indicate intermolt stage (a), premolt stage (b-f) and postmolt stage (g,h). Bar = 50  $\mu$ m. EE = epidermal edge, I = indent pattern of the epidermis, L = white layer at the edge of the epidermis, S = setae, SC = seta cone, SCn = newly-formed setal cones, Sn = newly-formed seta,  $\blacktriangleright$  = wavy edge of epidermis, \* = clear zone between cuticle and epidermis.

synthesis of new cuticle. Small and quite thin fiber-like projections between each setal cone and epidermal tissue were observed. These projections would later become new setae. In the next premolt stage, the D3 stage (Figure e), the clear zone between the setal cones and the epidermal tissue was clearly widened. A thin white layer at the edge of the epidermal tissue together with a highly wavy edge of the epidermal tissue could then be observed. The fiber-like projections connecting each setal cone with the epidermal tissue were still seen. At the final, the D4 stage of premolt (Figure f), the clear zone between the setal cones and the edge of the epidermal tissue was extremely clear and dominant. The typical characteristics of the epidermal tissue were clearly marked. As the white layer at its edge was reflecting the light, and formed sharp and serrate notches, young setae were clearly seen projecting from the impressions between the sharp notches. The pigments in the epidermal tissue were obviously indent and arranged in a paralleled-band fashion. The setal cones then started to deform. After ecdysis the shrimps immediately entered the post molt A stage (Figure g), in which the new cuticle and the setae were very soft and delicate. The setal cone could then not yet be seen. Only a few hours after ecdysis, the shrimps entered post molt B stage (Figure h) where the cuticle was hardening by mineralisation of inorganic compounds such as calcium and phosphorus (Roer and Dillaman, 1984; Cameron, 1985; Machado *et al.*, 1990; Compere *et al.*, 1993; Ziegler, 1997; Pratoomchart *et al.*, 2002a). In the B stage, young setal cones were developing. Different sizes of setal cones were thus observed at the base of the setae. When the development of the setal cones was completed, the shrimps entered C stage (Figure a). In late C stage (C 3-4) when the synthesis of all layers of new cuticle was complete, the shrimps were designated as in the intermolt stage.

### Concluding Remarks

The criteria used for molt staging in the black tiger shrimp included:

- 1) The degree of retraction of the epidermal tissue from the cuticle.
  - 2) The width of the clear zone between the setal cones and the epidermis.
  - 3) The characteristics of the epidermis.
  - 4) The presence of newly-formed setae.
  - 5) The formation of setal cones
- All of above criteria make up easily observed physical characteristics of each stage as follows:
- 1) Intermolt stage (C stage): mature setal cones and full-spread epidermis
  - 2) Premolt stage:
    - D0- a clear margin of the epidermal tissue at the base of setal cones
    - D1- a clear narrow zone between the setal cones and the epidermis
    - D2- a wider clear zone and a wavy edge of the epidermis
    - D3- a wider clear zone, highly wavy edge of the epidermis and a white thin layer at the edge of the epidermis
    - D4- an obvious wider clear zone, serrated edge of the epidermis, light-reflecting white layer at the edge of the epidermis, and paralleled-band fashion of the epidermis.
  - 3) Postmolt stage:
    - A- soft and delicate setae, absence of setal cones
    - B- presence of the young setal cones

### Further application.

The above conclusions can serve as standard criteria for upcoming experiments. For example, examination of physical and biological changes of organs and tissues through the molting cycle, effect of diseases on molting cycle progression, role of environment on molting cycle, etc. The forthcoming results will lead to a clearer understanding of the regulatory mechanism of the molting cycle, specially of *Penaeus monodon*.

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