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Practical key for molt stage determination of the snow crab, *Chionoecetes opilio*, by means of setal development observation in the endite of the maxilla.

by

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Abstract

Observation of setae morphology of the basal endite of the maxilla is successfully used for defining molt stages of the snow crab (Chionoecetes opilio). The basal endite of the maxilla is easily dissected and can be preserved for up to 48 hours in a 10 ml sampling vial filled with ambient salinity sea water at 2-3°C, during which period a reliable staging can be ensured. Frozen endites are not suitable for the observation of setae morphogenesis due to a pronounced epidermal retraction and new setae extrusion.

Three molt periods (postmolt, intermolt and premolt) can be identified. The premolt period is further subdivided into three stages (D_0, D_1 and D_{3-4}). For snow crab, prediction of the beginning of the molting season is critical due to the economic problems encountered by the fishing fleet when low quality molters are found in abundance in the catch. Identification of molt stages by the technique presented in this paper will allow such a prediction and provide a biological basis for fishery management.

Résumé

L'observation de la morphologie des soies de l'endite basal du maxille a été utilisée avec succès pour la lecture des stades de mue du crabe des neiges (Chionoecetes opilio). L'endite basal du maxille peut être facilement dissequé et préservé jusqu'à 48 heures dans de l'eau de mer à des températures de 2-3°C. Les endites congelés ne peuvent être utilisés pour la lecture des stades de mue due à une rétraction prononcée de l'épiderme et des nouvelles soies.

Trois périodes de mue (postmue, intermue et prémue) ont été identifiées. La période de prémue a été subdivisée en trois stades (D_0, D_1 et D_{3-4}). Pour la pêcherie du crabe des neiges, la prédiction du début de la saison du mue est très importante, puisque la présence en abondance des crabes de mauvaise qualité venant de muer diminue considérablement la valeur des prises. L'identification des stades de mue par la technique présentée dans ce manuscrit permettera de prédire l'arrivée des crabes blancs et fournira une base biologique pour la gestion des pêcheries du crabe des neiges.

Introduction

Information on molt cycle is essential for ensuring the quality of crustacean products (Miller and O'Keefe, 1981), for growth studies (Conan, 1985) and also for yield-per-recruit models (Caddy, 1977, 1979). The molt stages of decapod crustaceans have often been identified by external characteristics such as shell rigidity or color (Ennis, 1973; Haefner and Van Engels, 1975; Somerton, 1982; Munro and Therriault, 1983). However, these techniques tend to be subjective and are difficult to use for defining early premolt stages. Histological methods (Schwabe *et al.*, 1952; Skinner, 1962; Stevenson, 1968) can provide accurate results, but do not allow a quick determination of molt stages on large numbers of samples required, for example, for population surveys. Drach (1939) and Drach and Tchernigovtzeff (1967) described a technique based upon the observation of setal morphology, which permits quick molt stage determinations. This technique has been developed and adapted by numerous authors for a wide variety of crustacean species (Lyle and MacDonald, 1983 for review). However, this has not been applied to the snow crab, Chionoecetes opilio, (also known as spider crab, Watson, 1970; tanner crab, Somerton, 1981), which is the most important commercial crab in Eastern Canada (Elner, 1982). There are also large fisheries for this species in the Bering Sea (Otto, 1982) and Japan Sea (Sinoda, 1982). Increase in the catch of newly molted crabs, which are unsuitable for processing because of poor meat quality (Miller and O'Keefe, 1981), is detrimental to the commercial fishery. Therefore, predicting the incidence of molters in the fishery is essential to ensure the crab meat yield.

The purpose of this study is not to define detailed subdivisions of the molt cycle, but provide an accurate and fast method for recognizing periods in the molt cycle (postmolt, intermolt and premolt) and defining principal premolt stages. This subdivision can be further used for population dynamics studies, aspects of which are related to the commercial fishery of the species.

Material and Methods

Snow crabs (Chionoecetes opilio), ranging in size from 30 to 120 mm carapace width (C.W. : the widest part across the mesobranchial region), were caught in November 1984 with Japanese conical traps and with a Nephrops trawl in Chaleur Bay (48°03'00"N, 65°05'00"W), southern Gulf of St. Lawrence. The crabs were returned immediately to the laboratory for examination.

The basal endite of the maxilla (Fig. 1) was dissected from the crab with dissection scissors and preserved in a 10 ml sampling vial filled with ambient salinity sea water at 2-3°C. This appendage was chosen for defining the setal development stages because it is fairly transparent and is easily dissected from the animal. The basal endite of the maxilla was placed in a drop of ambient salinity sea water on a glass slide and observed at magnifications of x63-x150 under a compound microscope. A total of 238 basal endites were observed for defining molt stages within 24 hours of removal from the crabs. To check the maximum preservation period of endites during which a reliable molt staging can be conducted, a total of 140 endites were preserved by the same method, after defining molt stages. A series of 20 samples was then examined at intervals of 12 hours. To test the validity of staging frozen endites, a total of 70 endites were frozen in sea water for 10 days and defrosted at room temperature for examination.

To ensure that the sampling of the endites does not induce abnormal mortalities, a total of 168 crabs (74 test and 94 control) were kept in two 540 l tanks with running sea water at ambient water temperatures (2-7°C) and salinities (28.5-31‰) for a period of 45 days. All crabs were fed with frozen smelts (Osmerus mordax) twice a week, and mortality was checked once a day.

Results

(1) Observation of maxillae and setal morphogenesis

The maxilla has two bilobed endites (coxal endite and basal endite) on the basipodite and a simple lobed endite on the epipodite (Fig. 1). The basal endite bears two rows of dense setae at the distal end, and a few setae in the medial region of the ventral surface

(Figs. 2-4). The number of setae varies between individual crabs. No setae are existent on the dorsal surface. During the intermolt period, the endite is thickened and curved toward the dorsal side (Fig. 4).

Three molt periods (postmolt, intermolt and premolt) are identified in the snow crab, Chionoecetes opilio. The premolt period is subdivided into three stages (D_0, D_1 and D_{3-4}). Stage D_1 is further divided into three substages (D_1', D_1'' and D_1'''). The number of endites examined for each of these stages and substages is given in Table 1, and the terminology follows that of Lyle and MacDonald (1983).

Table 1. Number of endites observed in each molt stage and substage in Chionoecetes opilio.

A-B	C	D_0	D_1'	D_1''	D_1'''	D_{3-4}	Total
9	159	21	11	16	14	8	238

At these periods and stages, the morphology of the developing setae in the basal endite of the maxilla can be described as follows:

-Postmolt period (stage A-B): The cuticular formation is not completed. The setal shaft is thin-walled and the setal lumen appears very wide with a granular protoplasm (Figs. 3, 7a). The endite is thin and soft.

-Intermolt period (stage C): The cuticular formation is completed. The setal lumen becomes narrow and granular protoplasm is not conspicuous (Figs. 4, 7b). Tubular-like cuticular structure (hereafter refered to as cuticular tube) can be observed under the setal articulation of the setae lying in the near distal region of the endite (Figs. 4, 6). The endite is thick and hard. No epidermal retraction occurs.

-Premolt period (stage D): This period consists of three stages (D_0, D_1 and D_{3-4}). Stage D_0 (Fig . 8): The first sign of apolysis. The epidermis retracts from the cuticle at the base of the setae. Stage D_1: Stage D_1 has three substages (D_1', D_1" and D_1"'). Substages D_1' (Fig . 9): The epidermal retraction accelerates leaving a wide retracted zone. The tip of the new setae does not appear in the retracted zone. The setal shaft in the epidermis is not clearly visible. Substage D_1" (Fig . 10): The tip of the new setae appears in the retracted zone. The setal invagination progresses and at the end of this substage, a scalloped edge (Aiken, 1973) can be observed at the tip of the epidermis. The setal shaft in the epidermis becomes visible. Substage D_1"' (Fig . 11): The fold of setal invagination is conspicuous showing a tubular sheath at the tip of the epidermis. The setal shaft in the epidermis is visible but neither its proximal ends nor the barbule development can be clearly observed.

Stage D_{3-4} (Fig . 12): The retraction of the new endite continues. At the end of this stage, the new endite is particularly apparent under the connecting part of two lobes of the old endite. The proximal ends of the setal shafts are closed and the setal articulation is completed. The new setae are extruded by more than half their length into the retracted zone.

(2) Endite preservation after removal from the crabs

Up to 48 hours after removal from the crabs, a reliable staging can be made from the endites preserved in a 10 ml sampling vial filled with sea water at 2-3°C. After this time, molt staging becomes difficult, due to degeneration of the endite. Frozen endites do not change their apparent epidermal structure in the postmolt and intermolt periods, but a pronounced epidermal retraction and new setae extrusion are observed in the premolt period.

(3) Effect of the endite sampling on mortality

No obvious difference in feeding behavior was observed between the crabs without a pair of the basal endites and the control crabs both maintained in the aquarium. During the 45 days in the aquarium, 3 individuals out of 74 crabs without endites on the maxilla died. Whereas, among the 94 control crabs, 2 crabs died. The mortality ratio was not significantly different between control and test groups ($p > 0.05$) on a contingency table.

Discussion and Conclusions

The determination of molt stages of snow crabs, *Chionoecetes opilio*, by observation of setal development is generally in accordance with those described by Drach (1939), and Drach and Tchernigovtzeff (1967).

The postmolt period (stage A-B) and intermolt period (stage C) can be distinguished by observing the thickness of the setal lumen and its granular appearance (Mills and Lake, 1975; Reaka, 1975; Van Herp and Bellon-Humbert, 1978; Lyle and MacDonald, 1983). The formation of the internal cone at the base of the seta is also used to define the postmolt and intermolt periods (Drach, 1944; Scheer, 1960; Kamiguchi, 1968; Peebles, 1977). However, the morphology of this structure varies considerably among the families and is nonexistent in certain families (Drach and Tchernigovtzeff, 1967; Reaka, 1975). This structure is not observed (probably nonexistent) in the snow crab. For the species without this structure, other tegumental formations can be observed to define the postmolt and intermolt periods, for example, by considering stage B as a progressive formation of tegument and stage C as an achievement of tegumental formation (Drach and Tchernigovtzeff, 1967). In the snow crab, the cuticular tube can be clearly observed in stage C under the setal articulation of the setae lying in the near distal region of the endite. This can be used to distinguish between the postmolt and intermolt periods.

Due to the convexity of the endite and the existence of two rows of dense setae at the tip of the endite, an artifact (in the form of a line at the distal region of the endite) is often observed, which could cause stage C to be misread as stage D_0 with an epidermal retraction. However, the transition from stage C to stage D_0 is characterized by the appearance of a clear epidermal retracted zone at the tip of the endite unlike the opaque line of the artifact.

Stage D_1 is divided into three substages (D_1', D_1'' and D_1''') by observing the formation of setal invagination (Stevenson, 1968, 1972; Mills and Lake, 1975; Reaka, 1975; Peebles, 1977; Van Herp and Bellon-Humbert, 1978; Lyle and MacDonald, 1983). Drach and Tchernigovtzeff (1967) described the barbule formation as a general characteristic of substage D_1'". However, this characteristic cannot be used as a criteria of this stage due to scarcity of the barbules on the setae of the basal endite of snow crab.

Stage D_2 is characterized by the secretion of new cuticle in the region between the new setae (Drach and Tchernigovtzeff, 1967). However, this characteristic is often impossible to observe (Stevenson, 1972). In such a case, histological sections of the exoskelton are required to define stage D_2 (Drach and Tchernigovzeff, 1967; Stevenson et al., 1968; Aiken, 1973). This could not be defined by setagenic observation in the basal endite of the maxilla in the snow crab, as it is in some other crustaceans (Van Herp and Bellon-Humbert, 1978; Lyle and MacDonald, 1983).

The final stage in the premolt period (stage D_{3-4}) is characterized by the formation of setal articulation (Lyle and MacDonald, 1983), closed proximal ends of the setal shaft, and an extrusion of more than half the length of the new setae. Histological sections are required to define stages D_3 and D_4 (Scheer, 1960; Kurup, 1964; Drach and Tchernigovtzeff, 1967; Van Herp and Bellon-Humbert, 1978). Therefore, these stages are redefined as D_{3-4} and used as the final stage in the premolt period by restricting molt staging to setal development observations.

The endite can be preserved in ambient salinity sea water at low temperatures (2-3°C) for up to 48 hours after removal without changing the structure of the cuticle and epidermis. Freezing samples as suggested by Lyle and MacDonald (1983) however, may cause the endites to be misread, especially at the premolt stages, due to an amplified retraction of the epidermis and a pronounced setae extrusion. Aiken (1973) suggested that the pleopods of the American lobster (*Homarus americanus*) be examined fresh for the same reason. It was concluded that molt staging of the endite of the maxilla in the snow crab should be carried out on fresh material and within 48 hours of removal.

The removal of the basal endites does not appear to increase the mortality of crabs and can be performed within 5 to 10 seconds on each animal. By taking monthly samples, this technique allows the determination of the molting season and can also be used in tagging experiments. It is also suitable for studying the molt cycle in an aquarium. For this type of study, the molt stage can be checked twice by cutting off each lobe from the basal endites.

The present technique minimizes subjectivity and possible misreading of the molt stages and, in contrast to histological techniques, it reduces the sampling time, especially when processing a large number of animals.

Little is known about the molt cycle of snow crabs. In the southern Gulf of St. Lawrence, some snow crab fisheries are closed seasonally, due to the appearance of too many white crabs (molters) in the commercial catch (Bailey and Cormier, 1983). The incidence of white crab in the commercial catch not only depreciates the value of crab meat but may also cause high mortalities of released crabs(Watson, 1971). Kon (1980) reported that the molting season of snow crabs (*C. opilio*) varies in relation to their carapace width classes in the Japan Sea. If this is also the case for the stocks in the southern Gulf of St. Lawrence, the appearance of white crabs could be observed in different seasons. For the snow crab fishery, prediction of the beginning of the molting season is critical, due to the economic problems encountered by the fishing fleets when low quality white crabs are found in abundance in the catch. The prediction of the incidence of molters in the fisheries could lead to seasonal closures of crab fishing in order to avoid catching the white crabs in abundance. A survey of molt stages on natural populations of snow crab throughout the year by the technique presented herein will provide a better understanding of molt cycle and provide a biological basis for fishery management.

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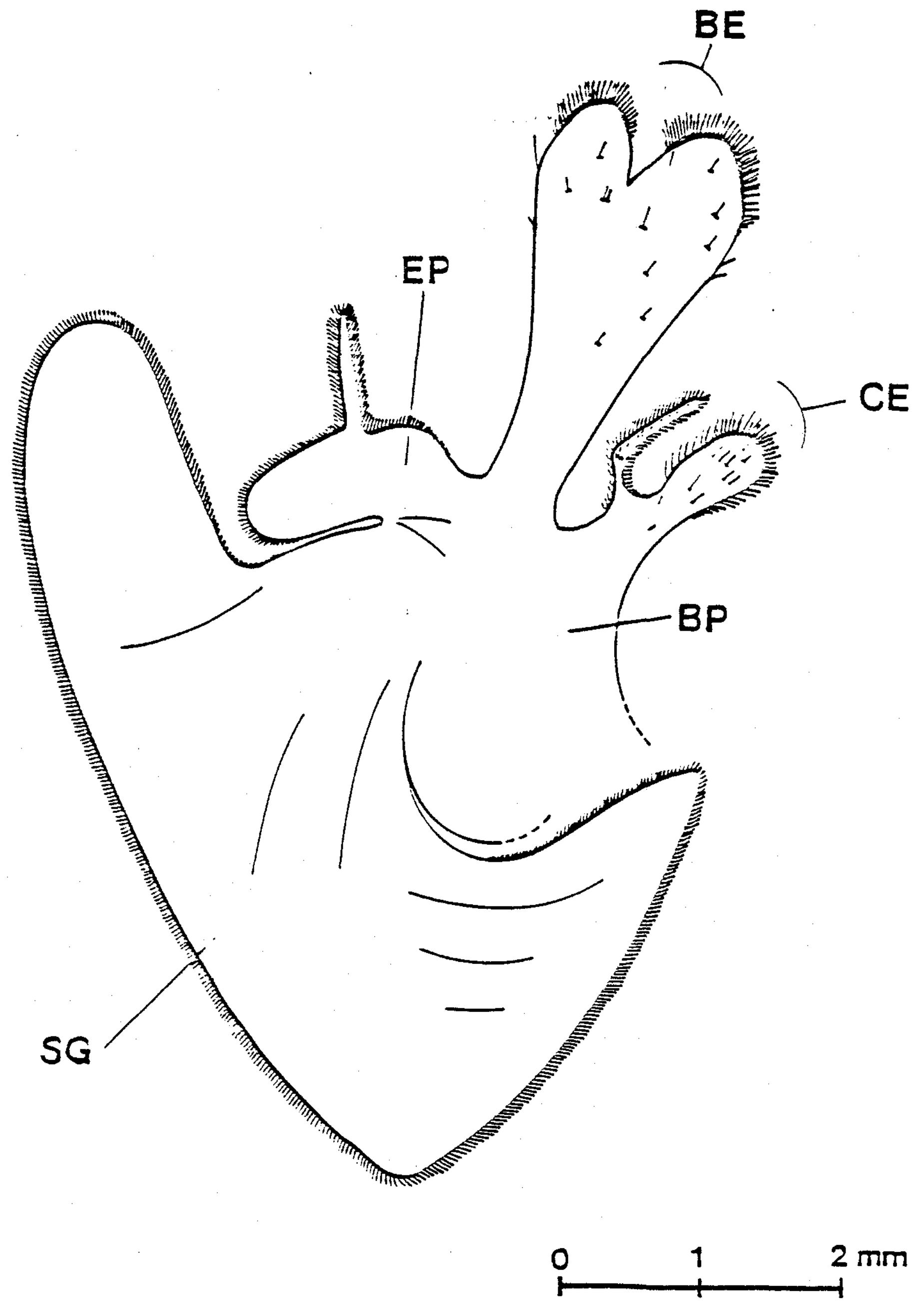
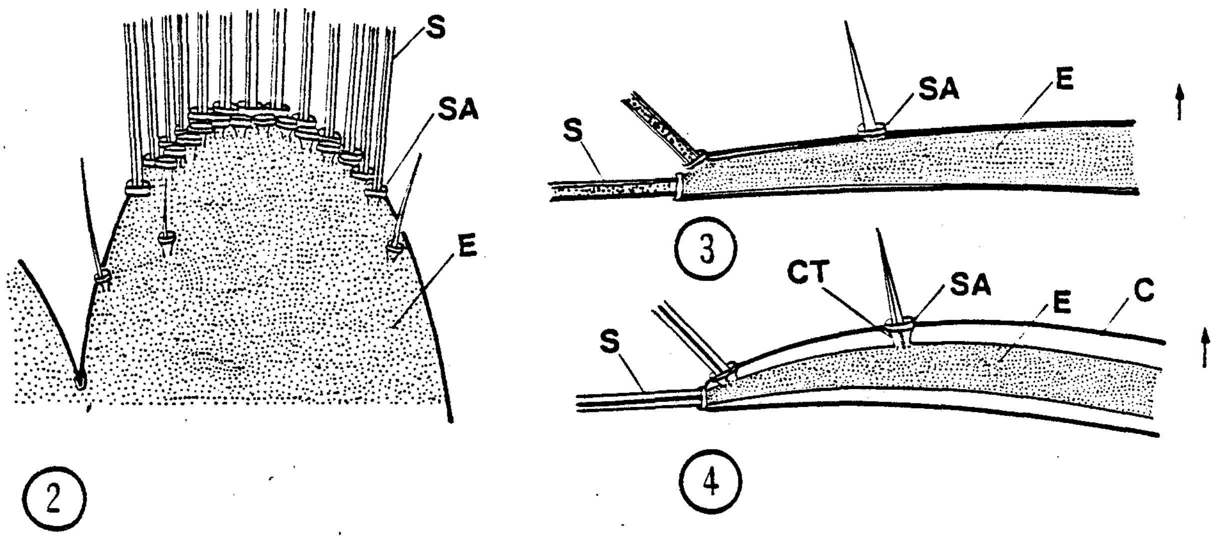


Fig. 1. Ventral view of the maxilla of Chionoecetes opilio showing the basal endite used for molt staging. (female, 62 mm in carapace width)

BE - basal endite, BP - basipodite, CE - coxal endite, EP - endopodite, SG - scaphognathite.



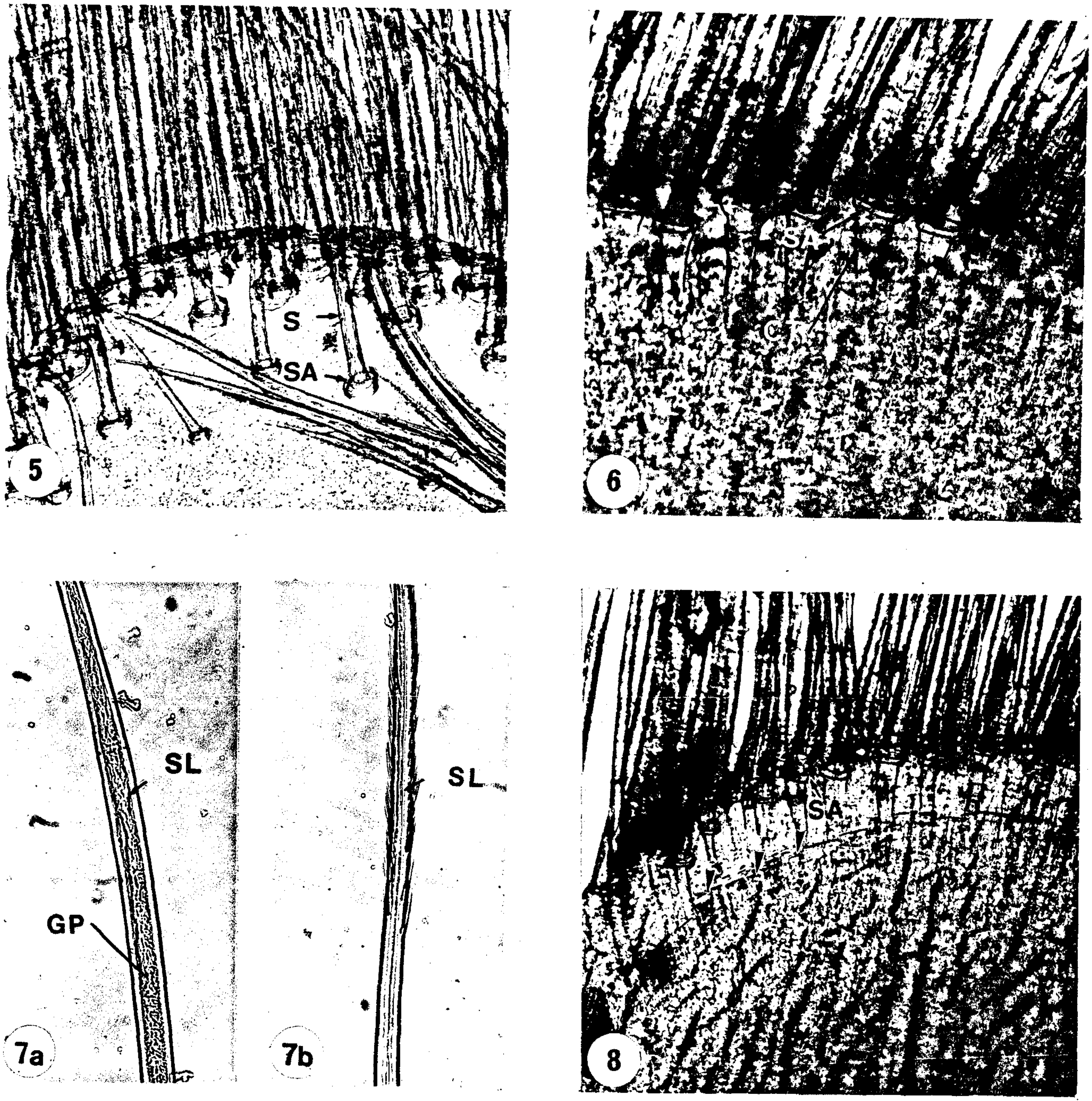
Figs. 2 - 4. Diagrams of the basal endites of the maxilla of <u>Chionoecetes opilio</u>.

2: General view of the endite (ventral view). The setae in the near distal region are not drawn;

3: Lateral view of the endite (postmolt period). Arrow indicates ventral side;

4: Lateral view of the endite (intermolt period). Arrow indicates ventral side.

CT - cuticular tube, E - epidermis, GP - granular protoplasm, S - seta, SA - setal articulation.



Figs. 5-8. Setal development in the basal endite of the maxilla in <u>Chionoecetes opilio</u>.

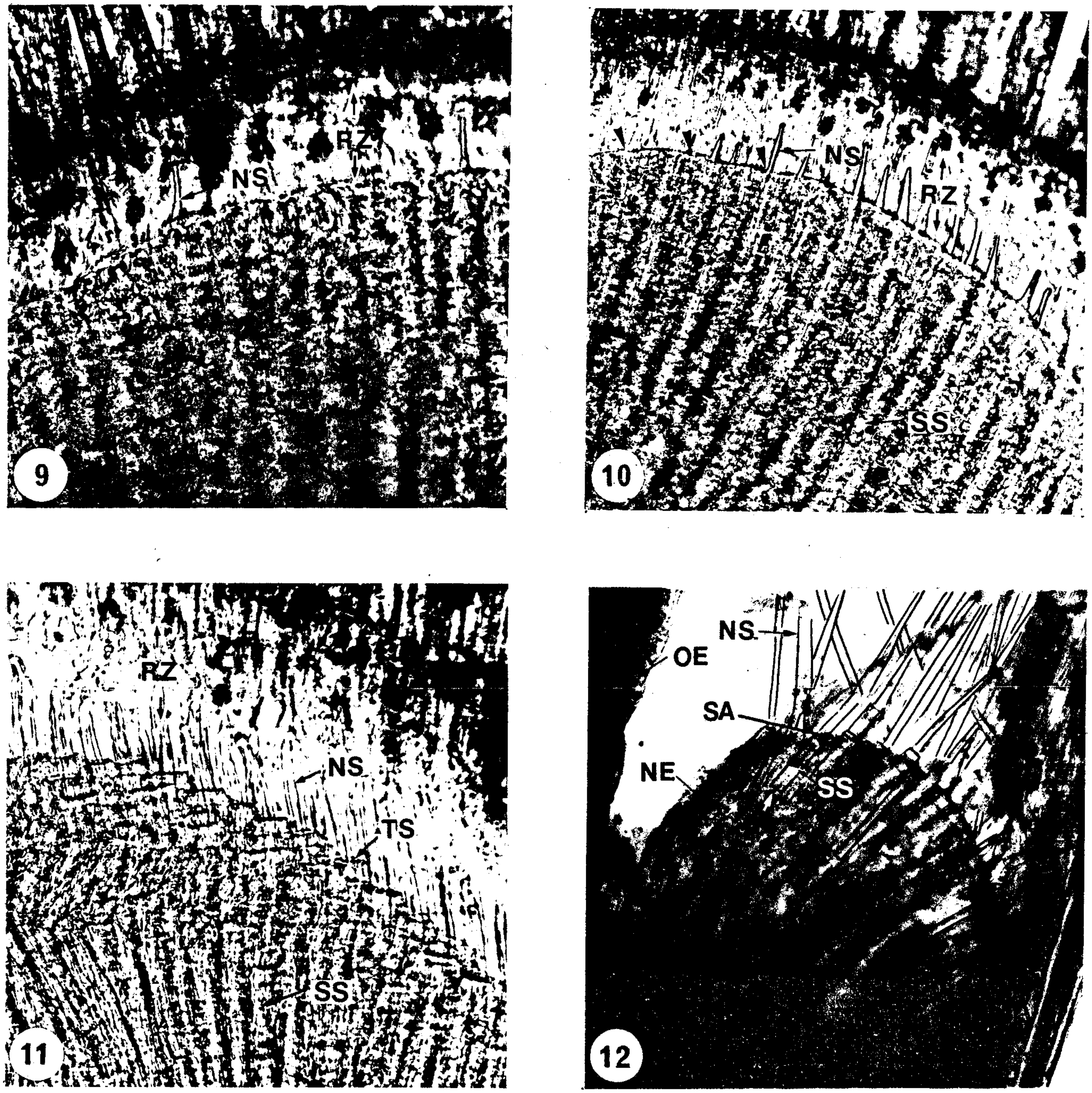
5: Stage A-B, the cuticular formation is not completed and the cuticular tube under the setal articulation is not distinguishable;

6: Stage C, the cuticular formation is completed showing a cuticular tube under the setal articulation of the setae lying in the near distal region of the endite;

7: Stages A-B and C, (a) - The setal lumen appears very wide a ganular protoplasm, (b) - The setal lumen becomes narrow and granular protoplasm is not conspicuous;

8: Stage D_0, the epidermis retracts from the cuticle at the base of the setae (arrows).

CT - cuticular tube, GP - granular protoplasm, S - seta, SA - setal articulation, SL - setal lumen.



Figs. 9-12. Setal development in the basal endite of the maxilla in Chionoecetes opilio.

9: Stage D_1', the epidermal retraction accelerates leaving a wide retracted zone. The tip of the new setae does not appear in the retracted zone;

10: Stage D_1", the tip of the new setae appears in the retracted zone. The setal invagination continues at the tip of the epidermis (arrows);

11: Stage D_1'", formation of tubular sheath at the tip of the epidermis is distinguishable;

12: Stage D_{3-4}, the setal articulation is completed and the proximal ends of the setal shaft are closed (arrow). The new setae are extruded more than half their length into the retracted zone.

NE - new endite, NS - new seta, OE - old endite, RZ - retracted zone, SA - setal articulation, SS - setal shaft, TS - tubular sheath.