Maine Department of Marine Resources
Setal (pleopod) Staging

Citations:

Original methods and staging criteria: Aiken, D. E. 1973. Proecdysis, setal development, and molt prediction in the American lobster (*Homarus americanus*). Journal of the Fisheries Board of Canada, 30: 1337–1344.

Atlantic Veterinary College at the University of Prince Edward Island, AVC Lobster Science Centre, 2001, "Pleopod Staging SOP #FM-02"

Factor, J. R. 1995. Biology of the lobster, *Homarus americanus*. San Diego, CA: Academic Press

Table 3 from Aiken 1973 "Criteria for staging proecdysis from pleopods of *Homarus*."

Molt stage[a]	Pleopod stage	Description
C_4	0	Epidermis closely applied to cuticular nodes at tip of pleopod; no amber zone or epidermal retraction at pleopod tip (Fig. 4B).
D_0	1.0	First indication of apolysis — amber or double-bordered region forms at the pleopod tip. Chromatophores often show signs of reorganization but there is no epidermal retraction from the cuticle (Fig. 4C).
D_0	1.5	Epidermis retracting from terminal cuticular nodes; may have double-bordered appearance (Fig. 4D, E, F).
D_0	2.0	Epidermal line clearly formed and retracting from lateral cuticular nodes (Fig. 4G, H).
D_0	2.5	Maximum epidermal retraction — not touching any lateral cuticular nodes (Fig. 4I).
D_1'	3.0	Invagination papillae form at site of future setae; epidermis becomes scalloped (Fig. 4J, K).
D_1''	3.5	Invagination papillae clearly formed but shafts of new setae not well defined (Fig. 4L).
D_1'''	4.0	Shafts of developing setae visible but proximal ends not clearly defined (Fig. 4M). Shafts now invaginated to maximum length.
D_2'	4.5	Shafts visible full length but proximal ends are bifurcate instead of blunt (Fig. 4N, O). Barbules becoming visible on setal shafts.
D_2''	5.0	Shafts of developing setae thick, proximal ends blunt (Fig. 4P, Q).
D_3'	5.5	Shafts of setae very thick and dark, proximal ends blunt. Classify as D_3'' if folds or ripples are visible in cuticle on upper surface of pleopod (Fig. 4R).

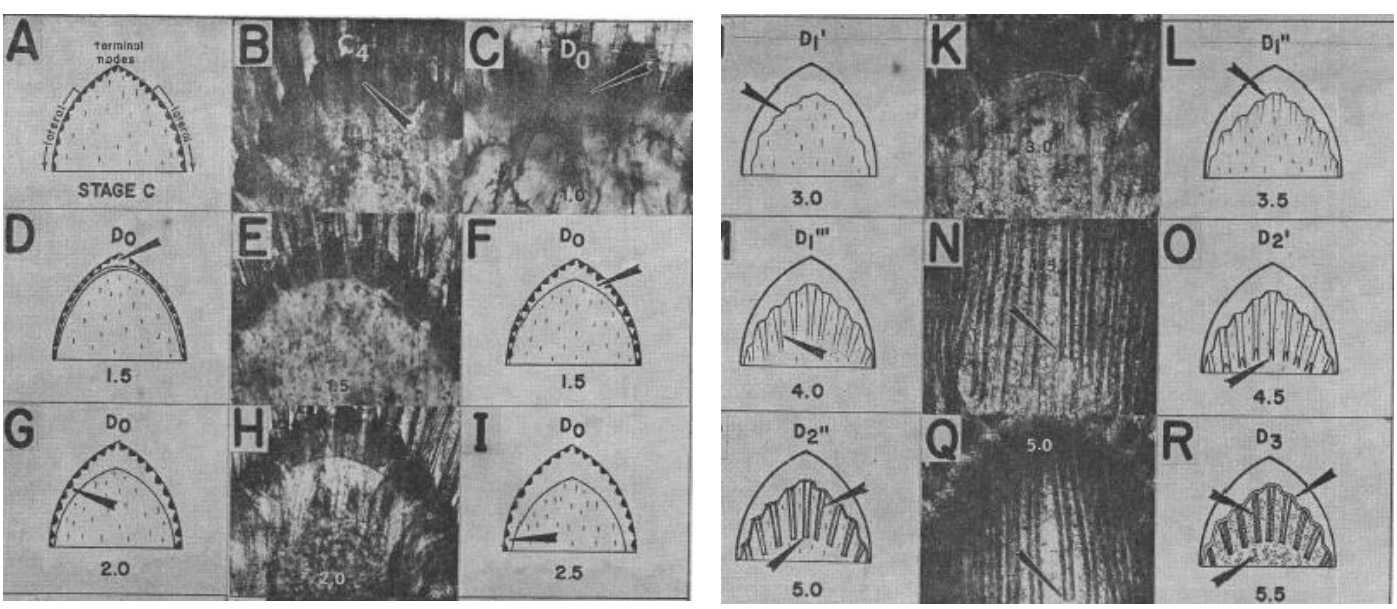


Figure 4 from Aiken (1973) "Morphology of *Homarus* pleopods during intermolt (A,B) and proecdysis (C-R)"

Setal (pleopod) Staging

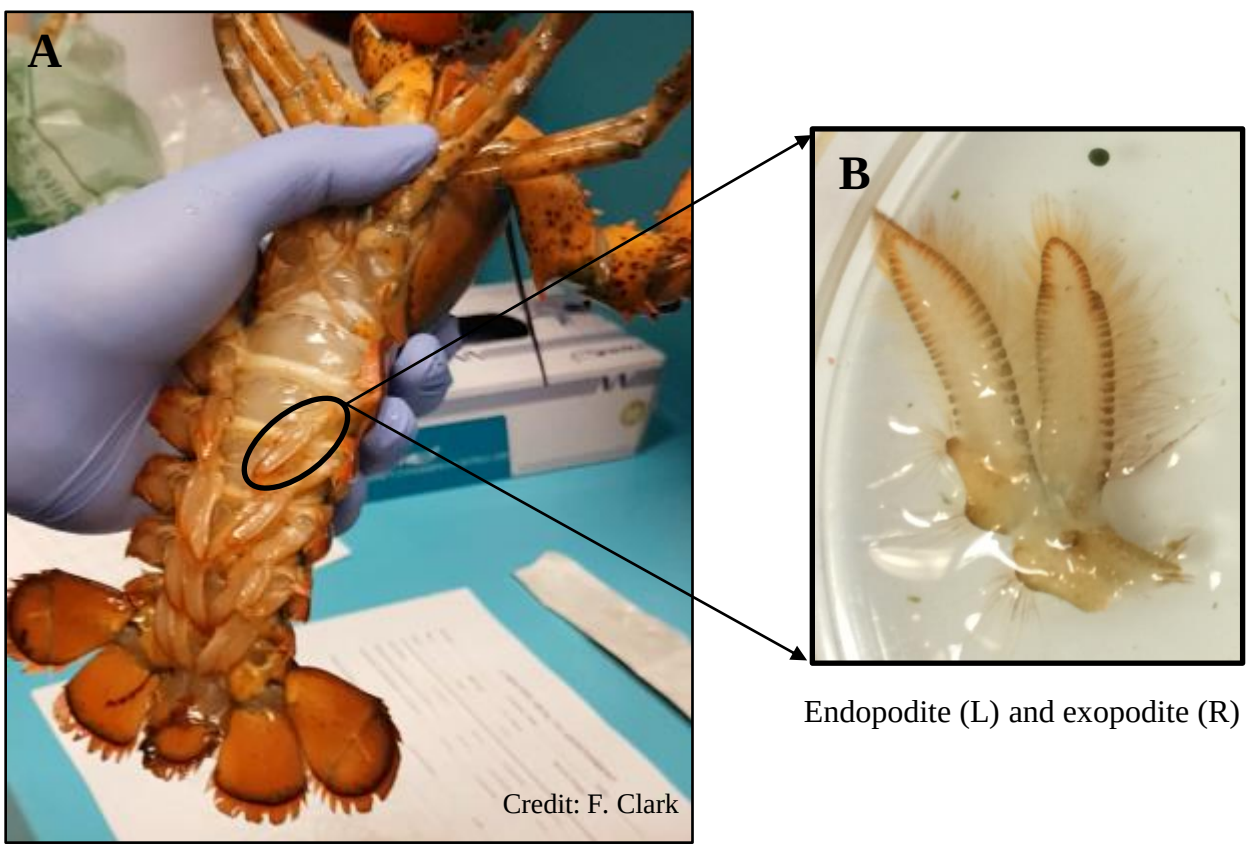
Suggested supplies for pleopod collection:

- Dissecting microscope with undermount lighting
- Camera mounted on microscope
- Microscope slides or glass dish
- Small, dissection scissors
- Kim wipes or similar wipes for microscope slides
- Forceps
- Cold, filtered seawater (FSW) in a squirt bottle

Pleopod collection and prep:

1) Put a small amount of FSW on a microscope slide or glass dish.
2) Hold the lobster in one hand so the ventral side is facing upwards.
3) Using scissors, cut the second pair on the right side (Fig. 1A). - If missing or deformed, collect the third pleopod set on the same side. For consistency and ease of interpretation, attempt to collect the same location on each lobster.
4) Gently grasp the pleopod by the base with forceps and place in the FSW.
   - Cut pleopods can be stored in chilled FSW for 12 hours
5) Examine and determine stage from the endopodite (Fig. 1B).

Figure 1. The ventral side of a lobster with the second set of pleopods circled (A) and the pleopod endopodite and exopodite (B).



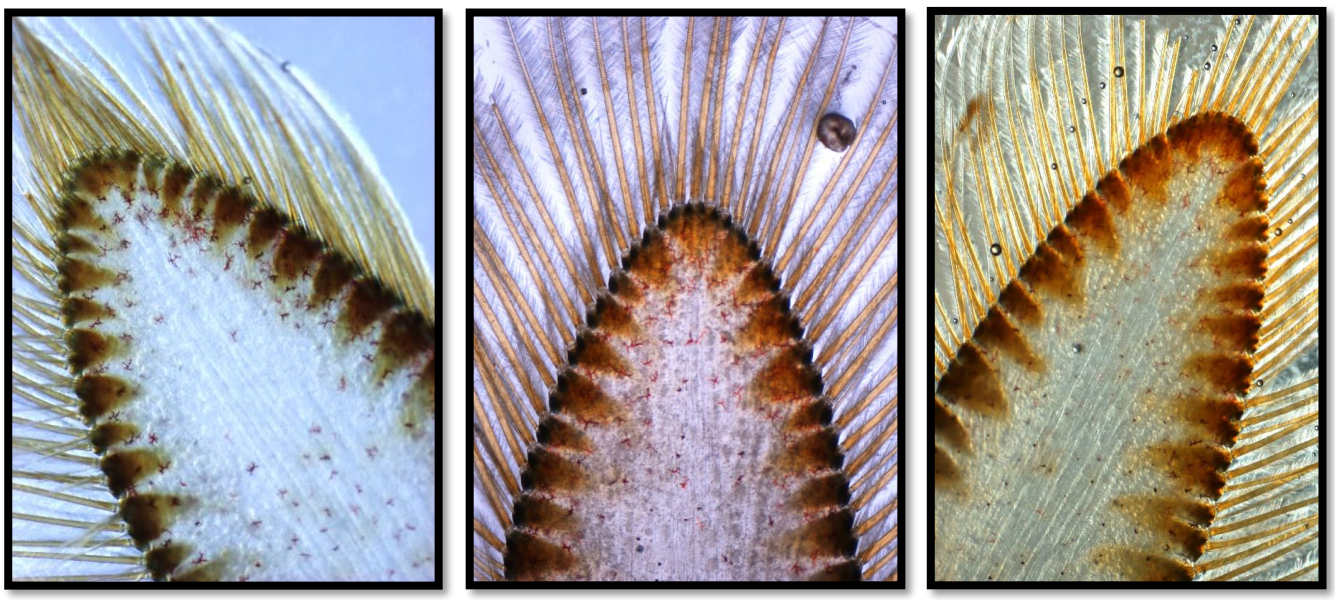
Endopodite (L) and exopodite (R)

Setal (pleopod) Staging

*All images taken at 2-3.5x

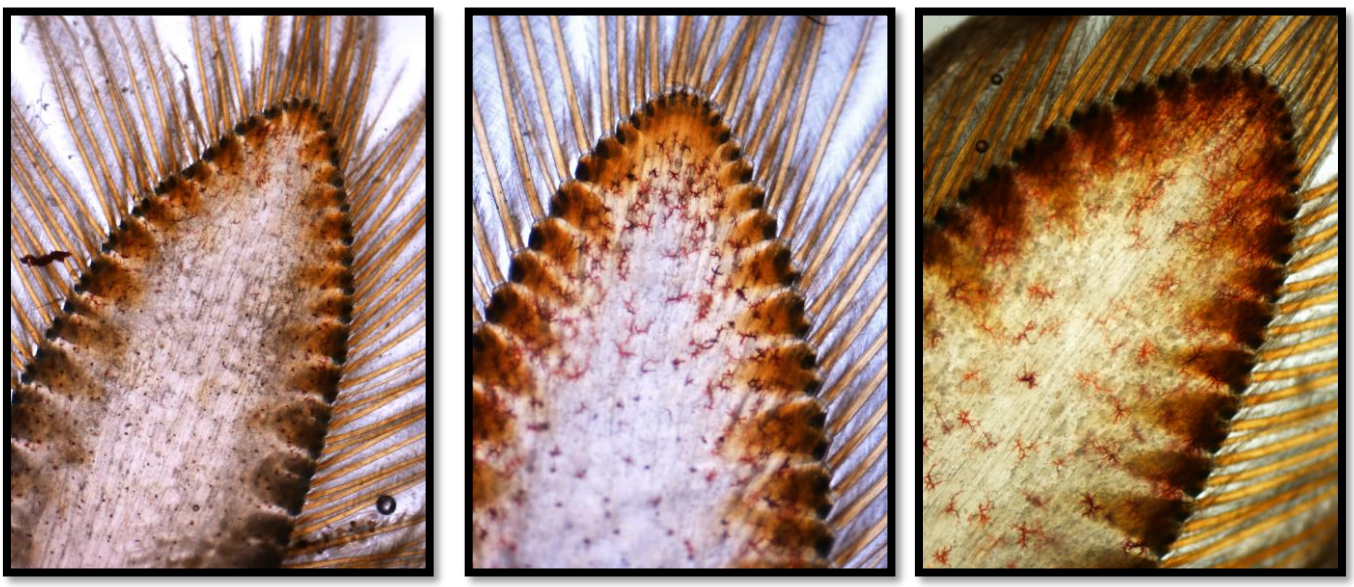
Image credits: MEDMR

Stage 0 (Molt stage C_4, intermolt):



- No separation between the epidermis and the tip of the pleopod
- No fluid “amber zone” representing the start of apolysis

Stage 1-1.5 (Molt stage $D_{0'}$ and $D_{0'''}$, start of premolt):



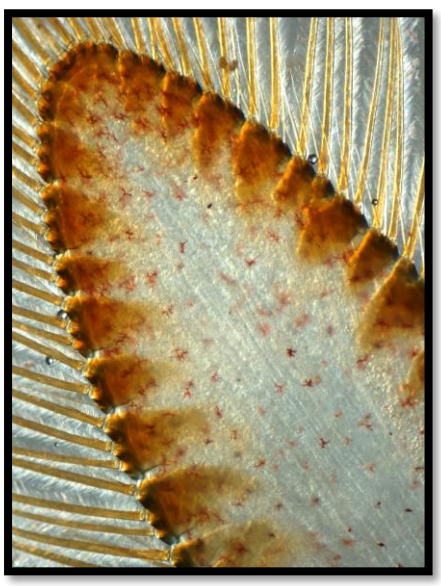
- First separation between the epidermis and the tip of the pleopod, may give a “double border” appearance in parts
- First appearance of “amber zone” representing the start of apolysis
- Coloration/pigments may begin to reorganize at this point

Setal (pleopod) Staging

* All images taken at 2-3.5x

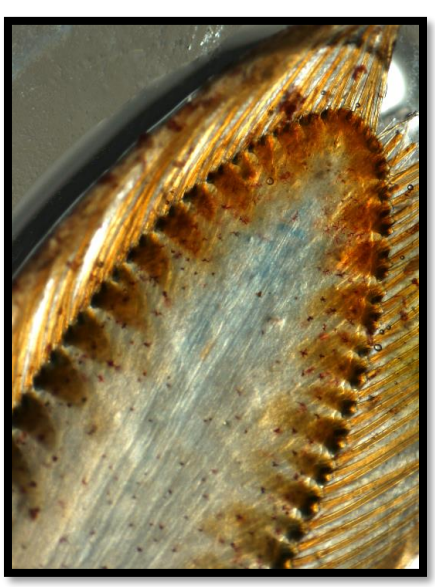
Image credits: MEDMR

Stage 2-2.5 (Molt stage $D_{0"}$ and $D_{0'''}$, start of premolt):



-Retraction of epidermis is distinct at the tip and upper half of pleopod
- In stage 2.5, there is a distinctive line indicating no epidermal contact

Stage 3-3.5 (Molt stage $D_{1'}$ to $D_{1"}$, premolt):



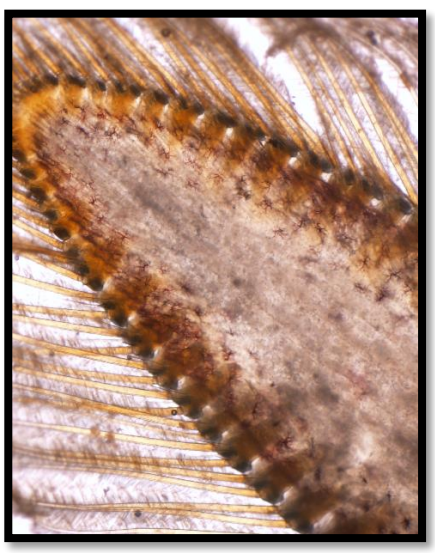
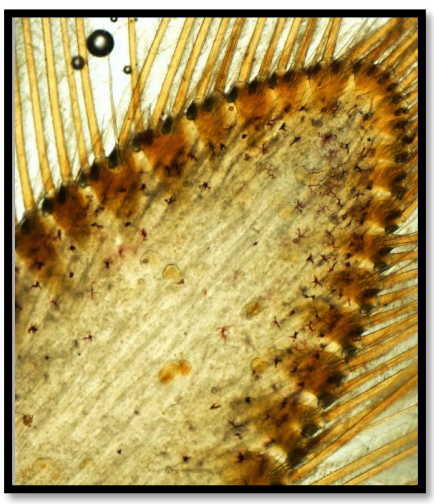
- Epidermis appears “scalloped” as new setae begin to form
- Shafts/base of new setae are present but not well defined

Setal (pleopod) Staging

* All images taken at 2-3.5x

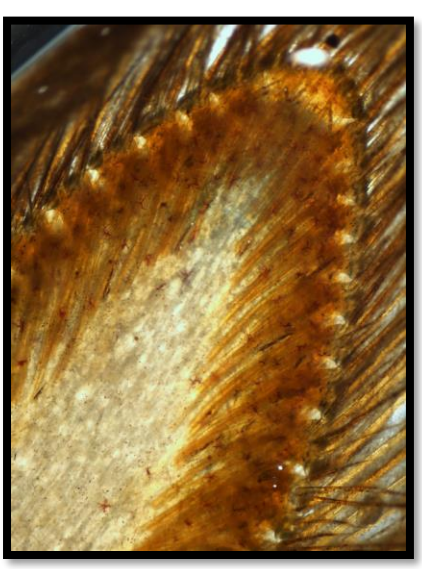
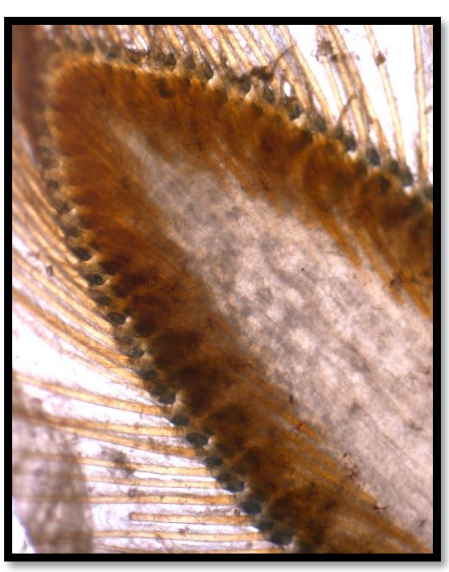
Image credits: MEDMR

Stage 4-4.5 (Molt stage D_{1}‴ to D_{2}', premolt):


- Shafts of new setae are now visible in all parts of the pleopod, but the proximal ends are difficult to distinguish
- In 4.5, the ends of the developing setae are more visible and bifurcate

Stage 5-5.5 (Molt stage D_{2}" to D_{3}', premolt, molting imminent):

- New setae are visible to the naked eye and are a brown/orange color
- Shafts of setae are thick, and the proximal ends blunt
- Changes in the cuticle are also evident on the edges of the carapace, indicating molting is imminent

*** Other visual indicators of molt status (shell hardness, darkening of abdomen, blue color at edges of shell) are described in Factor (1995).**