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TECHNIQUES FOR PREPARING CRUSTACEANS FOR SCANNING ELECTRON MICROSCOPY

Bruce E. Felgenhauer

A B S T R A C T

Several techniques for preparing the internal and external surfaces of crustaceans for SEM are described in detailed flow-chart form. Suggestions are given for improving fixation, cleaning, and overall appearance of specimens.

The study of gross morphological detail as well as fine structures of crustaceans has been substantially enhanced by the many recent technological advances in scanning electron microscopes (SEM), and, as a result, the use of this instrument in all aspects of crustacean biology is on the increase. No matter how good an instrument may be, however, the results still depend on the quality of the material photographed. One of the major problems in preparing crustaceans for SEM is the removal of accumulated debris. An additional problem is that the crustacean cuticle provides an excellent habitat for epibiotic organisms ranging from bacteria and fungi to symbiotic mites (Bauer, 1975; Felgenhauer and Schram, 1978; Holmquist, 1985, and references therein). Apart from aesthetic considerations, these organisms may cover and obscure important structures, such as the terminal pores of chemoreceptors, or cause difficulties in the identification of setal types. Marine crustaceans are particularly troublesome, since they may exude appreciable quantities of mucus at the time of fixation.

Several authors have offered good suggestions for specific problems in preparing crustaceans for SEM (e.g., Abele, 1971, gonopods of brachyuran crabs; Scotto, 1980, larval crustaceans). This note describes several general techniques that have proven to be particularly successful in my own work (e.g., Felgenhauer and Abele, 1983, 1985) for freeing the body surfaces of crustaceans of unwanted organisms and debris. Several other methods are presented for examining the internal anatomy of crustaceans. All micrographs presented in this note were taken with a Cambridge S4-10 or a JEOL 840 scanning electron microscope at accelerating voltages of 3-20 kV.

I. BASIC SPECIMEN PREPARATION

Fresh material is always preferable to specimens previously fixed in ethanol or Formalin. Below is an outline of a simple protocol that consistently produces excellent results (Fig. 1A, B).

- 1a. Fix fresh material in 3% glutaraldehyde at room temperature for 3 h in whatever buffer is best for the tissue being prepared. For fresh-water forms, I prefer a 0.1 M phosphate buffer at a pH of 7.0, and for marine crustaceans I use Millipore-filtered sea water or 0.1 M sodium cacodylate at a pH of 8.0.
- 1b. If Formalin- or ethanol-fixed tissue must be examined, hydrate it to distilled water and continue beginning at step 3 below.
2. Wash tissue in 3 changes of the chosen buffer for 5 min each to remove excess fixative.
3. Accomplish secondary fixation (postfixation) of the material in 1-2% osmium tetroxide (OsO_4) in buffer choice for 2 h. Always use OsO_4 under a

fume hood and with proper laboratory safety precautions, e.g., gloves, apron, and goggles.

4. Accomplish further osmication of tissue (optional—see General Suggestions) by means of an osmium-binding agent such as thiocarbohydrazide. Procedures for this important step are outlined by Malick *et al.* (1975); a modified version is outlined by Tyson and Sullivan (1979).
5. Rinse in buffer (3 changes, 5 min each), wash in distilled water (3 changes, 5 min each), and dehydrate in a graded ethanol series (distilled water to 25% ETOH, 35%, 50%, 70% (specimens may be stored at room temperature in 70% for extended periods without damage to the tissue), 80%, 90%, 100%; three 5-min changes each) to the transitional fluid amyl acetate or acetone; critical-point dry; sputter-coat with gold-palladium, mount, and observe.

II. REMOVAL OF DEBRIS/EPIBIONTS

1. Prepare material as described in steps 1–4 of Basic Specimen Preparation. *Do not* begin dehydration series.
2. Wash material in three 5-min changes of distilled water. Next, place material in a weak solution of the anionic surfactant TWEEN-80 (two drops concentrate to 100 ml of distilled water) for 15 min. (This step may not be necessary if material is only slightly fouled.)
3. Sonicate material for 10 s (easy does it!—compare Fig. 1E with Fig. 1F) in the TWEEN-80 solution. Sonication time may need to be reduced for small specimens (e.g., for body length of <1 mm).
4. Wash tissue carefully in three 5-min changes of distilled water to remove the surfactant.
5. Dehydrate, critical-point dry, mount, sputter-coat, and observe. Specimens should be stored in a vacuum desiccator. Results of this technique can be seen if Fig. 1C and Fig. 1D are compared.

III. REMOVAL OF MUCUS (MODIFIED FROM MARISCAL, 1974)

1. Prepare specimens as described in steps 1–4 of Basic Specimen Preparation (Section I). *Do not* begin dehydration series.
2. Transfer specimens from distilled water to 16% glycerol solution (in distilled water). Place the vessel on a shaker table overnight to draw off the mucus.
3. Transfer the material to a 20% ethanol solution and place it on a shaker table for 6–10 h to remove the glycerol. This solution should be changed completely several times during this process.
4. Dehydrate material to 70% ETOH and sonicate (careful!) for 10 s.
5. Complete specimen preparation as described in step 5, Section I.

IV. REMOVAL OF BACTERIA AND FUNGI

Frequently bacteria and fungi will cover cuticle surfaces and obscure important features (Fig. 2A, B). These infestations are often not noticeable until the specimen is examined in the scanning electron microscope. Bacteria and fungal hyphae are particularly difficult to remove by mechanical means. If live specimens of the infested material are still available, it may be possible to remove the bacteria and fungi. Prokaryotes can be eradicated by the use of broad-spectrum antibiotics such as streptomycin or tetracycline. Dosage and length of treatment vary depending upon the severity of the infestation. For most problems treatment for

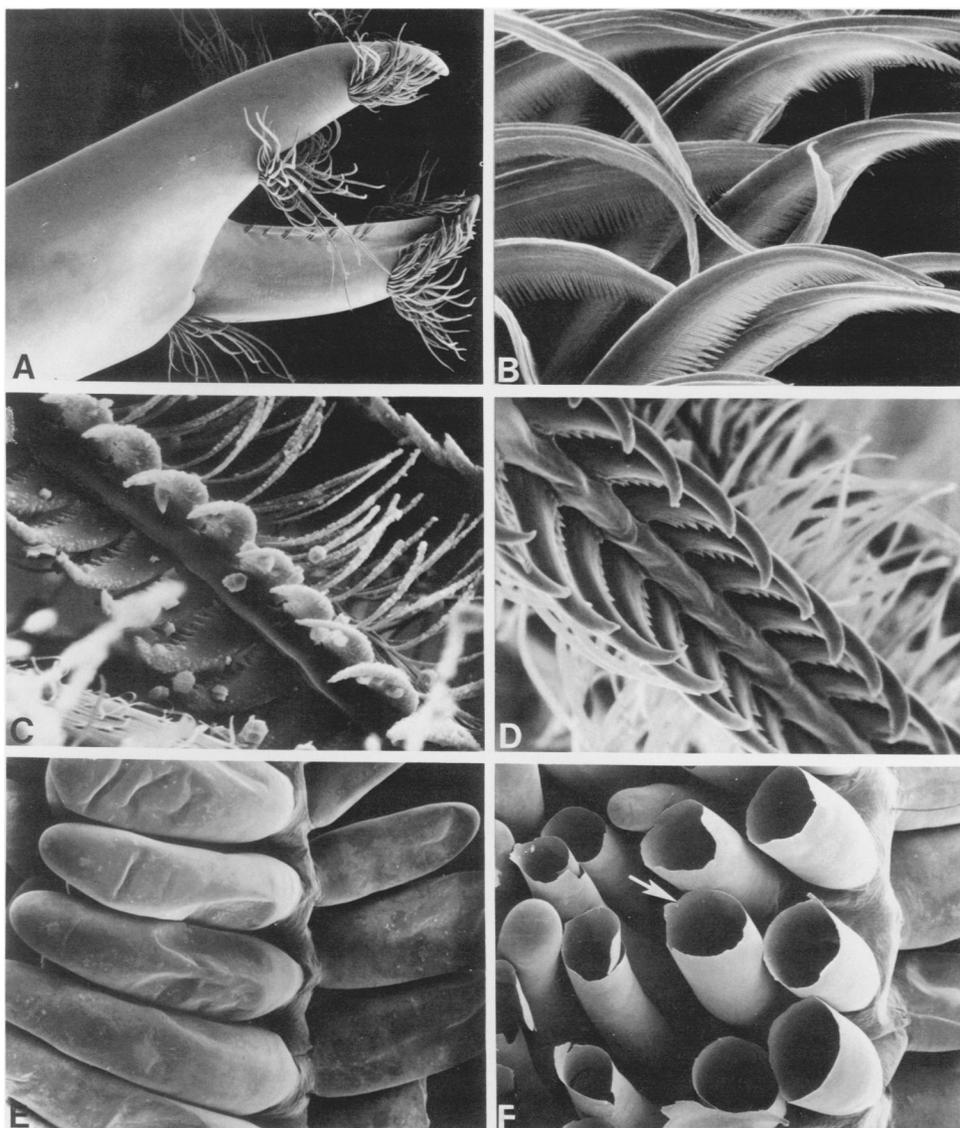


Fig. 1. Scanning electron micrographs of crustaceans. A, first cheliped of the caridean shrimp *Saron marmoratus* Olivier, $\times 75$; B, plumose setae from the second maxilliped of the atyid shrimp *Potimirim glabra* (Kingsley), $\times 250$; C, fouled serrate seta from the first pereopod of *Atya innocous* (Herbst), $\times 600$; D, same serrate setae as in C following cleaning procedure described in Section II, $\times 600$; E, trichobranchiate gill lamellae of *Nephrops* sp., after 10-s sonication, $\times 200$; F, same gill lamellae as in E after sonication for 20 s. White arrow indicates breakage of the thin gill cuticle caused by over-sonication, $\times 200$.

two or three days in a solution of 250 mg of either antibiotic in 5 gal (18.9 l) of water is sufficient to clear the infestation.

For eukaryotic organisms, such as fungal hyphae, a two-day treatment in a 1–2% solution of cupric sulfate is very effective. Many other compounds and an-

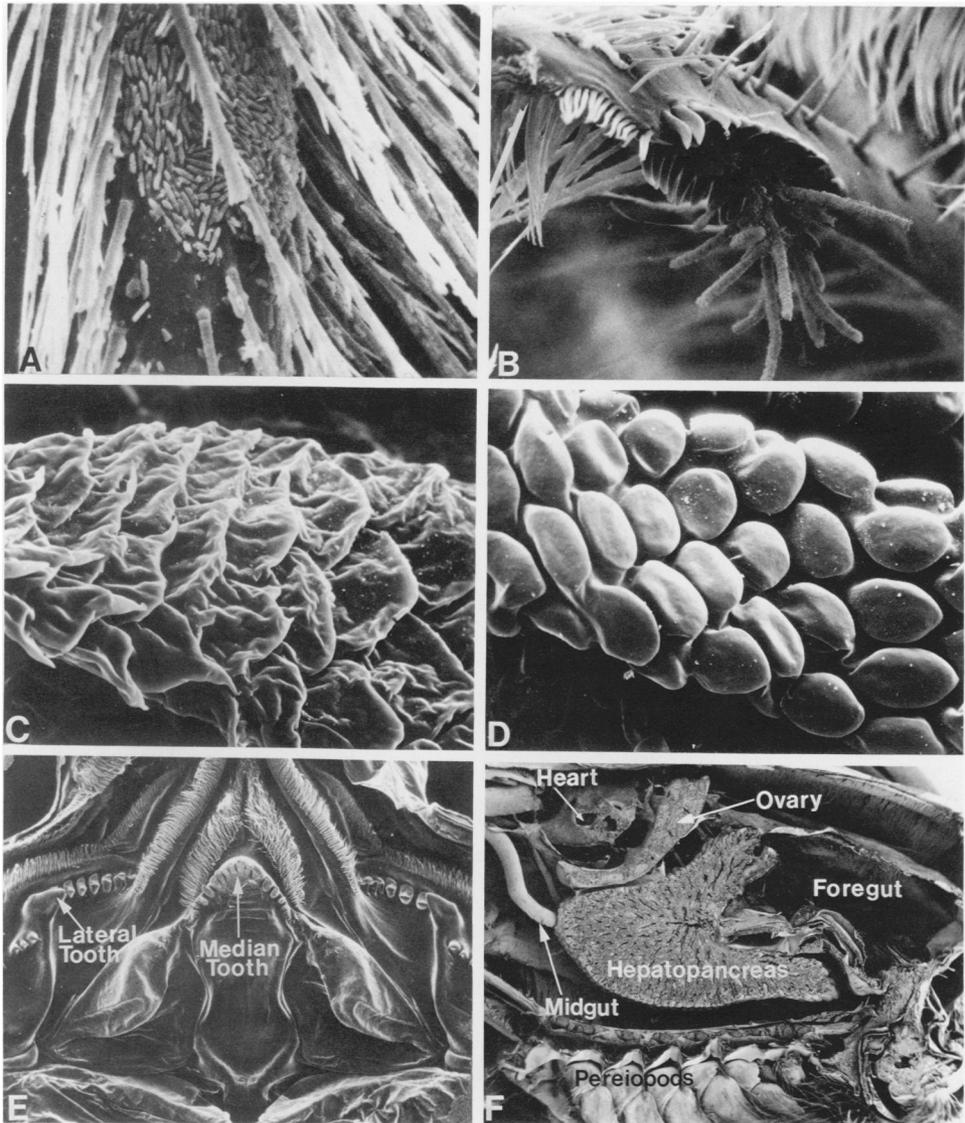


Fig. 2. Scanning electron micrographs of crustaceans. A, bacterial infestation from the branchiostegite of the grapsid crab *Sesarma reticulatum* Say, $\times 600$; B, fungal infestation of plumose setae of the third maxilliped of *Atya innocous*, $\times 400$; C, results of air drying the convoluted membrane within the foregut of *Atya innocous*. Note distorted and collapsed cuticle surface, $\times 800$; D, convoluted membrane of *A. innocous* after critical-point drying, $\times 800$; E, internal features of the foregut of *Penaeus setiferus* (Linnaeus), $\times 200$; F, sagittal paraffin-carved thoracic region of the pelagic caridean shrimp *Oplophorus* sp., $\times 100$.

tibiotics can be used to remove fungus. I offer the one above because it seems to work best.

After treating material for either type of infestation, follow the basic SEM preparation in Section I.

GENERAL SUGGESTIONS

I recommend highly following each of the above procedures in its entirety. Investigators who have had difficulties with their material invariably have skipped one or more steps in the protocol. Each of these procedures is admittedly time consuming, but the results are worth the effort.

Crustaceans that are properly fixed and dried when observed in the SEM resemble most closely the living condition. Many investigators circumvent postfixation with osmium tetroxide for reasons of safety or time. Osmium tetroxide, like most substances used in electron microscopy, is dangerous (always use under a hood, since the vapor alone may fix tissues), but it is also an integral part of the fixation process. In addition to its role in stabilizing lipids, it increases the specimen's contrast and stability under the electron beam, thereby reducing annoying charging. The use of an osmium-binding agent such as thiocarbohydrazide greatly reduces charging for particularly delicate specimens. I recommend its use for all tissues.

Another shortcut commonly followed is air drying the specimens rather than critical-point drying them. The surface tension of the dehydrating agent may substantially distort the specimen surface. Structures such as plumose setae or thin cuticle usually collapse when air dried (see Fig. 2; compare C with D).

Recently, SEM has been employed to examine soft anatomy as well as endoskeletal elements. Oshel (1985) introduced paraffin carving of crustaceans, and the details of this technique can be found in his paper. It is an effective and useful technique for observing crustacean anatomy in three dimensions (Fig. 2F). A sharp scalpel can be used, but I prefer to use a standard microtome for this technique for several reasons. First, the measured thickness of the 10–15- μm paraffin sections makes it possible to know the exact location within the specimen of the image viewed. Second, the investigator has the advantage of both light microscope and SEM views of the same areas of tissue. Finally, if one wishes to cut in another plane, it is easy to re-embed the tissue and cut in another direction.

Techniques for preparing whole structures such as the foregut (Fig. 2E) have been discussed in detail by Felgenhauer and Abele (1985) and hence will not be repeated here.

Many excellent studies concerning preparative techniques for Crustacea exist in the literature. I felt the need, for myself and others, to present these methods in one concise paper. Because it would be profitable to all to keep this collection up to date, I would welcome information on new methods and suggestions that may have been overlooked in this note.

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LITERATURE CITED

- Abele, L. G. 1971. Scanning electron micrographs of brachyuran gonopods.—*Crustaceana* 21: 218–219.
- Bauer, R. T. 1975. Grooming behavior and morphology of the caridean shrimp *Pandalus danae* Stimpson (Decapoda: Natantia: Pandalidae).—*Zoological Journal of the Linnean Society* 65: 281–303.

- Felgenhauer, B. E., and F. R. Schram. 1978. Differential epibiont fouling in relation to grooming in *Palaemonetes kadiakensis*.—*Fieldiana (Zoology)* 72: 83–100.
- , and L. G. Abele. 1983. Ultrastructure and functional morphology of feeding and associated appendages in the tropical fresh-water shrimp *Atya innocous* with notes on its ecology.—*Journal of Crustacean Biology* 3: 336–363.
- , and ———. 1985. Feeding structures of two atyid shrimps with comments on caridean phylogeny.—*Journal of Crustacean Biology* 5: 397–340.
- Holmquist, J. G. 1985. The grooming behavior of the terrestrial amphipod *Talitroides alluaudi*.—*Journal of Crustacean Biology* 5: 334–340.
- Malick, L. E., R. B. Wilson, and D. Stetson. 1975. Modified thiocarbonylhydrazide procedure for scanning electron microscopy: routine use for normal, pathological, or experimental tissues.—*Stain Technology* 50: 265–269.
- Mariscal, R. N. 1974. Scanning electron microscopy of the sensory epithelia and nematocysts of corals and a corallimorpharian sea anemone.—*Proceedings of the Second International Coral Reef Symposium 1*. Great Barrier Reef Committee, Brisbane, pp. 519–532.
- Oshel, P. E. 1985. Paraffin carving: A preparative technique for scanning electron microscopy of crustaceans.—*Journal of Crustacean Biology* 5: 327–329.
- Scotto, L. E. 1980. Studies on decapod Crustacea from the Indian River region of Florida XIV. A method for rapid preparation of brachyuran larvae for scanning electron microscopy.—*Crustaceana* 38: 99–101.
- Tyson, G. E., and M. L. Sullivan. 1979. Antennular sensilla of the brine shrimp, *Artemia salina*.—*Biological Bulletin* 156: 382–392.

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