Marine Biology 40, 261-276 (1977)

MARINE BIOLOGY

Antifouling Adaptations of Marine Shrimp (Crustacea: Decapoda: Caridea): Functional Morphology and Adaptive Significance of Antennular Preening by the Third Maxillipeds

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Abstract

Preening (cleaning, grooming) of the antennules and other cephalothoracic appendages by the third maxillipeds was observed in several species of shrimp. Distribution and ultrastructure of serrate grooming setae on the third maxillipeds, which scrape the antennules, was studied with light and scanning electron microscopy. The motor patterns of antennular cleaning were similar for all species. Antennular preening was the most frequent grooming behavior observed, but the duration of other grooming behaviors was greater. Tidepool shrimps (*Heptacarpus pictus*) experimentally prevented from grooming the antennules by ablation suffered fouling of the olfactory hairs of the antennules with their subsequent breakage and loss; antennules of controls remained clean and undamaged. Antennular preening, a frequent and widespread behavior of caridean shrimps and other decapod crustaceans, is suggested as having high adaptive value in keeping sensory sites free of epizoic and sedimentary fouling which might render them inoperative.

Introduction

A characteristic behavior of caridean shrimp and other decapod crustaceans is the grooming of the antennules (first antennae) by setal combs on their third maxillipeds. This cleaning behavior has been described in detail for the shrimp *Pandalus danae* Stimpson (Bauer, 1975). Ablation experiments indicated that the behavior prevents settlement of fouling organisms and debris on the antennules, which are important sites of chemoreception in decapod crustaceans.

The purpose of this study was to extend and compare observations of this grooming behavior to other species of caridean shrimps. This report deals with preening by the third maxillipeds, while subsequent reports will cover other grooming behaviors, such as gill cleaning, general body grooming and the role of cleaning behavior in reproductive activities. Distribution and ultrastructure of serrate grooming setae on the third maxillipeds of these species are described with light and scanning electron microscopy. Experiments showing the consequences of third maxilliped removal were performed primarily on the tidepool shrimp *Heptacarpus pictus* (Stimpson). The general question asked by experimental work was whether or not preening of the antennules keeps them free of epizoic and sediment fouling. The adaptive value of antennular and other grooming behaviors by the third maxillipeds in marine shrimps is discussed.

Materials and Methods

Shrimps observed for grooming behaviors were usually captive specimens in recirculating aquaria or on watertables with running seawater. Observations were made during the day or evening with laboratory lights on. No blinds or shields were used to screen the observer from the shrimps. *Heptacarpus pictus* was observed in the field during the course of many collecting trips.

Limb movements of smaller shrimps such as *Heptacarpus pictus* (2 to 3 cm total length) were best observed in small glass aquaria, using a dissecting microscope for viewing. The housing containing the oculars and objectives was removed from the microscope stand, turned with objective lenses forward, and handheld, somewhat like a telescope or binoculars. Shrimps could be observed at

Table 1. Information on caridean species in which antennular preening has been directly observed

Species observed	Locality of collection	Number of specimens observed (approximate)	Approximate observation time (h)
Hippolytidae			
Heptacarpus pictus	Bird Rock; Beach and Tennis Club, La Jolla, California (tidepools in rocky intertidal)	500-1000	100
H. stylus (Stimpson)	University of Washington's Friday Harbor Laboratories, San Juan Island, Washington (in algae off the Laboratories' floating docks)	20	2-3
H. paludicola (Holmes)	Argyle Lagoon, San Juan Island; Mission Bay, San Diego, California; San Quentin Bay, Baja California (in *Zostera* sp. beds)	100	20
H. palpator (Owen)	Mission Bay, San Diego (among fouling organisms beneath floating docks)	10	2-3
H. taylori (Stimpson)	Loring Street, La Jolla (rocky intertidal)	25	5
H. brevirostris (Dana)	Friday Harbor Laboratories (in algae off the floating docks)	25	4-5
Hippolysmata californica Stimpson	La Jolla (shallow subtidal rock crevices)	10	5
Pandalidae			
Pandalus danae	San Juan Island (in algae off the Friday Harbor Laboratories' floating docks and subtidal dredging)	100	50
P. platyceros Brandt	San Juan Islands (subtidal dredging)	5	1
P. hypsinotus Brandt		5	1
P. montagui tridens Rathbun		5	1
Pandalopsis dispar Rathbun		5	1
Crangonidae			
Crangon nigricauda	Mission Bay, San Diego (burrowed in sand)	1	5
Paracrangon echinata Dana	Friday Harbor Laboratories (subtidal dredging)	20	2
Alpheidae			
Alpheus sp.	La Jolla (subtidal dredging)	3	5-10
Alpheus sp.	San Felipe, Baja California (under intertidal rocks)	10	5
Betaeus macginitieae	La Jolla (under subtidal sea urchins)	30	10
B. harfordi (Kingsley)	La Jolla (commensal with abalones, *Haliotis* sp.)	10	2
Palaemonidae			
Palaemon ritteri	San Felipe and Puertocitos, Baja California (in schools in intertidal rock pools)	50-100	20

Table 2. Species in which presence of antennular preening is inferred from morphology of third maxillipeds (specimens from Scripps Institution of Oceanography's Benthic Invertebrate Museum except where noted)

Family	Species
Hippolytidae	*Hippolyte* spp.; *Spirontocaris* spp.; *Eualus* spp.; *Caridion gordoni* (Bate) (from United States National Museum)
Crangonidae	*Crangon nigromaculata* Lockington
Glyphocrangonidae	*Glyphocrangon* spp.
Palaemonidae	*Palaemon* spp.; *Palaemonetes* spp.
Pasiphaeidae	*Pasiphaea emarginata* Rathbun
Rhynchocinetidae	*Rhynchocinetes* sp.
Nematocarcinidae	*Nematocarcinus ensifer* Smith
Oplophoridae	*Acanthephyra* sp.
Atyidae	*Caridina nilotica* (Roux) (from United States National Museum)
Stylodactylidae	*Stylodactylus* sp. (second maxilliped? see text)
Procarididae	*Procaris hawaiana* Holthuis (specimens from John Maciolek, University of Hawaii)
Gnathophyllidae	*Gnathophyllum americanum* Guerin (from United States National Museum)
Processidae	*Processa* sp.

varying magnifications through the sides of the aquarium without much disturbance. Focusing was accomplished by moving towards and away from the shrimp.

Rapid movements were analyzed photographically, using a 50 mm single lens reflex camera equipped with an electronic flash unit (1/1500 sec duration). Magnification of subjects was done by the use of extension tubes. By repeated photographs of a particular movement, the exact positioning of limbs used in the behavior could be reconstructed. Illustrations of grooming behavior were drawn by projecting photographic transparencies onto paper with a Bausch and Lomb Trisimplex Micro-Projector. The images were then traced directly.

Morphology of the third maxillipeds and other thoracic legs involved in cleaning were studied with light microscopy, camera lucida drawings and scanning electron microscopy (methods used in SEM are given in Bauer, 1975). Species most closely studied in this fashion included *Heptacarpus pictus* (Hippolytidae), *Palaemon ritteri* Holmes (Palaemonidae), *Betaeus macginitieae* Hart (Alpheidae), *Alpheus* sp. from the Gulf of California, *Crangon nigricauda* Stimpson and *C. nigromaculata* Lockington (Crangonidae), and *Procaris hawaiana* Holthuis (Procarididae). Other species examined for grooming structures are listed in Tables 1 and 2.

Setal groups which appeared, on the basis of morphology, to be possible grooming structures were watched for movements directed against a shrimp's own body. In the two species most intensively observed (*Heptacarpus pictus, Palaemon ritteri*), cleaning behaviors described below and elsewhere (Bauer, in preparation) were seen many times in a large number of individuals (Table 1). Similar movements observed fewer times in other species which used the same limbs and setal groups were assumed to be homologous behaviors. In like manner, species in which only the morphology of homologous limbs was studied were considered to have similar grooming behaviors if the morphology of homologous limbs was close to species observed alive. There was never any case in the course of these studies in which a setal group identified by direct observation as a grooming structure in one species was used for another function in a different species.

In general, quantitative description of cleaning behaviors (i.e., the construction of ethograms) was not done in this study. One exception was ethograms of all grooming behaviors of *Heptacarpus pictus*. Individual behaviors were given verbal signals (e.g. A_1 = an act of antennular grooming). Five gravid females were observed for 10-min intervals in laboratory aquaria and their behavior recorded on tape. Tapes were played back and the amount of time spent in each behavior recorded using a stopwatch. This method is not very precise, as it takes the observer time to note the inception of a behavior and then to verbalize its symbol. Also, the shrimp can engage in more than one grooming behavior at a time. Nonetheless, these simple ethograms can give a general idea of the frequency and duration of cleaning behaviors.

Shrimps were prevented from grooming the antennules by ablating the third maxillipeds near the bases with scissors. Exposure to environmental fouling occurred in cages through which seawater could circulate. Cages were 4 to 5 l plastic buckets with the tops covered by fine plastic screening. Buckets were perforated with many small (2 to 3 mm) openings; rocks and shells were placed in the buckets as substrate for the shrimp (*Heptacarpus pictus*). Standard 25 x 75 mm glass microscope slides and 50 x 70 mm rectangles of asbestos board were attached by rubber bands to the sides of the cages. These settling plates were intended to sample biota settling out in the vicinity of the cages to check for differential settlement between the shrimp's exoskeleton and an inanimate substrate. The cages were hung into the settling tanks of the Scripps Institution of Oceanography's seawater system. Seawater is carried from the end of the SIO pier down a flume and into these tanks before being filtered for use in the SIO facilities.

Control shrimp always had the first walking legs ablated. This pair of limbs was chosen for removal because it was never observed to participate in cleaning behaviors. Experimental and control shrimps were either placed together in the same cages or in separate but similar cages in the same settling tank.

The first experiment was conducted from January 22 to February 19, 1974. Fifty experimental shrimp (third maxillipeds removed) and 50 controls were placed in cages in the settling tanks. In addition, 25 more experimentals and 25 controls were kept on laboratory watertables to be observed for compensatory cleaning of the antennules by other appendages and for signs of fouling. The second experiment was from May 16 to June 5, 1974 with 60 experimentals and 60 controls placed in settling tank cages. The last experiment was from June 5 to June 19, 1974, with 42 experimentals and 35 controls set out in cages.

Upon termination of an experiment, shrimp were preserved in 10% buffered seawater formalin, except in the first experiment, where seawater Bouin's was used. Examination for fouling was done microscopically, both with antennules taken from living individuals and on preserved material. Descriptions of fouling on antennules are from experimentals and controls which had not molted, unless otherwise noted. Determination of whether or not molting had occurred was done by examining the characteristics of the amputated limb bud. Absence of molting during the course of the experiment was shown by a lack of setae on the regenerating limb bud, and very often further confirmed by the presence of scabbing about the base of the *limb due to incidental injury during* ablation which would be removed by molting.

Results

Structure of the Third Maxillipeds

The third maxillipeds are pediform limbs held out in front of the shrimp. Primitively, the endopod of the limb has the 7 segments characteristic of a typical thoracic limb (*Procaris hawaiana*, Fig. 1A). In higher carideans, there has been a coalescence of segments, propodus with dactylus, while the basis, ischium and merus are fused into a single segment

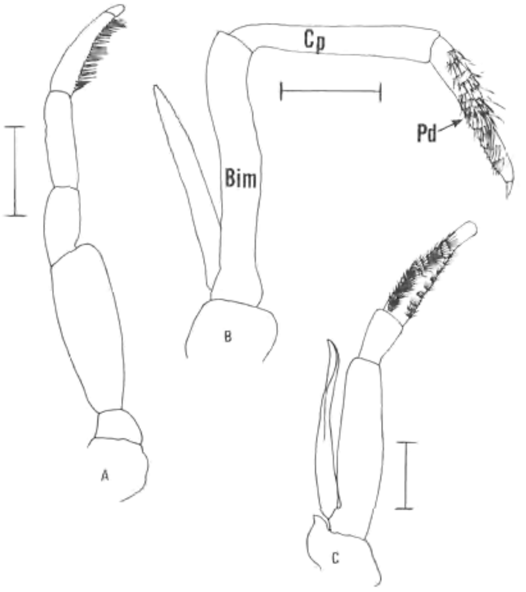

Fig. 1. Location of grooming setae on third maxillipeds of (A) *Procaris hawaiana*, (B) *Palaemon ritteri*, (C) *Alpheus* sp.; medial views of third maxillipeds are shown; only grooming setae are illustrated. Bim: basi-ischial-meral segment; Cp: carpus; Pd: propodus-dactylus. Scale bar = 1.0 mm

(Balss, 1944). An even further reduction in the flexibility of the limb is seen in some caridean families. The hippolytid, pandalid and crangonid species examined morphologically and observed behaviorally possess a much lower degree of mobility *at the carpal-propodal joint than the* alpheid and palaemonid species. Differences in the flexibility of the third maxillipeds are important in terms of the distribution of grooming setae, as will be discussed below.

Medial surfaces of the third maxillipeds show distributions of serrate setae on distal segments. These setae collectively comprise a rasping organ used in scraping the cuticle of appendages. Grooming setae are found on the propodus of *Procaris hawaiana*, the propodus-dactylus of *Alpheus* sp., *Betaeus macginitieae* and *Palaemon ritteri* (Fig. 1). *Heptacarpus pictus* (Fig. 2A) and *Pandalus danae* have rows of these setae on the carpus as well. In *Crangon nigromaculata*, the setae

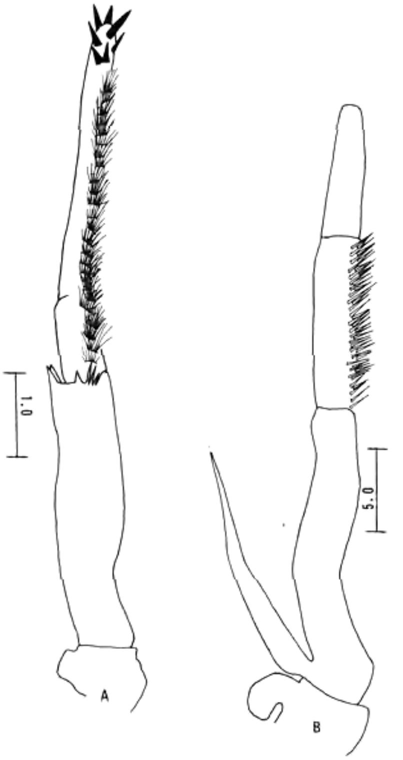

Fig. 2. Grooming setae on third maxillipeds of (A) *Heptacarpus pictus,* (B) *Crangon nigromaculata;* medial views. Scale bars in millimeters

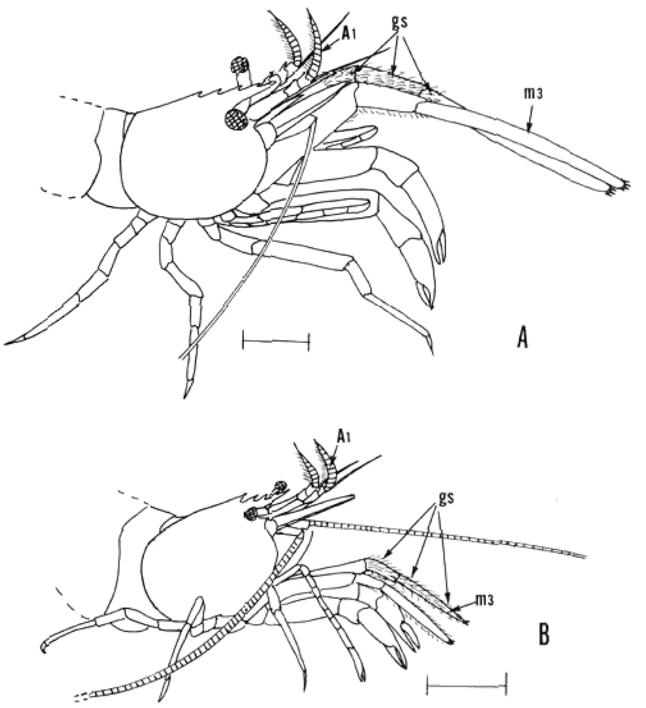

Fig. 3. *Heptacarpus palpator.* Comparison of relative size of third maxillipeds and distribution of grooming setae on these limbs between (A) large male and (B) female. A_1: antennule; gs: grooming setae; m_3: third maxilliped. Scale bars = 5.0 mm

are on the carpus only (Fig. 2B). Those shrimp which have the grooming setae on the propodus-dactylus alone can reach the lowered antennules and the pereopods with the propodus-dactylus because of the flexibility of the carpal-propodal joint. In *Heptacarpus* spp. and *Pandalus* spp., the third maxilliped is quite rodlike. The propodus-dactylus is unable to bend back sufficiently to clean the lowered antennule. Serrate setae must be in the path of the antennule when it is flexed to be cleaned, i.e., on the carpus and the proximal end of the propodus-dactylus. This is illustrated quite clearly in the ontogeny of *H. taylori* and *H. palpator* males. Large males of these species have enormously developed third maxillipeds (functional significance unknown) compared to smaller males and adult females (Fig. 3). In larger males, serrate setae are absent from most of the propodus-dactylus, being found only proximally. Most grooming setae are on the carpus and distal end of the basischial-meral segment. Females and smaller males have a more usual distribution of setae on the carpus and propodus-dactylus. In older males, the propodus-dactylus is too far away from the body to be used in grooming and so lacks grooming setae. The distal end of the pre-carpal segment has moved nearer to the region where the antennules are lowered and so grooming setae have developed there in larger males (Fig. 3A).

Crangon spp. observed in this study had grooming setae on the carpus alone, as noted above. In other shrimps observed, propodal groups of setae clean the walking legs and other pereopods. This behavior may be absent in burrowing *Crangon* spp., thus explaining the absence of propodal grooming setae. Leg cleaning has not been observed in these shrimp, although the time spent in observing *C. nigricauda* was limited (Table 1).

Members of the monotypic family Stylodactylidae may clean the antennules with the second maxillipeds instead of the third. Morphological examination of a *Stylodactylus* sp. shows that the third maxillipeds have a structure similar to that of the first two chelipeds: long and leglike, heavily decorated with plumose setae, perhaps as a feeding adaptation. The second maxillipeds project out from the mouthfield much like the third maxillipeds of other carideans. It is possible that the second maxillipeds of stylodactylids groom the antennules.

Structure of the Grooming Setae

Closely spaced rows of serrate setae which compose the rasping organ of the third maxillipeds are set perpendicular to the longitudinal axis of the segments on which they occur (Fig. 4). Typically, each setal row or group displays a fan-shaped arrangement. As with many other setal groups, the setae decrease in size toward either end of a row. Set in well-developed sockets, these setae have a considerable freedom of movement. Breakage of setae during grooming might be reduced by this characteristic. Setae set inflexibly might snap off when being rubbed against limb surfaces. It is also possible that the basal flexibility of setae allows them to conform to the shape of the surfaces being groomed. Setae are always directed distally on the segment. Elevation of the setae above the limb surface at some angle less than 90° exposes the tooth-like setules on the setae.

Grooming setae are naked proximally and bear double rows of blade or tooth-setules (Fig. 4C,E; Fig. 5). Tooth-setules are usually curved toward the tip of the seta, with this tendency increasing distally. Rows of setules are set apart from one another at some angle between 45° and 135°. Setules in one row are curved toward those of the opposite row. Setular rows are inserted upon the side of the seta which faces both distally and toward the limb segment. Thus, the orientation of a grooming seta and the placement of its tooth-setules expose the serrations to some surface which will be groomed. Tooth-setules vary in size and shape and may or may not be serrate themselves (Fig. 5 A-F).

Variations in grooming setae include the presence or absence of digitate scale-setules on the side of the setal shaft opposite the rows of tooth-setules. The shaft can either be naked (*Palaemon ritteri, Heptacarpus pictus, Crangon nigricauda*) or covered with scale setules (*Alpheus* sp., *Betaeus macginitieae, Pandalus danae*) (Fig. 5A, B, F). These setules are thin, multidigitate distally, and usually elevated above the setal shaft. Presumably, the presence of these scales increases the rasping surfaces of the seta. *Procaris hawaiana* setae show these scales in a very poorly formed condition (Fig. 5G). It seems evident that the numerous grooming setae, with their scale- and tooth-setules, present an array of serrations which form an effective rasping and scraping structure.

Antennular Preening

Perhaps the most important grooming function of the third maxillipeds is cleaning the antennules. These appendages bear the thin-walled olfactory setae, the aesthetascs. Antennules of caridean shrimp which were examined are of two basic morphologies. In *Pandalus* spp., *Palaemon* spp., *Betaeus* spp., *Alpheus* spp. and *Crangon* spp., the aesthetascs are somewhat sparsely distributed on the outer flagellum, when compared to hippolytids such as *Heptacarpus pictus* (Fig. 6A). In this latter shrimp, the outer flagellum is short and stout, with many closely spaced olfactory hairs giving it a brush-like appearance. Those shrimp with elongate outer flagella wave the antennules back and forth arrhythmically (flicking; Maynard and Dingle, 1963). Antennules of *Heptacarpus* spp. are held upright and rotated through 180° on the peduncle rapidly and synchronously. Fluttering of the outer flagella occurs in bursts, followed by periods of rest. Both behaviors facilitate movement of water about the aesthetascs. In the case of *Palaemon* spp. or *Betaeus* spp. for example, the simple waving of the outer flagellum is presumably sufficient to circulate water through the relatively low density of aesthetascs. In *H. pictus* the rapid rotation of outer flagella sets up observed semicircular currents passing through the aesthetascs from in front of and behind the antennule which exit laterally (Fig. 6B). Anomuran and brachyuran crabs with similar brush-like antennules flick them rapidly, aesthetasc brush forward, out in front of the crabs. Snow (1973), analysing these movements in *Pagurus alaskensis* (Benedict), concluded that this behavior facilitated movement of water about the aesthetascs. Thus, olfactory hairs are incessantly exposed to fouling by particulate matter and settling stages of sessile organisms carried along by water circulating about them.

Antennular grooming is a very frequent and noticeable behavior of some

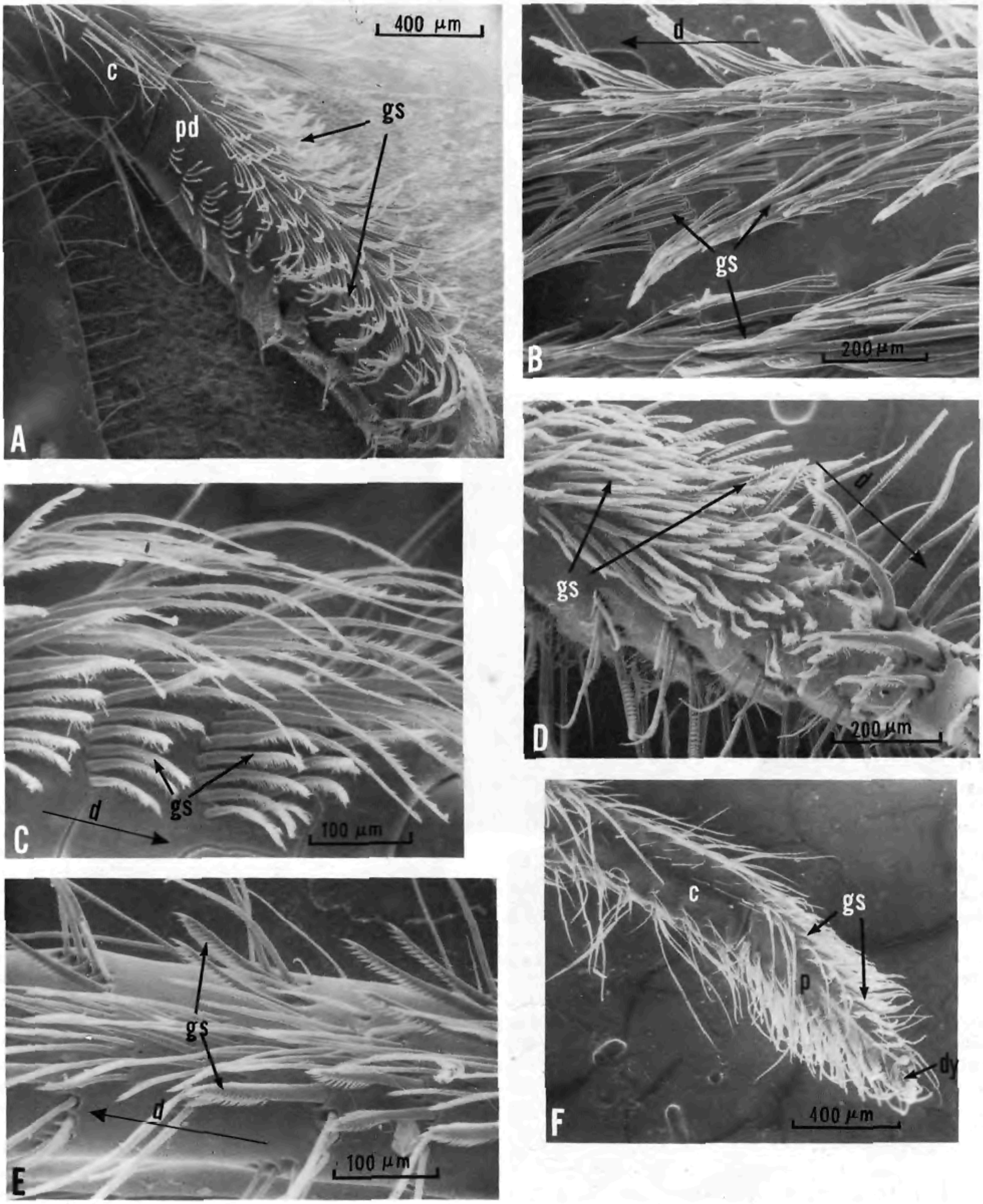

Fig. 4. Arrangement of serrate grooming setae on medial surfaces of caridean third maxillipeds. (A) Third maxilliped of Betaeus macginitieae; (B) portion of propodus-dactylus in Alpheus sp.; (C) portion of propodus-dactylus in *Heptacarpus pictus*; (D) propodus-dactylus in *Palaemon ritteri*; (E) portion of carpus in *Crangon nigricauda*; (F) terminal segments of third maxilliped in *Procaris hawaiana,* inferior view, medial surface toward top of photo. c: carpus; d: distal; dy: dactyl; gs: grooming setae; p: propodus; pd: propodus-dactylus

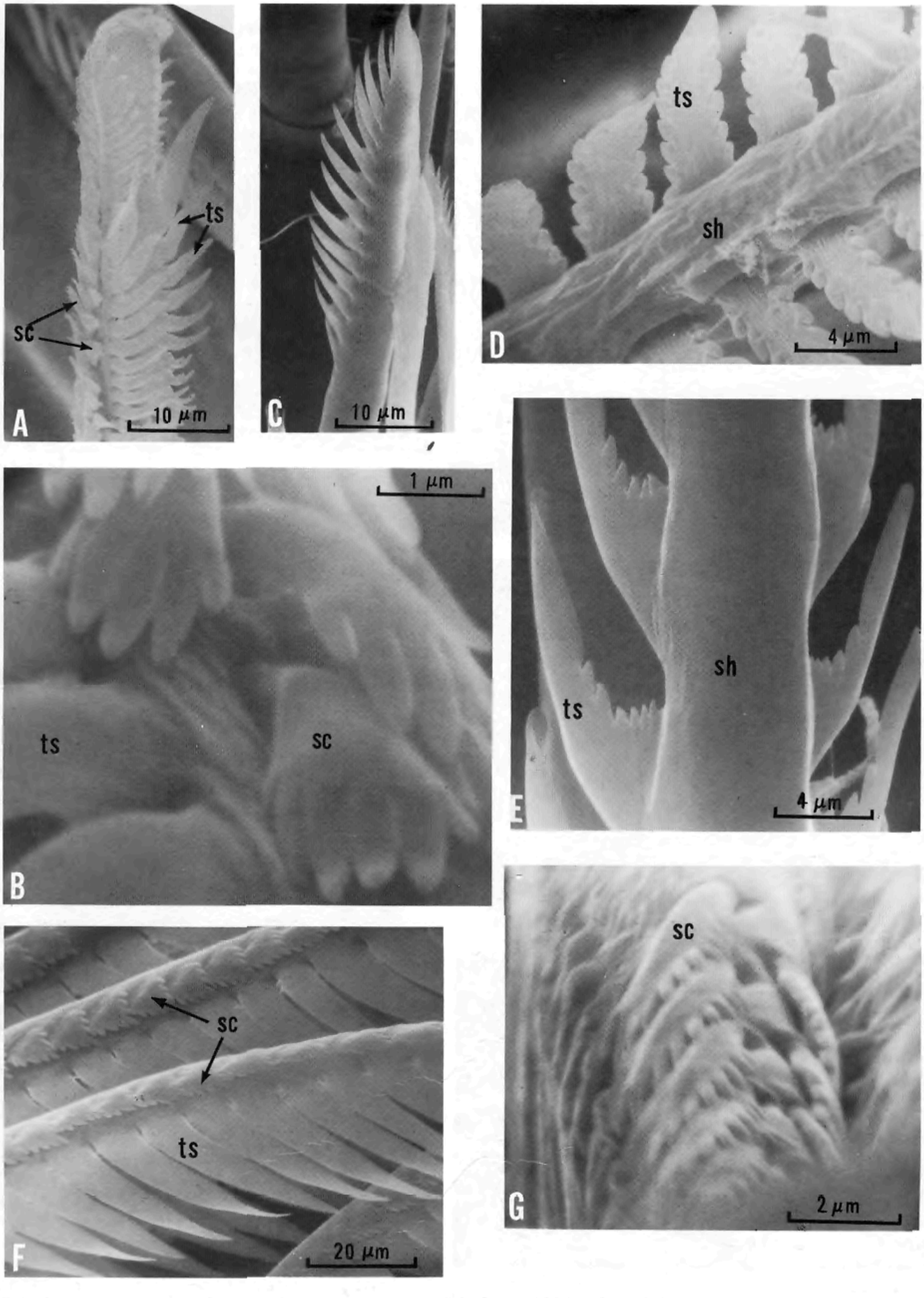

Fig. 5. Fine structure of grooming setae from third maxillipeds. (A) Terminal portion of grooming seta in *Betaeus macginitieae*; (B) close-up of scale-setules shown in (A); (C) grooming seta in *Crangon nigricauda*; (D) portion of grooming seta in *Palaemon ritteri*; (E) portion of grooming seta in *Heptacarpus pictus*; (F) grooming setae in *Alpheus* sp.; (G) portion of grooming seta in *Procaris hawaiana*, showing incompletely formed scale-setules. sc: scale-setule; sh: setal shaft; ts: tooth-setule

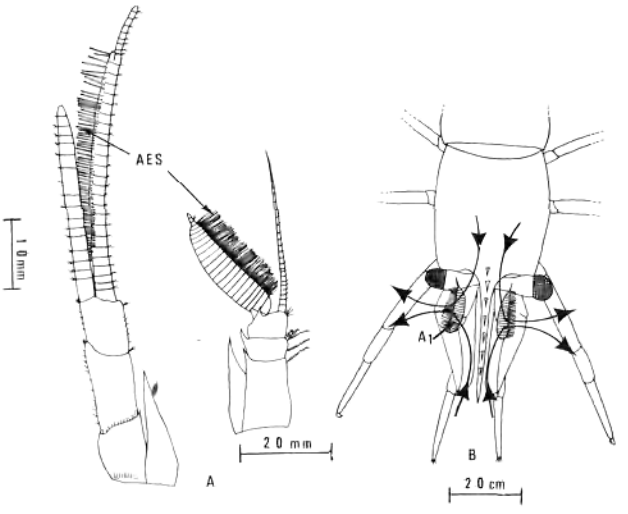

Fig. 6. Comparison of density of aesthetascs on antennules and mode of water circulation through aesthetascs. (A) Antennules of *Betaeus macginitieae* (left) and *Heptacarpus pictus* (right), illustrating greater density of aesthetascs on antennule of latter; (B) schematic representation of water currents (arrows) circulating through aesthetascs of *H. pictus* due to rapid semicircular rotation of the antennule. A_1: antennule; AES: aesthetascs

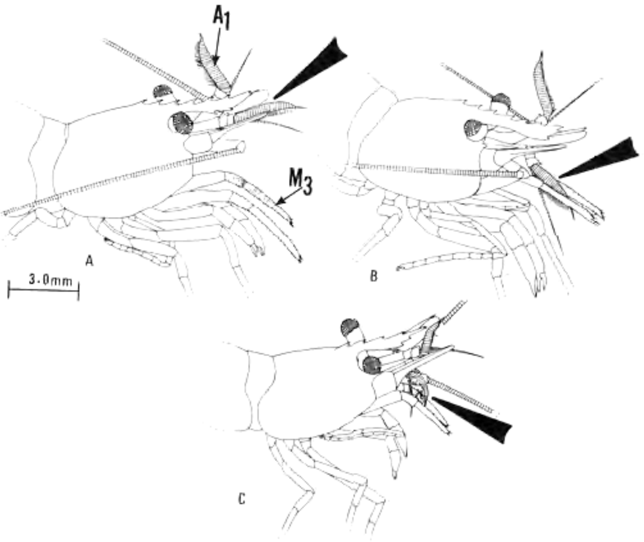

Fig. 7. *Heptacarpus pictus*. Antennular grooming. (A) lowering of antennule (arrowhead); (B) clamping of third maxillipeds about antennule (arrowhead); (C) antennule being raised and drawn through setal combs of third maxillipeds (arrowhead). A_1: antennule; M_3: third maxilliped

shrimp, and has been described in detail for *Pandalus danae* (Bauer, 1975). Motor patterns of this behavior are quite similar in all species of carideans observed in this study. The outer flagellum of an antennule is rotated on the peduncle so that the aesthetasc rows are facing anteriorly. Lateral movement of the antennal scale occurs so that the antennular flagella can be flexed ventrally. Simultaneously, the third maxillipeds are elevated and spread apart. They clamp together about the ventrally flexing antennule with their setal combs. Inner and outer flagella move back up dorsally through the grooming setae as the third maxillipeds are depressed (Fig. 7). Thus, the aesthetascs are scraped and combed through by the rasping setae. When being groomed, the aesthetascs are in the midsagittal plane. If they were held out laterally, these delicate setae might be crushed and torn by the grooming setae.

Antennules are usually cleaned alternately. Although the motor patterns of the behavior are characteristic, the number of cleaning acts in a bout is variable, from one to several times. Each act of antennular cleaning is a rapid movement, lasting less than 1 sec. Frequency of cleaning bouts is variable between species, which might be related to overall levels of activity. Shrimps which are most active appear to engage most frequently in grooming behaviors, a qualitative generalization which Jander (1966) found true for insect grooming behavior.

Carideans which have been examined morphologically but not observed behaviorally for antennular cleaning (Table 2) show similar distributions of grooming setae on the third maxillipeds. In all cases, the morphology of these setae is similar to that described and illustrated above. It is probable that the antennules are groomed in a similar fashion in these species as in those observed in this study. In one case (*Procaris* sp.), this prediction has been confirmed by another worker (A. Provenzano, personal communication).

Pereopod Cleaning

Terminal segments of the third maxillipeds scrape and brush the pereopods (chelipeds and walking legs). Walking legs swing forward, while the maxillipeds swing out and back to clamp around them. The maxillipeds slide forward as the leg pulls back to its normal posi-

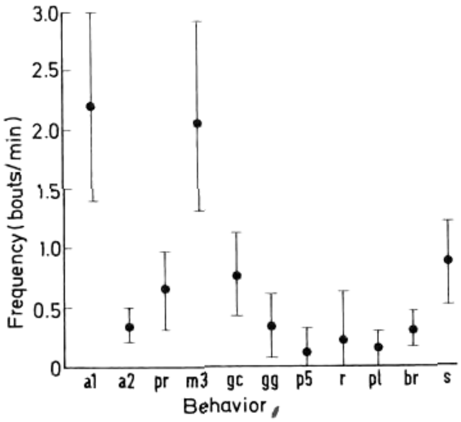

Fig. 8. *Heptacarpus pictus.* Frequency of various behaviors in gravid females. Mean, 95% confidence limits for 10-min observational periods of 5 different females. Bout = period of uninterrupted behavior, consisting of one or more acts of same behavior. a1: Antennular grooming; a2: antennal grooming; pr: cleaning of pereopods by third maxillipeds; m3: all grooming behaviors of third maxillipeds; gc: brushing of gills by second chelipeds; gg: general body grooming by second chelipeds; p5: grooming by last walking legs; r: resting, no activity; pl: fanning of the pleopods; br: brushing of brooded embryos by second chelipeds; s: food-searching behavior

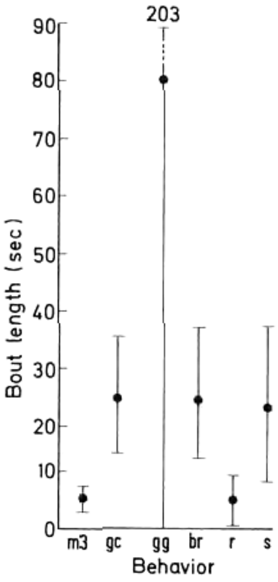

Fig. 9. *Heptacarpus pictus.* Duration of bouts of various behaviors of gravid females. Mean, 95% confidence limits for 10-min observational periods of 5 different females. Same behavioral symbols as Fig. 8

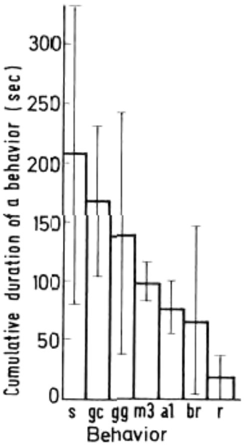

Fig. 10. *Heptacarpus pictus.* Summed duration of various behaviors in gravid females. Same observational data and notation as in Figs. 8 and 9

tion, scraping its surfaces between the maxilliped setae (illustrated for *Pandalus danae* in Bauer, 1975). Chelipeds are groomed in a similar fashion. During food-searching behavior, chelipeds probe and explore the substratum, while the tips of the walking legs are always in contact with it. Thus, distal parts of the pereopods are regularly exposed to fouling from sediment and debris covering the substratum, which must be removed by grooming.

Autogrooming

Autogrooming refers to the rubbing together of the third maxillipeds after a grooming behavior. It is a reciprocal cleaning of the third maxillipeds, and has been illustrated and described in detail for *Pandalus danae* (Bauer, 1975). I have observed this behavior in all *Heptacarpus* spp., *Palaemon ritteri, Alpheus* spp., *Betaeus* spp. and *Pandalus* spp. examined. It is likely that autogrooming of the third maxillipeds is as widespread as antennular grooming in caridean shrimp.

Behavioral Aspects of Grooming

Laboratory observations indicate that cleaning activities of the third maxillipeds differ in the frequency of occurrence and length of a bout of behavior from those of other cleaning limbs, e.g. general body preening by the cleaning chelipeds and last walking legs. Movements involved in cleaning the antennules are more frequent than any other cleaning activity (Fig. 8). Individual acts of preening by the third maxillipeds are rapid sweeping movements of less than 1 sec in duration. Bouts (more than one act) of cleaning are rarely longer than 1 min in *Pandalus danae*. In *Heptacarpus pictus*, a bout of antennular cleaning rarely consists of more than three acts. By contrast, brushing of the gills and brooded embryos by the second chelipeds in *H. pictus*, and general body cleaning by the chelipeds and last walking legs are prolonged (Fig. 9). Qualitative observations on *Pandalus danae*, *Palaemon ritteri* and the other shrimps observed in this study indicate that the frequency and duration of a particular cleaning behavior relative to other cleaning behaviors are similar to that of *H. pictus*. The overall frequency of grooming, however, differs considerably between species. Comparison with other behaviors shows that *H. pictus* spends much time in all types of grooming behavior (Fig. 10).

The form and occurrence of grooming behaviors noted in field observations on *Heptacarpus pictus* did not qualitatively appear different from those seen in captive individuals. Grooming is a relatively stereotyped behavior, and, as Lorenz (1958) has pointed out, such fixed motor patterns are little changed by captivity. However, the frequency and patterning of cleaning behaviors with other activities might be changed by laboratory confinement, but this was not a topic of this study.

Experiments on Adaptive Value of Antennular Grooming in Heptacarpus pictus

Experimental work on antennular preening consisted of ablation experiments designed to test the hypothesis that the selective pressure promoting this behavior is fouling. In the first experiment, fouling was first observed on the antennules of experimentals kept on laboratory watertables. A darkening of the antennules and an observed loss of the chemosensory hairs (aesthetascs) from the antennules indicated fouling. The experiment was terminated when extensive aesthetasc damage became apparent.

Antennules of non-molted experimental shrimps were heavily fouled by *Leucothrix* sp., a long-chained filamentous bacterium ubiquitous in marine environments (Johnson *et al.*, 1971; Sieburth, 1975). Strands of *Leucothrix* sp. were found on all regions of the antennular flagella, being especially concentrated in joints between flagellar segments (Fig. 11B, D). It was also quite abundant on the aesthetascs proper. *Leucothrix* sp. fouling is best observed in living material, as it tends to break off in preserved material. Olfactory setae of *Heptacarpus pictus* were often covered with a microbial coat composed of coccoid and bacilliform bacteria and associated debris (Fig. 12C). Diatoms were also common on the aesthetascs and other parts of the antennule (Fig. 12F). Particulate matter was aggregated among the bases of the aesthetascs, and groups or clusters of unidentified tear-shaped cells were quite abundant on the antennular flagellum, especially between the rows of aesthetascs (Fig. 11C; 12A, D, E). Hypotrichous ciliates could be observed crawling among heavily fouled and damaged aesthetascs. Non-molt control antennules were almost completely free of fouling (Fig. 11A, 12B). An occasional strand of *Leucothrix* sp., very rarely a diatom, and an occasional cluster of the unidentified cells were found on control antennules.

The most striking consequence of a lack of grooming was the complete loss of aesthetascs from many or most non-molt experimentals (Fig. 11A, C; Table 3). After 2 weeks, antennules of some or all non-molt experimentals were completely devoid of olfactory hairs; only blackened bases of the aesthetascs remained. Controls rarely showed aesthetasc breakage of any kind, and never were antennular segments rendered devoid in the normal length or number of olfactory hairs (Table 3). Observation of experimental shrimp kept on laboratory tables during the first experiment showed that this breakage first occurred in the distal segments of antennules, spreading sequentially to the proximal segments. Aesthetascs first broke at their tips, with breakage then proceeding toward their bases. During the June 5-19 experiment, all experimental and control shrimp were removed from the cages on three occasions and checked for aesthetasc damage. Injury was estimated under the dissecting microscope as the percentage of segments bearing aesthetascs with significant damage. (The term "significant damage" is defined in Table 3). Fig. 13 shows this loss in experimental shrimp over the course of this experiment. Controls in this experiment suffered little or no significant damage to the aesthetascs.

Examination of Table 3 reveals that experimental shrimp which had not molted always suffered the greatest injury to olfactory setae, both in terms of broken aesthetascs (Table 3C) and aesthetascs in which breakage had become significant (Table 3D). (In Columns C or D of Table 3, the confidence limits of means within

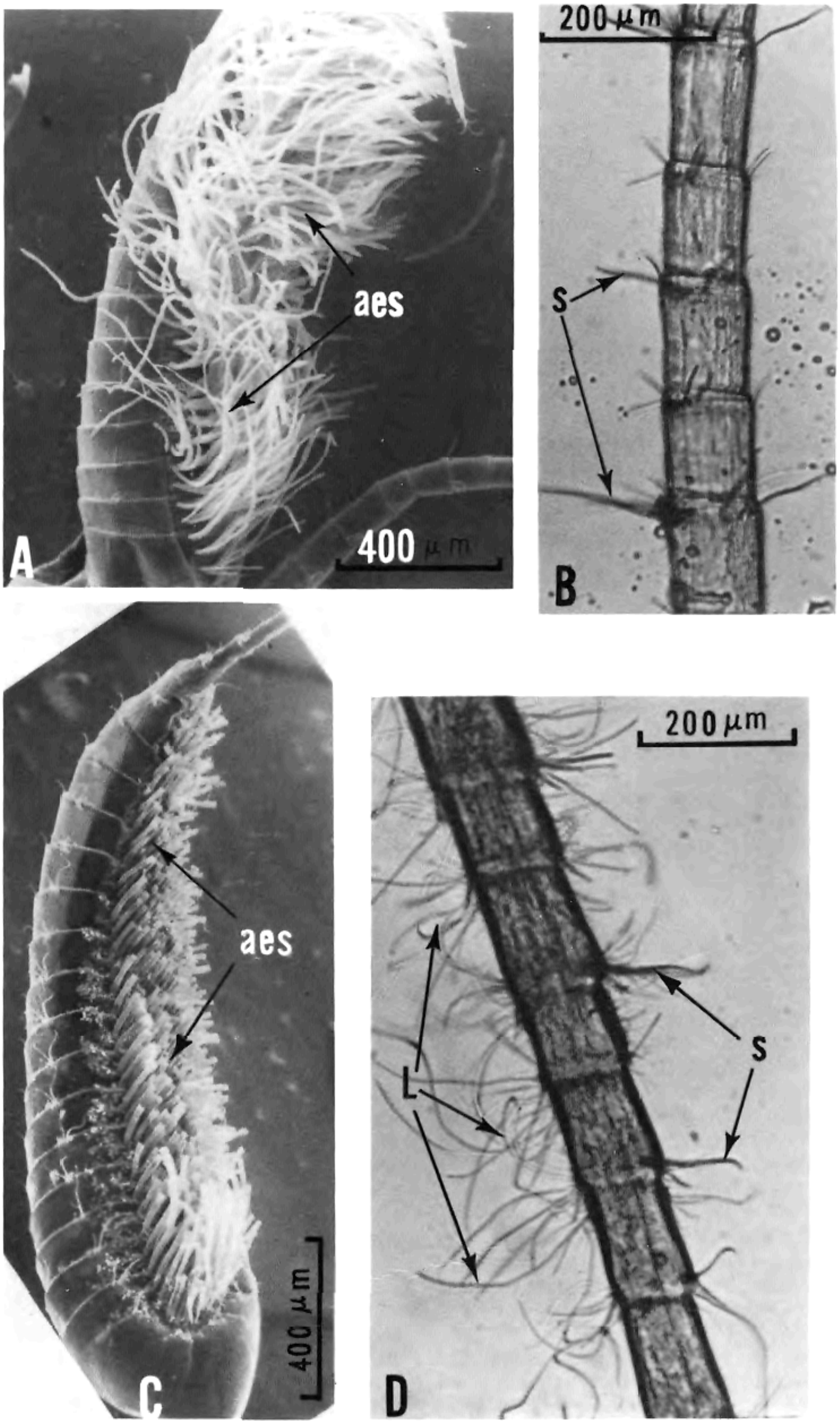

Fig. 11. *Heptacarpus pictus*. Comparison of fouled and unfouled antennules from third maxilliped ablation experiments. (A) Outer flagellum of antennule from control shrimp with unbroken aesthetascs (olfactory hairs); (B) inner flagellum of antennule from control shrimp; (C) outer flagellum from experimental shrimp — note broken aesthetascs; (D) inner flagellum from experimental shrimp fouled with the bacterium *Leucothrix* sp. (L); aes: aesthetascs; s: seta

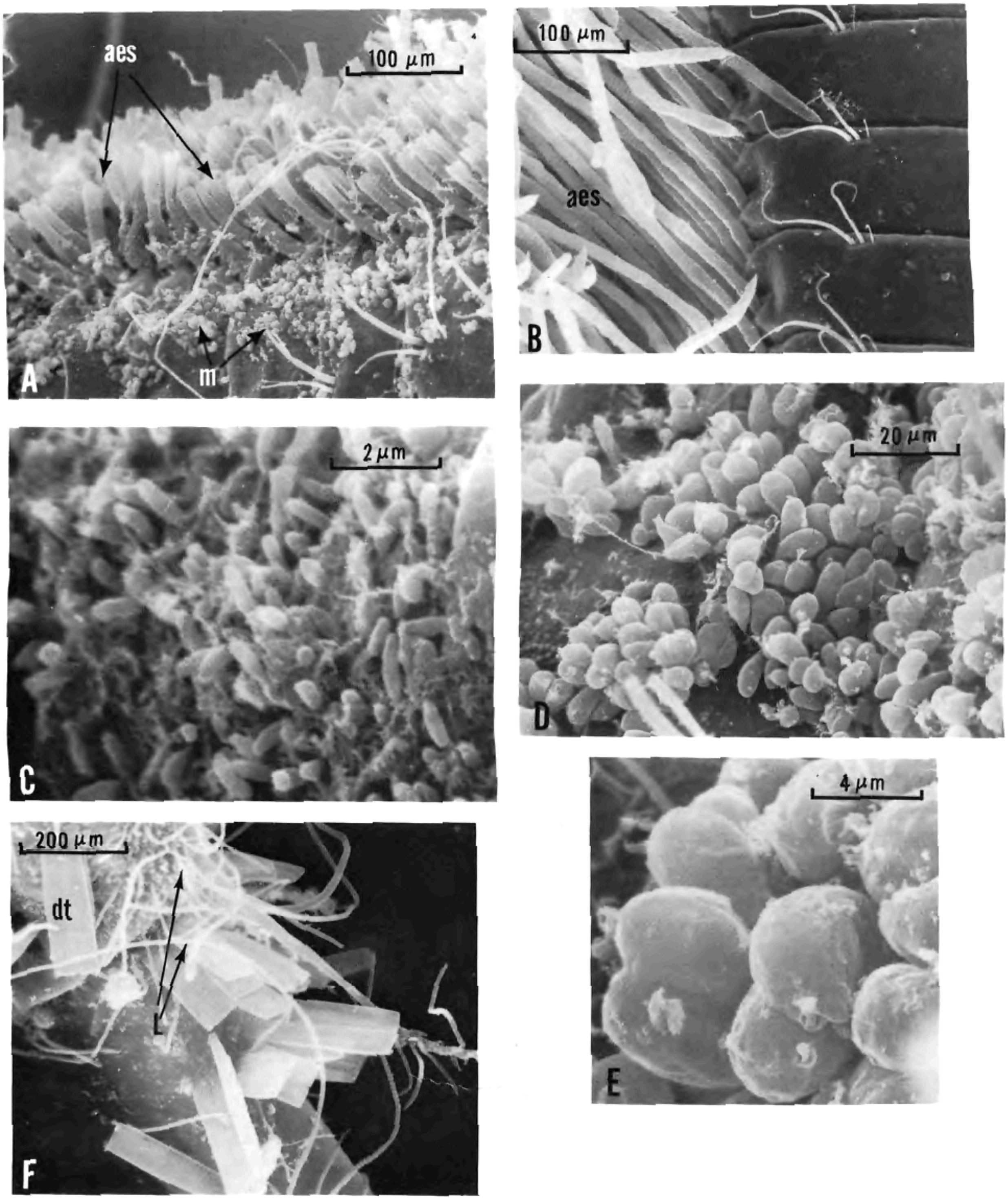

Fig. 12. *Heptacarpus pictus*. Fouling of antennules of experimental shrimp. (A) Portion of outer flagellum of experimental shrimp with damaged aesthetascs and fouling by clusters of microbial cells among and just below bases of the aesthetascs; (B) similar view from a control antennule – note lack of microbial cells and aesthetasc damage; (C) microbial fouling of experimental aesthetasc; (D) (E) views of microbial cell clusters illustrated in (A); (F) diatoms (dt) and *Leucothrix* sp. (L) strands fouling outer flagellum. aes: aesthetasc; m: microbial cells

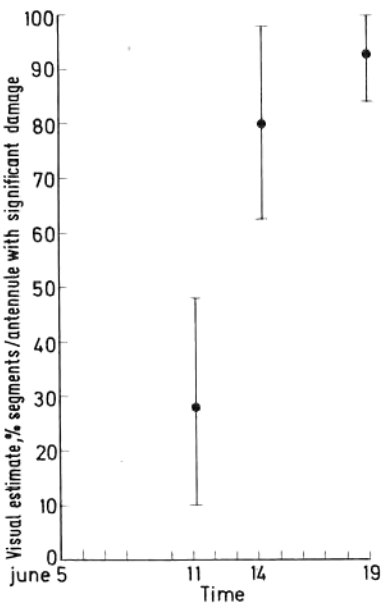

Fig. 13. *Heptacarpus pictus*. Time-course of damage to aesthetascs of experimental, non-molt shrimp in June 5-19, 1974, maxilliped-3 ablation experiment; means, 95% confidence limits are shown; June 11, N = antennules of 16 shrimp; June 14, N = 13; June 19, N = 14

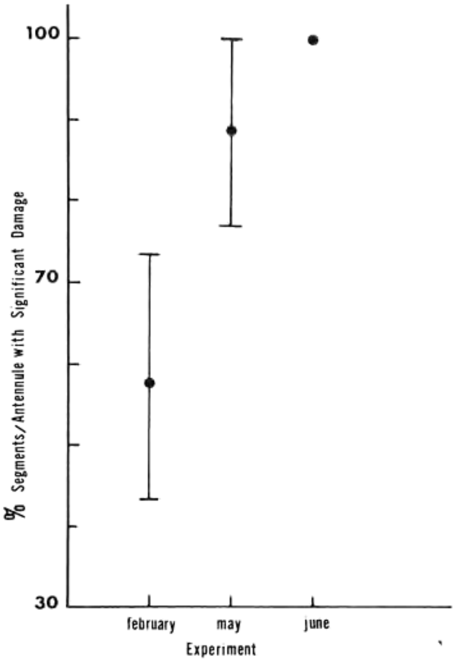

Fig. 14. *Heptacarpus pictus*. "Significant" aesthetasc damage (see Table 3) in third maxilliped ablation experiments (non-molt experimental shrimps); February (first experiment), N = 30 antennules, duration of experiment, 29 days; May (second experiment), N = 13, D = 21; June (third experiment), N = 21, D = 14

Table 3. *Heptacarpus pictus*. Aesthetasc damage in antennules resulting from ablation of third maxillipeds. Roman numerals refer to particular experiments: I, January 22-February 19 experiment; II, May 16-June 5 experiment; III, June 5-June 19 experiment; "broken aesthetascs" refers to one or more segments of an antennule showing some degree of breakage in at least half of the aesthetascs arising from that segment; "significant damage" refers to antennular segments in which more than one-half of the aesthetascs were missing for more than one-half their normal length

Treatment	% antennules with broken aesthetascs (A)			% antennules with significant damage (B)			% segments per antennule with any aesthetasc damage ($\bar{x}$, 95% confidence limits) (C)			% segment per antennule with significant damage ($\bar{x}$, 95% confidence limits) (D)			Number of antennules examined (E)		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Experimental, no molt	87	100	100	80	100	100	$\overline{82}$, 69-96	$\overline{100}$	$\overline{100}$	$\overline{58}$, 43-79	$\overline{90}$, 77-100	$\overline{100}$	30	13	21
Control, no molt	10	0	50	10	0	25	$\overline{4}$, 0-11	0	$\overline{6}$, 2-10	$\overline{2}$, 0-4	0	$\overline{3}$, 0-6	20	10	16
Experimental, molted	50	30	35	14	27	0	$\overline{45}$, 18-72	$\overline{29}$, 15-43	$\overline{20}$, 3-36	10, 0-27	18, 7-29	0	14	44	23
Control, molted	0	0	0	0	0	0	$\overline{1}$, 0-4	0	0	0	0	0	17	36	24

a column can be compared; where confidence limits do not overlap, the means are without further test significantly different at the 0.05 level of confidence.) Experimental shrimp which had molted during the experiments showed the next highest degree of damage in both categories. Comparison of experimental molted and experimental non-molted shrimp in the first experiment by analysis of variance (necessary because confidence limits overlap) shows that the means are significantly different at the 0.05 level. Experimental molted means are higher than those of controls which had not molted, but the difference is not significant except in the second experiment, where these controls showed no damage whatsoever. Experiments in which control molted and control non-molted showed statistically significant differences in aesthetasc damage were the first experiment, where the molted controls showed no damage; and the last experiment, where control non-molted showed slight aesthetasc damage in both categories.

Aesthetasc damage was significantly greater in the second and third experiments than in the first, even though the latter was longest in duration (Fig. 14). Qualitatively, *Leucothrix* sp., diatom and other microbial fouling was greatest in the last experiment (June 5-19), with even some of the non-molt control shrimp showing occasional, but not heavy, fouling.

Settling plates attached to cages containing experimental and control shrimp were examined for fouling. In all experiments, a similar type of fouling as that found on ungroomed antennules was present, i.e., ciliates, *Leucothrix* sp., debris. There was no qualitative difference between settlement on plates and the antennules of experimental shrimp which had not molted.

Compensatory cleaning of antennules by limbs other than the third maxillipeds appeared in experimental shrimp. Antennules could be observed being lowered down as in antennular grooming. Occasionally, the shrimp would reach up with the first cheliped and quickly swipe at the lowered antennule. The movements were brief and appeared to be ineffective in the sense that the antennule was not noticeably wiped with a setal group. Fouling of experimental antennules indicated the ineffectiveness of this compensatory grooming, which was also observed in ablation experiments with *Pandalus danae* (Bauer, 1975).

Discussion

These experiments clearly show that shrimp which do not preen the antennules will develop fouling on them, whereas shrimp which groom them will not. Setal scraping of the olfactory hairs prevents the settlement of or removes microbial growth from these chemosensory setae and the rest of the antennular surfaces as well. Molting removes growth from the antennules, as is indicated by the fact that experimentals which had molted showed a significantly lower degree of fouling than those which had not. Not only does fouling take place, but, in *Heptacarpus pictus*, actual destruction of the olfactory hairs occurs. Apparently, the aesthetascs can be regenerated after being destroyed, as experimental shrimp which had recently molted had full complements of aesthetascs.

Increased microbial fouling with subsequently increased aesthetasc damage seen in the later experiments (Fig. 14) may have been due to increased water temperatures, with subsequently higher microbial activity and fouling. The mean surface water temperature off the Scripps Institution of Oceanography pier was 13.1°C for the duration of the January 22-February 19 experiment but increased to 17.4° and 18.0°C, respectively during the course of the later two experiments.

The mechanism of breakage of aesthetascs in *Heptacarpus pictus* might be speculated on from making the observations that such breakage occurred in *H. pictus* but not in fouled antennules of *Pandalus danae* (Bauer, 1975), and that the two shrimp circulate water about the aesthetascs in different ways. *H. pictus* rotates its antennules (and aesthetascs) in rapid semicircular bursts, while *P. danae* slowly waves or flicks its antennules. As the aesthetascs are drawn through the water, they displace a volume of the medium and so encounter a force or resistance to movement. Epizoites, such as diatoms or the long-stranded *Leucothrix* sp., must also be moved along through a dense medium (water), and their movement is resisted as well. In *H. pictus* the aesthetascs are pushed through the water much faster than in *P. danae* and might experience a greater drag. Additional drag produced by the epizoites might have created a strain or stress greater than the mechanical strength of the aesthetasc, resulting in damage. It is also possible that some chitinivorous microorganism fouling the antennules of *H. pictus* but not *P. danae* is responsible for eroding away the aesthetascs of the former shrimp. However, informal ablation experiments with *Palaemon ritteri* in La Jolla resulted in the same sort of diatom and *Leucothrix* sp. fouling on the antennules as with *H. pictus*. *Palaemon ritteri* has antennules similar in morphology and movement to *Pandalus danae,* and the aesthetascs of *Palaemon ritteri* showed no breakage after a 2-week exposure to fouling. It seems likely that the breakage of fouled *H. pictus* olfactory hairs is related to the rapid rotation of the antennular flagellum upon which they are borne.

Although the term "maxilliped" ("jawfoot") implies primarily a feeding function, my observations indicated that the endopod of the third maxilliped is an important grooming appendage in caridean shrimp. Antennules, chelipeds and walking legs are scraped free of fouling material by the third maxillipeds. The third maxillipeds are often involved in grooming the flagellae of the second antennae in conjunction with a cleaning behavior of the first cheliped, but this interaction will be included in a subsequent report on the grooming activities of the chelipeds. Grooming activities of the third maxillipeds are quite conservative within the Caridea and other Decapoda. All caridean species I have examined groom the antennules in a similar fashion.

The third maxillipeds groom appendages which have concentrations of sensory receptors. Antennules bear the aesthetascs, which are known, on the basis of behavioral, structural and electrophysiological evidence, to be the major sites of olfaction in decapod crustaceans (references cited in Snow, 1973). Behavioral evidence indicates that chemoreceptors are located on the dactyls and chelae of the pereopods (Barber, 1961). The most likely environmental pressure which seems responsible for the grooming of these appendages is fouling, which could prevent reception of stimuli. In this study, *Heptacarpus pictus* aesthetascs were completely destroyed by fouling. If these setae are the major site of olfaction, such a loss in nature would be quite detrimental to these tidepool scavengers. In the case of *Pandalus danae*, where actual breakage of the aesthetascs did not occur, olfaction could be impaired by reduction of aesthetasc surface or perhaps by interrupted circulation of water through the aesthetasc rows. Tactile and other receptors dependent on deflection for reception of a mechanical stimulus could be inactivated by clogging due to fouling by sediment or an epizoite. It is suggested here that one of the most important selective pressures for the preening of cephalothoracic appendages by the third maxillipeds is maintenance of sensory sites.

Differences in the behavioral organization of the cleaning appendages might be related to their functions. High frequency of cleaning the antennules might be due to the immediate necessity of keeping sensory sites free of fouling. On the other hand, general grooming limbs, particularly the preening chelipeds, groom large, diversified areas of the body. Cleaning chelipeds of shrimp nip and brush at regions of the body which vary considerably in topography. The probable function of general body cleaning is prevention of epizoic and debris fouling which could interfere with swimming or mobility of limb joints and body segments (Bauer, 1975). Bouts of cleaning are not so frequent as those of sensory cleaning, as short-term fouling might be better tolerated on general body surfaces (e.g. the landing of a larval epizoite might be tolerated, but not its attachment and growth). Since areas being cleaned by the general grooming limbs are large and movements necessary to clean them diversified, the bouts of behavior have a greater duration than those of the third maxillipeds.

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Date of final manuscript acceptance: September 24, 1976. Communicated by J.S. Pearse, Santa Cruz