Antifouling adaptations of marine shrimp (Decapoda: Caridea): gill cleaning mechanisms and grooming of brooded embryos

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Gills in the branchial chambers of caridean shrimps, as well as the brooded embryos in females, are subject to fouling by particulate debris and epizoites. Important mechanisms for cleaning the gills are brushing of the gills by the grooming or cleaning chelipeds in some species, while in others, setae from the bases of the thoracic legs brush up among the gills during movement of the limbs (epipod-setobranch complexes). Setae of cleaning chelipeds and of epipod-setobranch complexes show similar ultrastructural adaptions for scraping gill surfaces. Ablation of the cleaning chelipeds of the shrimp *Heptacarpus pictus* results in severe fouling of the gills in experimentals, while those of controls remain clean. Embryos brooded by female carideans are often brushed and jostled by the grooming chelipeds. In *H. pictus*, removal of the cleaning chelae results in heavier microbial and sediment fouling than in controls.

KEY WORDS: – shrimp – gills – grooming – cleaning – epipods – setobranchs – fouling – Decapoda – Caridea.

CONTENTS

INTRODUCTION

Grooming behaviour is a frequent activity of caridean shrimp which appears to prevent epizoic and sediment fouling of the body (Bauer, 1975; Bauer, 1977, Bauer, 1978). This report deals with two specialized components of grooming behaviour, gill cleaning and female care of brooded embryos. The functional

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morphology of these behaviours and the ultrastructure of setae and processes utilized in these behaviours is·described. Experiments were performed which suggest the adaptive value of these behaviours.

Gills of caridean shrimp, like those of other decapod crustaceans, are enclosed within a branchial chamber. Gills are thus protected from injury while a rapid flow of water can be drawn across them in the narrow chamber by the pumping action of the scaphognathite (gill bailer). However, the enclosure of the gills creates a disadvantage in that gill lamellae in a confined space form a sediment trap. Particulate matter borne by the respiratory current can collect among gill filaments. Since the gills are thin outgrowths of the exoskeleton, there can be no ciliary-mucoid mechanisms to entrap and carry away this sediment, as in corals for example. In addition, fouling organisms might grow on and smother respiratory surfaces. Perhaps in response to these environmental pressures, it appears that carideans and other decapods have evolved a number of mechanisms to retard or prevent fouling. Although Vuillemin (1967) has discussed methods of gill cleaning in the Decapoda, those of caridean shrimp have not been studied in any detail. In this study, two major gill cleaning mechanisms of the Caridea were investigated: active brushing of the gills by the cleaning chelipeds and passive brushing by the epipod-setobranch system.

Female caridean shrimp, like other decapod crustaceans (except the Penaeidea), carry developing embryos attached beneath the abdomen which, in marine forms, hatch as advanced planktonic larvae. One possible function of incubation is to protect the embryos from predation and chance environmental injury to which the eggs and larvae of copiously spawning invertebrates such as coelenterates and echinoderms are subjected. Most decapods exhibit some sort of parental care in aeration and cleaning of embryos which can be termed brooding behaviour. Like the gills, the mass of embryos serves as a filter which accumulates debris from water circulating through them. In several species of shrimp, females pick and brush among the mass of embryos with the cleaning chelipeds. In this study, the survival value of this component of brooding behaviour was investigated in a caridean species, *Heptacarpus pictus* (Stimpson).

Observations on brushing of the gills by cleaning chelipeds in Caridea have been reported for various palaemonid species (Doflein, 1910; Schone, 1961), and for the gnathophyllid *Hymenocera picta* Dana (Wickler & Seibt, 1970). A study on brooding behaviour in a caridean has been conducted by Phillips (1971) (*Palaemon serratus* (Pennant)).

METHODS

Nineteen species of caridean shrimp were observed alive in this study, chiefly in laboratory seawater aquaria (Table 1). If the shrimps were small, it was convenient to observe cleaning behaviour through a hand-held dissecting microscope. Grooming activities were recorded by light photography. Transparencies made with a 35 mm camera using an electronic flash of 1/1500 sec duration could record the exact position of limbs during grooming. Drawings of the various behaviours were made by projecting the transparencies on to paper, where they were traced. Information on the location of collection and the time spent observing each species can be found in a previous report on antennular preening in Caridea (Bauer, 1977).

Table 1. Gill cleaning mechanisms of caridean species observed alive (+, present; −, absent)

Species	Brushing by cleaning chelipeds	Epipod-setobranch systems
Alpheidae		
Betaeus macginitieae Hart	−	+
B. harfordi (Kingsley)	−	+
Alpheus sp. (from San Diego)		
Alpheus sp. (from San Felipe, Baja California)	−	+
Crangonidae		
Crangon nigricauda Stimpson	+	−
Paracrangon echinata Dana	−	−
Hippolytidae		
Heptacarpus pictus (Stimpson)	+	+, reduced
H. brevirostris (Dana)	+	+, reduced
H. palpator (Owen)	+	+, reduced
H. paludicola (Holmes)	+	+, reduced
H. stylus (Stimpson)	+	−
H. taylori (Stimpson)	+	+, reduced
Lysmata californica Stimpson	+	+
Palaemonidae		
Palaemon ritteri Holmes	+	−
Pandalidae		
Pandalus danae Stimpson	−	+
Pandalus spp.	−	+
Pandalopsis dispar Rathbun	−	+

Most behavioural observations were made on *Heptacarpus pictus* (Hippolytidae), a small tidepool species of the southern California coast; *Betaeus macginitieae* (Alpheidae), a subtidal species commensal with sea urchins; *Palaemon ritteri*, a tidepool species from the Gulf of California, Baja California; and *Pandalus danae*, a large subtidal schooling species of the Pacific Northwest.

Structure of appendages and processes was recorded by light microscopy with camera lucida drawings. Ultrastructure of grooming setae and processes was recorded with scanning electron microscopy, with the methods outlined in Bauer (1975).

In addition to species observed alive, grooming behaviours were inferred from the morphology of several species using preserved material (Table 2). These specimens were largely from the Benthic Invertebrate Museum of the Scripps Institution of Oceanography, where most of this research was carried out. Some preserved material was obtained through the courtesy of the United States National Museum.

Experiments on the adaptive value of gill brushing were performed on two species, *Heptacarpus pictus* and *Pandalus danae*. In experiments with *H. pictus* shrimp had the cleaning chelipeds ablated, while controls had the first walking leg removed. Both experimentals and controls were placed in 4–5 l plastic buckets (cages) covered at the top with fine plastic mesh and perforated by many

2–3 mm holes through which sea-water could circulate. The cages were hung in the settling tanks of the Scripps Institution of Oceanography (SIO) seawater system. Seawater flows down the SIO pier in a flume which empties into the settling tanks before filtration and use in the SIO facilities. Attached to the cages were settling plates (standard glass microscope slides and 50 × 70 mm rectangles of asbestos board). Three ablation experiments were conducted: 8–25 February 1974 (25 experimentals, 25 controls); 6–19 April 1974 (26 experimentals, 26 controls); and 22 July–1 August 1974 (20 experimentals, 20 controls). All individuals were gravid females carrying embryos in the early stages of development. These individuals were chosen to prevent interruption of the experiment by molting (brooding females do not molt) and also to test the effect of a lack of grooming of brooded embryos. At the end of the experiment, the shrimps were preserved in seawater Bouin's solution (first experiment) or 10% seawater formalin (second and third experiments).

To quantify fouling of the gills, gills were removed from the preserved animals, mounted and cleared in Turtox CMC-10 medium, and viewed under a compound microscope at ×400 so that the gill filled the entire field of vision. A sensitive exposure meter (Lafayette Model F 49) was used to measure light transmission through the gills. A reading was taken with the gill in (gill reading) and out (blank reading) of the field of vision. Percent transmission is the gill reading/blank reading × 100. A gill reading and a blank reading had to be taken with each gill, due to voltage fluctuations which caused variation in illumination.

A similar experiment was conducted with *Pandalus danae* at the University of Washington Friday Harbor Laboratories in Spring, 1973. This is the "general grooming" experiment described in Bauer (1975: 62).

RESULTS

Gill cleaning by the chelipeds

Cheliped brushing of the gills is a major behaviour in all the hippolytid species examined, in *Palaemon ritteri,* and in *Crangon nigricauda.* Chelipeds involved in brushing the gills are the pair used in general body cleaning. In carideans, either the first or the second pair of chelipeds are the cleaning chelipeds. The cleaning chelipeds of *Heptacarpus pictus* (second chelipeds) and of *Palaemon ritteri* (first chelipeds) are shown in Fig. 1. The general structure of grooming chelipeds and their distribution in the Caridea are given in Bauer (1978).

Setae in the brushes on the chelal fingers are compound in those species which were observed cleaning the gills (Fig. 2). These setae are multiscaled in the hippolytids and *Palaemon ritteri,* naked proximally, with setules arising distally on the setal shaft. Digitate scale setules are much like those described from the grooming setae of the third maxillipeds of *Betaeus macginitieae* (Bauer, 1977) and *Pandalus danae* (Bauer, 1975) except that the scale setules completely surround the shaft of these setae. In *Crangon* spp., the setae are serrate rather than multiscaled. In those shrimp which were not observed to clean the gills with chelipeds (*Pandalus* spp., *Betaeus* spp., *Alpheus* spp.), the setae in the tufts on the chelal fingers were not compound or only slightly so (Fig. 3; Bauer, 1975: pl. 5C, D). It appears that the multiscaled setae are an adaptation for rasping, possibly evolved in conjunction with gill brushing, since the shrimp which do not clean the gills
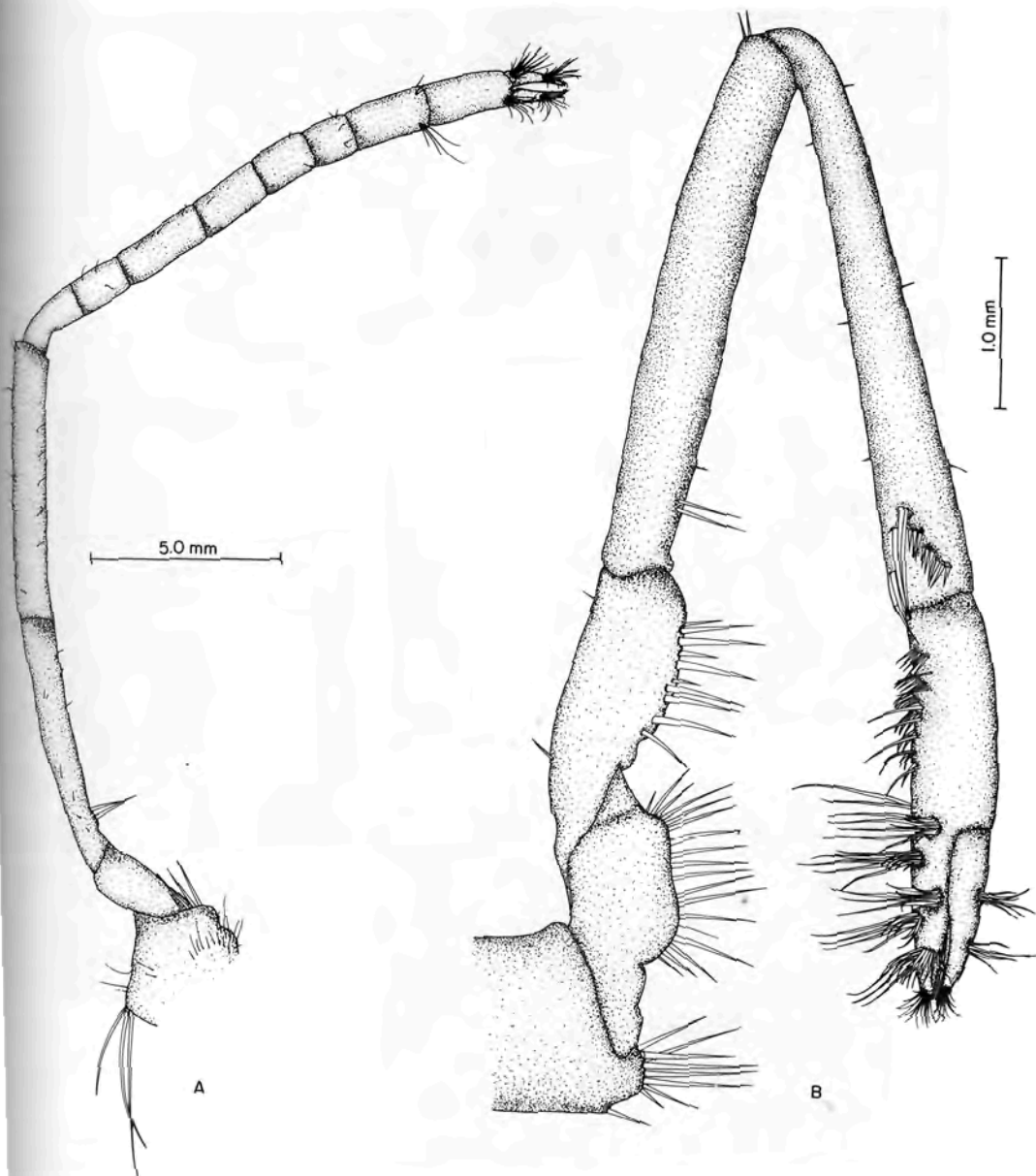
Figure 1. Cleaning chelipeds of two caridean species: A, the second cheliped of *Heptacarpus pictus*; B, the first cheliped of *Palaemon ritteri*.

engage in general body grooming but the setae on their chelae are not compound.

Heptacarpus pictus cleans its gills with the second chelipeds. The shrimp stands and reaches back under the cephalothorax and up into the gill chamber with the chelipeds. While each cheliped usually cleans the branchial chamber on its side, each can and does reach across into that of the opposite side of the body. Chelipeds form a characteristic U-shape, rapidly thrust up and down within the
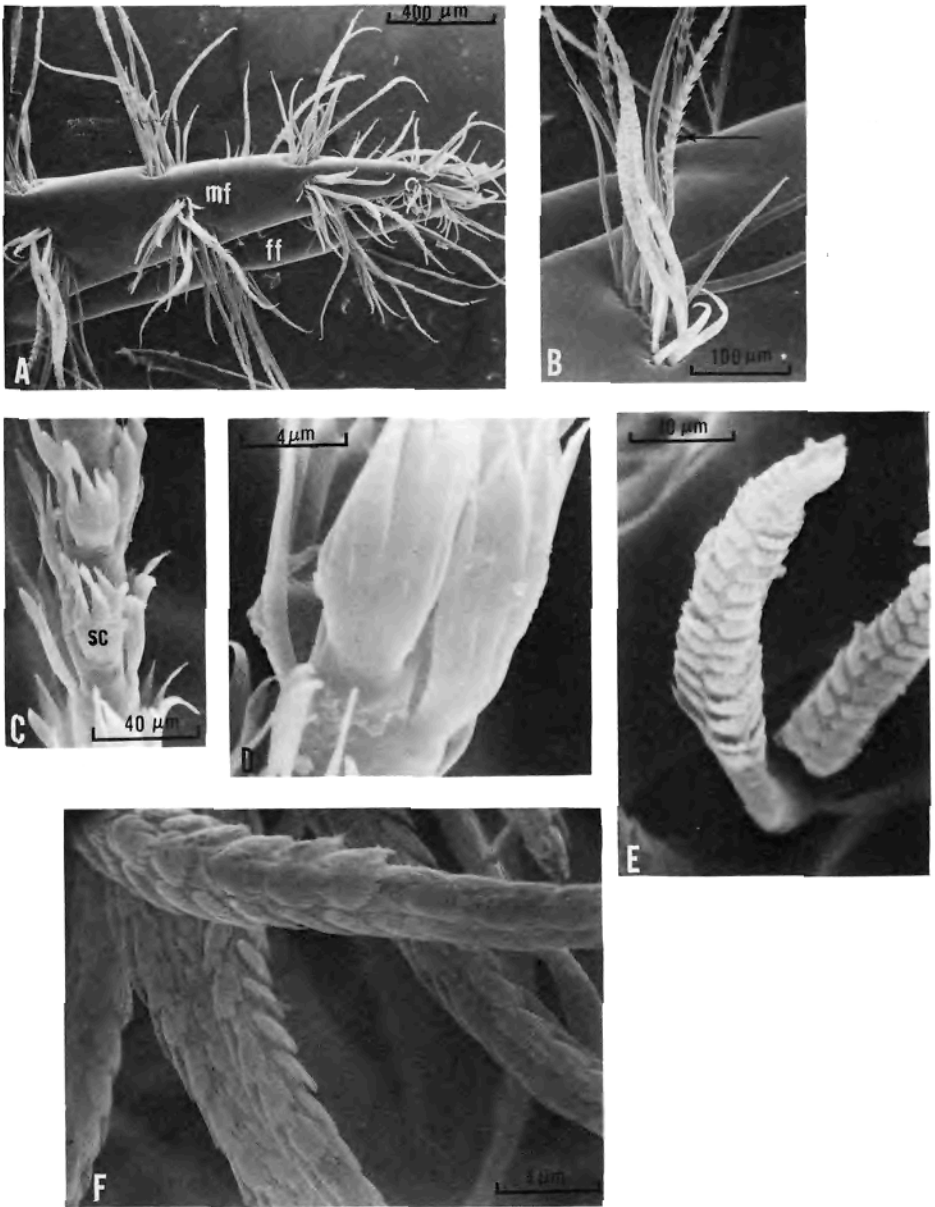
Figure 2. Gill brushing adaptations of the cleaning chelipeds: A, first chela of *Palaemon ritteri*, with tufts of multiscaled setae; B, tuft of setae from A; C, D, close-ups of the seta indicated with an arrow in B, showing the digitate scale setules; E, F, multiscaled setae from the cleaning chela 2 of *Heptacarpus pictus*; ff, fixed finger; mf, movable finger; sc, scale setule.

gill chamber (Fig. 4A). At the same time, fingers of the chelae snap rapidly open and shut. By these actions, the tufts of compound setae are brushed through and over the gill plates. Since the branchiostegite of this small shrimp is translucent (as is that of *Palaemon rittei*, see below), these cheliped movements have been directly observed. The chelipeds are inserted into the branchial chambers from between the bases of any two pereopods posterior to them. From any one
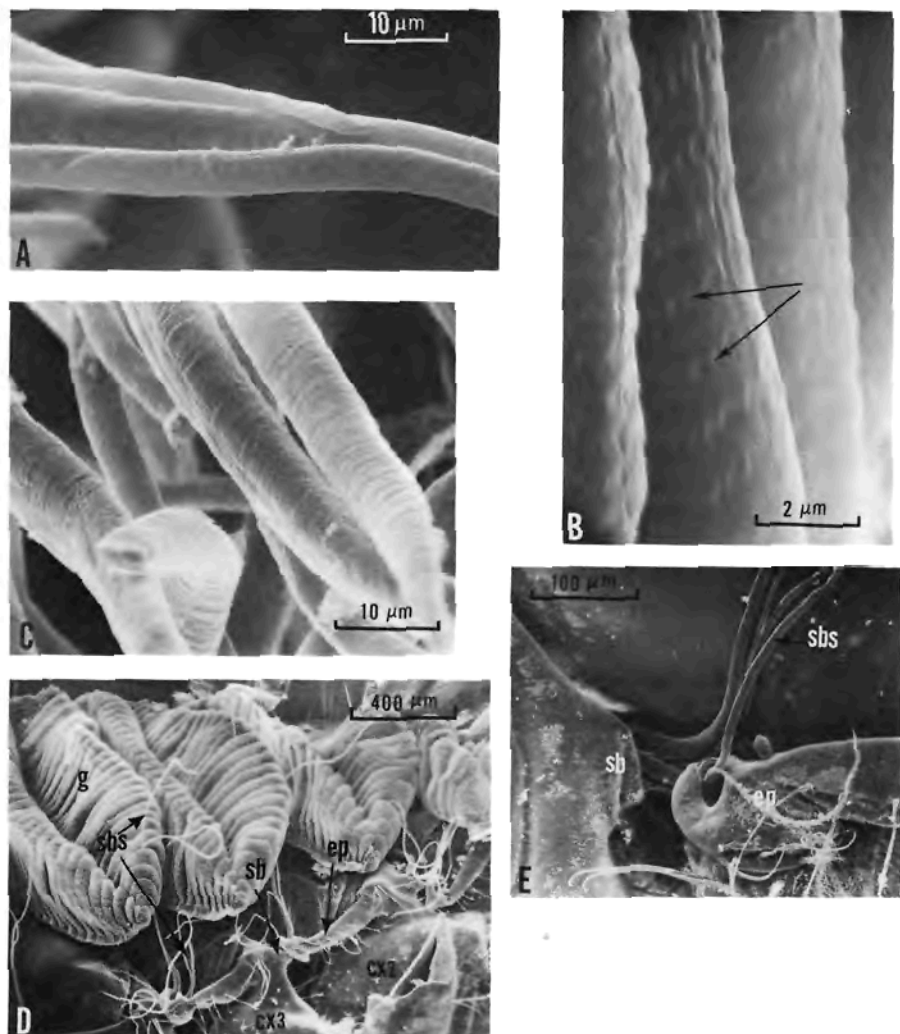
Figure 3. Gill cleaning characteristics of shrimps not brushing the gills. A–C. Setae from the cleaning chelae of shrimps that do not brush the gills: A, setae from chela 2 of *Betaeus macginitieae*; B, close-up of the setae in A, showing contours (arrow) that might be incipient scale setules; C, setae from the cleaning chelae of *Alpheus* sp.—note lack of scale setules; D, view of the right branchial chamber of *Betaeus macginitieae*, gill cover removed, showing the gill and the epipod-setobranch complexes; E, epipod-setobranch, with the hook of the epipod displaced from the bases of the setobranch-setae; cx 2,3, coxae of the second and third pereopods; ep, epipod; g, gill; sb, setobranch; sbs, setobranch-setae.

insertion point, a chela can reach both posteriorly and anteriorly in the chamber by bending the subdivided carpus. Chelipeds are only rarely inserted through the anterior (excurrent) opening of the gill chamber.

Palaemon ritteri brushes the gills with the first chelipeds. A frequent sequence is for the right cheliped to reach back into the posterior gills of the right branchial chamber from below while the anterior gills are simultaneously cleaned by the left cheliped, which reaches in front of the mouthfield, entering the right gill chamber anteriorly (Fig. 4B). When the gills of the opposite side are cleaned in
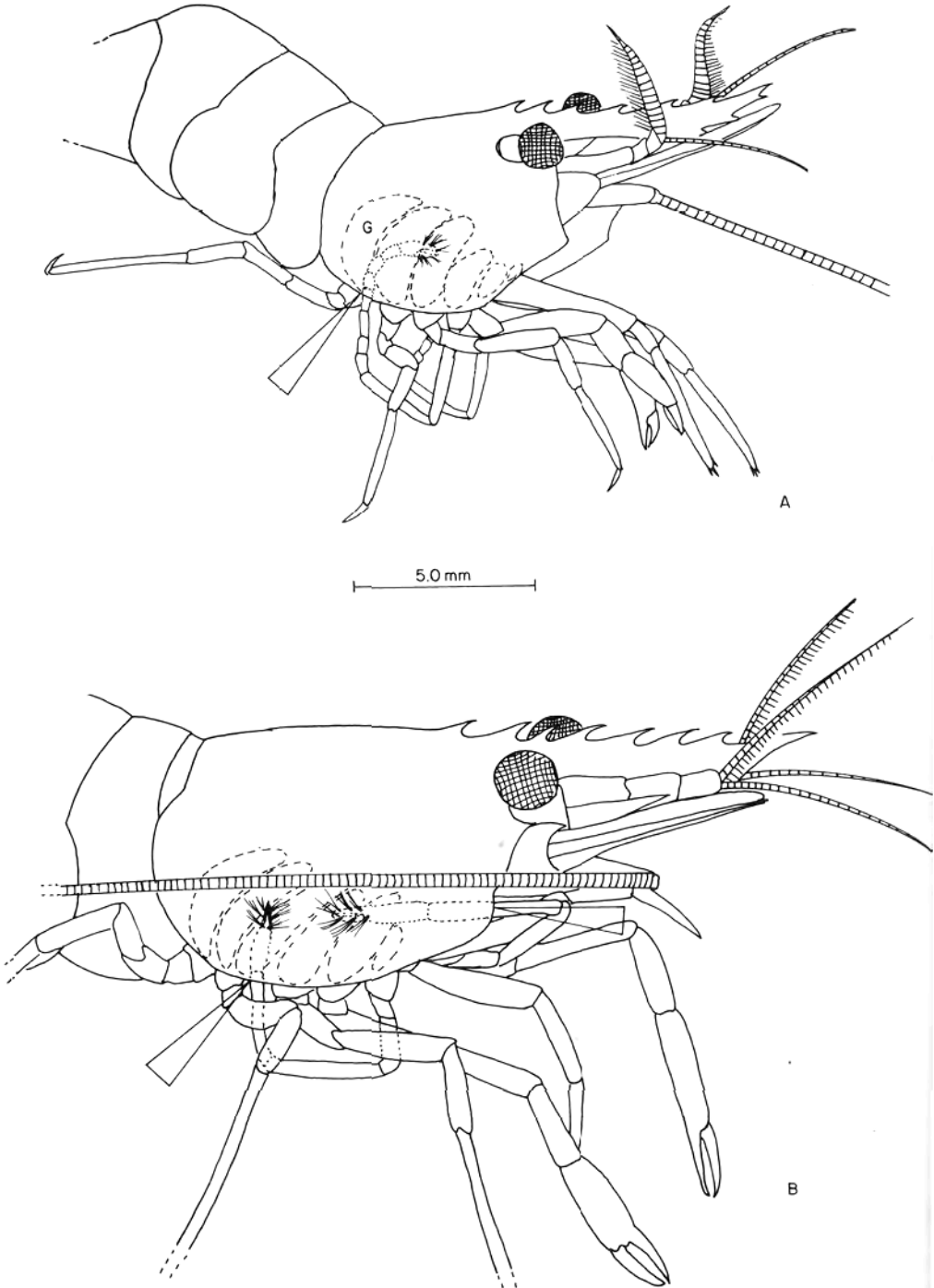
Figure 4. Brushing of the gills by the cleaning chelipeds: A, second cheliped of *Heptacarpus pictus* inserted into the branchial chamber (arrow) with the chela brushing over the gills; B, gills of the right branchial chamber being cleaned by both chelipeds 1 in *Palaemon ritteri*. The left first cheliped is inserted into the gill chamber anteriorly (arrow), while the right first cheliped is inserted along the ventral margin of the chamber (arrow); G, gill.

this fashion, the sequence is reversed. However, each cheliped can and does clean all of the gills on its side.

Gill cleaning by the epipod-setobranch complex

Another mechanism which appears, on the basis of morphology, to be a gill-cleaning device is the epipod-setobranch complex. Setobranchs are papillae on the anterior edges of thoracic legs found in many caridean shrimp and some other decapods. From these tubercles, long compound setae extend up among the gills. The term "setobranch" was coined by Borradaile (1907) to denote the setiferous papilla, but the word has been incorrectly applied to the setae of the papilla (e.g., Thomas, 1970). Huxley (1880) called homologous setae of a crayfish "coxopoditic" setae, but since there are often other types of setae on the coxa, I believe the more specific term "setobranch-seta" should be applied to them, in order to avoid confusion. Setobranchs and their setae are closely associated with unique hooked epipods in the Caridea.

In *Pandalus danae* and *Betaeus macginitieae,* epipod-setobranch complexes are well developed and will be used to illustrate the typical morphology of such a system (Figs 3D, E, 5, 6). A setobranch tuft from the coxa of a pereopod extends up between two gills to lie under and along the gill plates. A majority of setobranch-setae from a given leg extend up to the gill(s) anterior to it, but some are associated with the gill(s) of the same limb. The third maxillipeds and the first four pereopods each bear a blade-like epipod (mastigobranch of many authors) with a hook at the posterior end. Each epipod hook snaps around the base of the setobranch tuft on the limb posterior to it. Thus, the last walking leg has no epipod, for there is no setobranch posterior to it. Conversely, the third
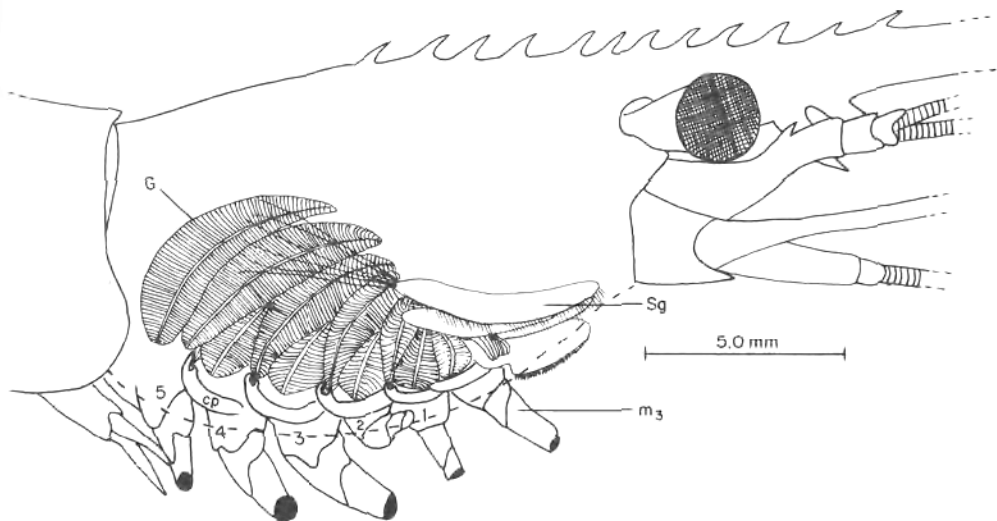

Figure 5. View of the exposed gill chamber of *Pandalus danae,* figuring gills, epipods, and other elements of the branchial system and its cleaning apparatus; 1–5, bases of pereopods 1–5; cp, epipods (on maxilliped 3 and pereopods 1–4); G, gill; m_3, base of the third maxilliped; Sg, scaphognathite (gill bailer); setobranchs are hidden by the hooked ends of the epipods, and their setae extend up beneath the gills; the ends of the long setae extending from the scaphognathite are attached to the posterior gills.
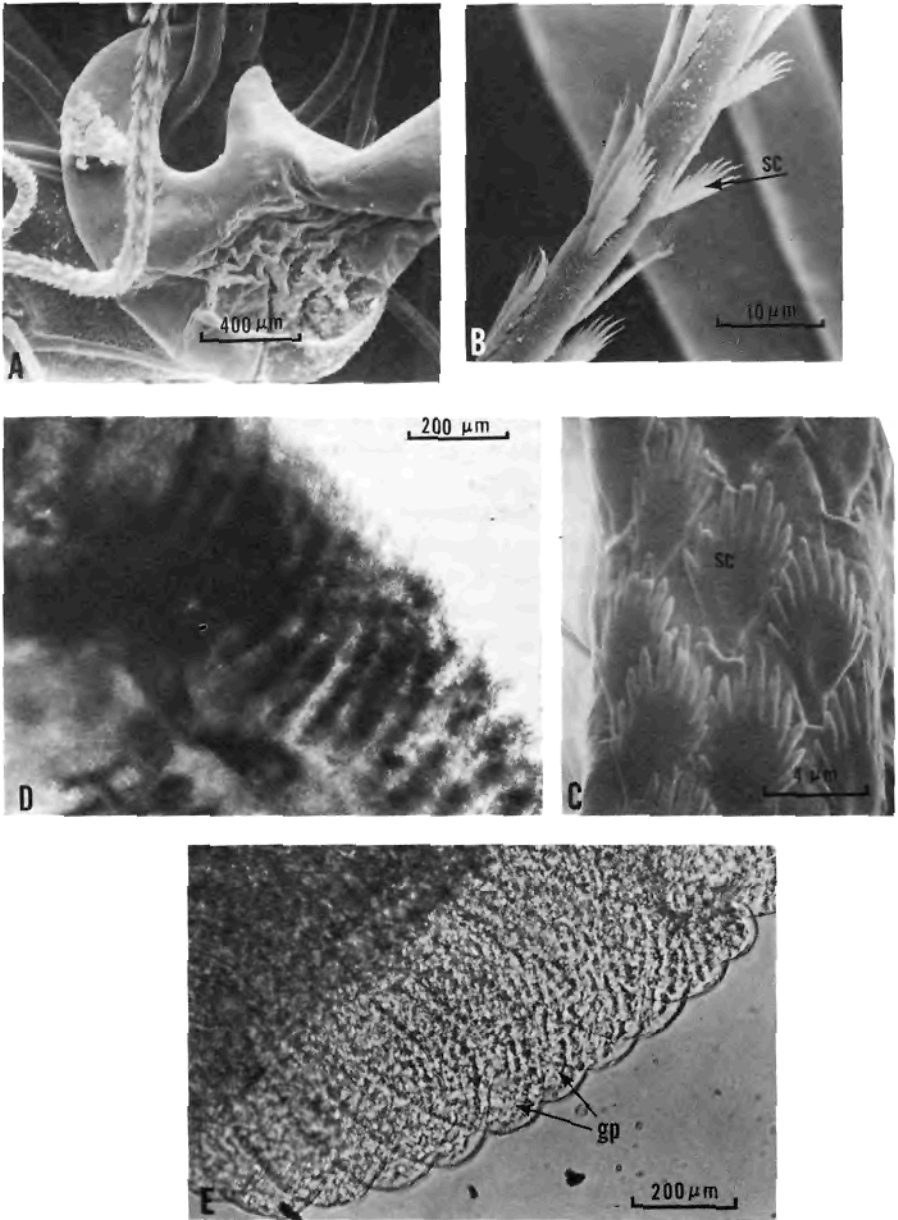
Figure 6. Characteristics of epipod-setobranchs and results of gill fouling experiments: A, end of an epipod of *Betaeus macginitieae*, hooked around setobranch-setae; B, C, setobranch-setae of this species, showing digitate scale setules; D, gill of an experimental shrimp (*Heptacarpus pictus*) with the cleaning cheliped removed, fouled with particulate debris; E, unfouled gill of a control shrimp; sc, scale setule; gp, gill plate.

maxilliped has no setobranch, as there is no hooked epipod anterior to this limb. (The epipod of maxilliped 2 is thin, laminar and apparently respiratory in function.)

Examination of dead and preserved shrimp shows that when a setobranch-bearing limb is moved posteriorly, the setobranch-setae are drawn back and thus

along and over gill plates, presumably fluttering and scraping the gills in life. Debris removed could be swept away in the respiratory current. Since the setobranch-setae are surrounded by the epipod hook on the appendage anterior to it, they are drawn through the hook as a group. When the setobranch-bearing limb moves forward again, the setae are guided back to their original position through the epipod hook. If an epipod hook is removed from its setobranch tuft, the setae in the group spread apart and become separated from the gills. Thus, the epipod hooks appear to keep a setobranch setal group together and guide the setae back to the gill after being drawn away during leg movement.

Additional evidence that setobranch-setae assist in gill cleaning can be derived from their morphology. Their setulation is very much like that of the setae of chelipeds which brush gills, e.g., chela 1 of *Palaemon ritteri*, chela 2 of *Heptacarpus pictus*. As with these setae, the setal shafts of setobranch-setae are surrounded by multidenticulate setae (Fig. 6B, C). Thus, a rasping brush arising from the coxa of the leg can assist in cleaning the branchiae.

In the shrimp observed, there is a negative correlation between the possession of cheliped brushing and epipod-setobranchs for gill cleaning (Table 1). Thus the alpheids *Betaeus* spp. and *Alpheus* spp., and *Pandalus danae* were never observed cleaning the gills with the chelipeds, and all of these shrimp have well developed epipod-setobranchs. The crangonid *Crangon nigricauda* and the palaemonid *Palaemon ritteri* completely lack pereopodal epipods and setobranchs, and both clean the gills by cheliped brushing. In the Hippolytidae, there is variability in this character, with the tendency being for reduction of epipod-setobranchs and all possessing cheliped brushing of gills. Thus, in *Lysmata californica*, the epipod-setobranch system is fully developed and concomitant with cheliped brushing, while in *Hippolyte* it is completely absent and *Hippolyte* brushes the gills with chelipeds (C. K. Barry, personal observation), as in all hippolytids observed. In the related genera *Heptacarpus, Eualus, Lebbeus* and *Spirontocaris*, there are degrees of epipod-setobranch loss, from a maximum of four epipods to complete loss (see distribution of epipods in spirontocarids in Rathbun, 1904). where epipod-setobranchs are lost, they are lost in sequence from posterior to anterior and not at random. In *Heptacarpus pictus* from La Jolla (but not in all of those from San Luis Obispo, California), there is the interesting case in which the setobranch of one limb is apparently absent (10 individuals examined) but the epipod hook on the limb anterior to it is present and apparently functionless, as there are no setobranch-setae for it to hook around (no setobranch on pereopod 3, but epipod with hook on pereopod 2). This has not been found in any other heptacarpid species in which reduction of epipod-setobranchs takes place, i.e., the epipod-setobranchs are usually lost as a pair.

Examination of other carideans (Table 2) reveals a similar pattern in the distribution of epipod-setobranchs and cheliped brushing. If the cleaning cheliped had tufts of multiscaled setae similar to those of *Heptacarpus pictus* or *Palaemon ritteri*, then that cheliped was considered to be involved in cleaning the gills. In general, as Table 2 shows, cheliped brushing based on this criterion was not concurrent with epipod-setobranchs. Behavioural observations are necessary to confirm or deny these inferences.

Epipod-setobranchs of the primitive species *Procaris hawaiana* may be instructive in determining how the hook-like epipods of caridean genera such as *Heptacarpus, Betaeus, Alpheus, Pandalus,* and *Lysmata* (Fig. 7B–D) developed. In *P.*

Table 2. Gill cleaning mechanisms of caridean species inferred from the morphology of preserved specimens (+, present; −, absent)

Species	Brushing by cleaning chelipeds	Epipod-setobranch systems
Atyidae		
Caridina nilotica (Roux)	−	+
Crangonidae		
Crangon nigromaculata (Lockington)	+	−
Glyphocrangonidae		
Glyphocrangon sp.	−	−
Gnathophyllidae		
Gnathophyllum americanum Guerin	+	−
Hymenocera elegans Heller	+	−
Hippolytidae		
Spirontocaris spp.	+	+, reduced
Eualus spp.	+	+, reduced
Caridion gordoni (Bate)	−?	+
Nematocarcinidae		
Nematocarcinus ensifer Smith	+?	−
Oplophoridae		
Acantheyphyra sp.	−	+
Palaemonidae		
Palaemon spp.	+	−
Palaemonetes spp.	+	−
Pasiphaeidae		
Pasiphaea emarginata Rathbun	−	−
Procarididae		
Procaris hawaiana Holthuis	−	+, reduced
Processidae		
Processa sp.	+	−
Rhynchocinetidae		
Rhynchocinetes sp.	+	−
Stylodactylidae		
Stylodactylus sp.	−	−

hawaiana, epipods have hooks on their medial faces which clamp around the setobranch tufts (the epipod on pereopod 4 does not have a hook and, correspondingly, there is no setobranch on pereopod 5). Epipods of *P. hawaiana* consist of a blade from which extends a thin laminar process which separates two successive gills in the branchial chamber. Hooked epipods could easily be derived from this type of epipod by loss of the dorsal (apparently respiratory) process (Fig. 7A).

Experiments on the adaptive value of cheliped brushing in Heptacarpus pictus

Experimental and control females from the February and April ablation experiments were examined for gill fouling, as experimentals had the cleaning
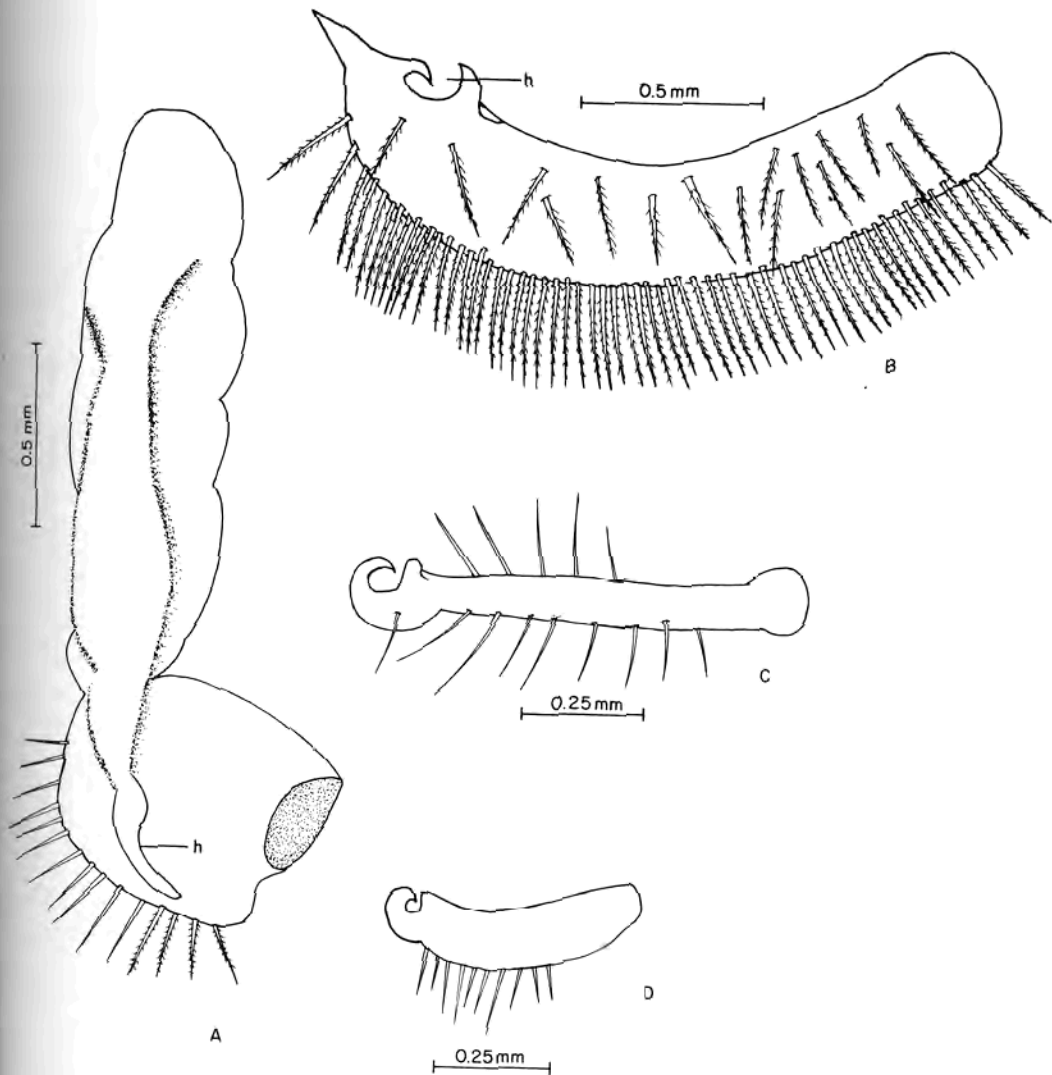
Figure 7. Thoracic epipods of epipod-setobranch systems in carideans. A, Epipod of the third pereopod of *Procaris hawaiana*; B, epipod of the fourth pereopod of *Pandalus danae*; C, pereopodal epipod of *Betaeus macginitieae*; D, pereopodal epipod of *Heptacarpus pictus*; all views are lateral except A, which is medial; h, hook.

chelipeds ablated, while the controls did not (first walking leg ablated). Within a few days of cheliped removal, it was observed that gills of experimental shrimp had darkened, a result of sediment and detrital fouling of the gills. Heavy fouling, both by particulate debris and by epizoites, was apparent within two weeks (Fig. 6D). Control gills remained clean (Fig. 6E). Sediment and detrital particles were entrapped between adjacent gill plates of experimentals. Particulate matter was heavily concentrated beneath the gills, against the body wall as well as above the dorsal edges of the gills. Epizoic fouling consisted of heavy concentrations of *Leucothrix* sp., a long-chained bacterium, diatoms, other unicellular algae, and stalked ciliates. Hypotrichous ciliates were found moving
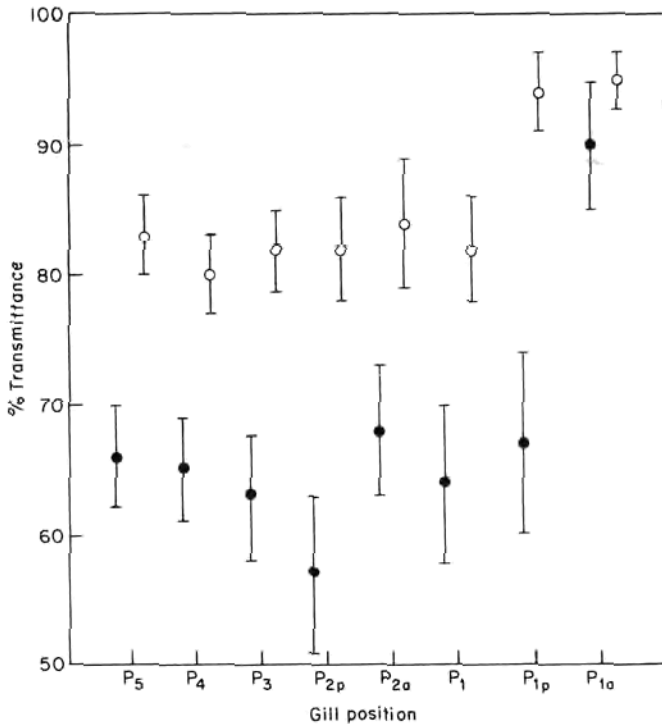
Figure 8. Transmission of light through control (unfouled) and experimental (fouled) gills from the April cheliped 2 ablation experiment; P_x, gill of pereopod x; a, anterior half of gill; p, posterior half of gill. Number of gills examined: P5, experimental and control = 16 each; P4 ≈ 16 each, P3 = 16 each, P2p = 15 each, P2a = 14 control, 15 experimental, P1 = 14 each; means, 95% confidence limits are shown.

among the debris between gill leaves. Although controls never showed sediment fouling, individuals near a molt showed some *Leucothrix* sp. and microbial fouling, but never in the concentration seen on experimental gills. Individuals which molted and regenerated the cleaning chelipeds during the experimental period showed no gill fouling.

Fouling was measured quantitatively by relative transmission of light through the gills and the results are given in Figs 8 and 9. Lowered transmission of light and the greater variability of measurement in experimentals is due to sediment fouling, i.e., the size and optical density of particles on experimental gills varied, while such particles were absent from controls. Low variability is thus expected in controls, where only optical density of gill tissue prevents passage of light. It can be noted that, in the April experiment, the P1 (pereopod 1) gill and the anterior half of the P2 gill were less fouled than the posterior gills. These gill regions lie beneath the scaphognathite (gill bailer), and its pumping action may blow or scrape off sediment. This difference is not so apparent in the February experiment (Fig. 9); however, when the fouling of the posterior and the anterior halves of the small first gill were measured (at ×1000), the fouling was found mainly on the posterior half. In April measurements, the whole gill was measured at ×400, and thus the difference between the gill halves was not distinguished. Qualitatively, the ventral anterior half of the P2 and the anterior half
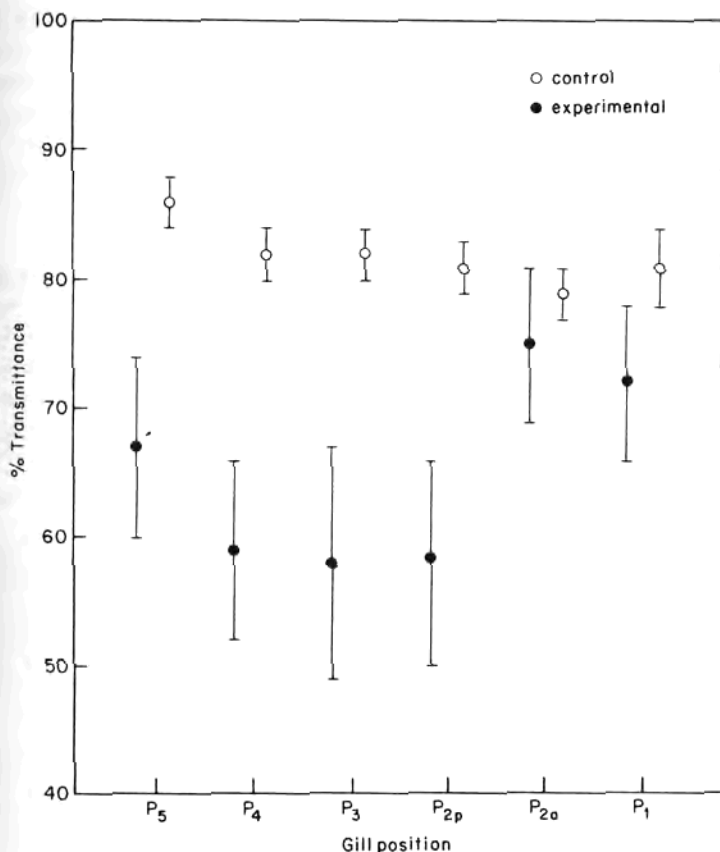
Figure 9. Transmission of light through control and experimental gills from the February cheliped 2 ablation experiment. Number of gills examined for P5 = 14 experimental, 15 control; P4, 15 experimental, 16 control; P3, 16 each; P2P, 15 experimental, 14 control; P2A, 15 experimental, 13 control; P1, 13 experimental, 15 control; P1p, P1a, 13 each; means, 95% confidence limits are shown.

of the P1 gills were relatively free of fouling in experimentals. Both gills have setobranch-setae reaching up to them; their cleaning action, plus the rocking movement of the gill bailer, may account for the distribution of fouling on the anterior gills.

Shrimps with fouled gills were much more sensitive to stress than controls. During examination of living animals for fouling, shrimp were placed, out of water, in a dish under a dissecting microscope. Experimentals with fouled gills frequently died during this handling or were moribund for a time when replaced in seawater before recovering. Controls showed no such trauma. Shrimps with fouled gills behaved sluggishly and soon died when placed in poorly aerated water, while control shrimps with clean gills did not. Measurements of oxygen consumption of shrimps with fouled and unfouled gills at varying levels of dissolved oxygen are necessary to confirm impaired respiration due to gill fouling.

In *Pandalus danae*, behavioural observations did not indicate that this shrimp used its chelipeds in cleaning the gills. Instead, it was inferred from morphology

that the epipod-setobranch system was the alternate mechanism to cheliped brushing. In the general grooming experiments on *P. danae* (Bauer, 1975), Treatment 2 and 3 animals had the grooming chelipeds removed. The gills of these shrimp and of controls showed no fouling. These results support the conclusion that *P. danae* does not clean its gills with the chelipeds.

Cleaning of brooded embryos

Females brooding eggs insert the setose chelipeds into the egg mass and, with rapid brushing and picking movements, jostle and apparently scrape individual eggs (Fig. 10). Fingers of the chelae rapidly snap open and shut during these bouts, apparently to bring the brushes of serrulate setae on the fingers into play. Eggs have not been observed being removed from a healthy egg mass, but the shrimp is certainly capable of doing so. Female *Heptacarpus pictus* deprived of males will spawn and attach unfertilized eggs to the pleopods but will soon discard them by cleaning movements (Bauer, 1976). *Heptacarpus pictus* is thus capable of ridding the egg mass of dead or diseased eggs whose infection might spread to the rest of the embryos.

Cleaning of brooded embryos by the cleaning chelipeds was observed in all hippolytid species, *Palaemon ritteri,* all the alpheid species, but not in the pandalids. Lack of this behaviour in *Pandalus danae* may simply be a result of the
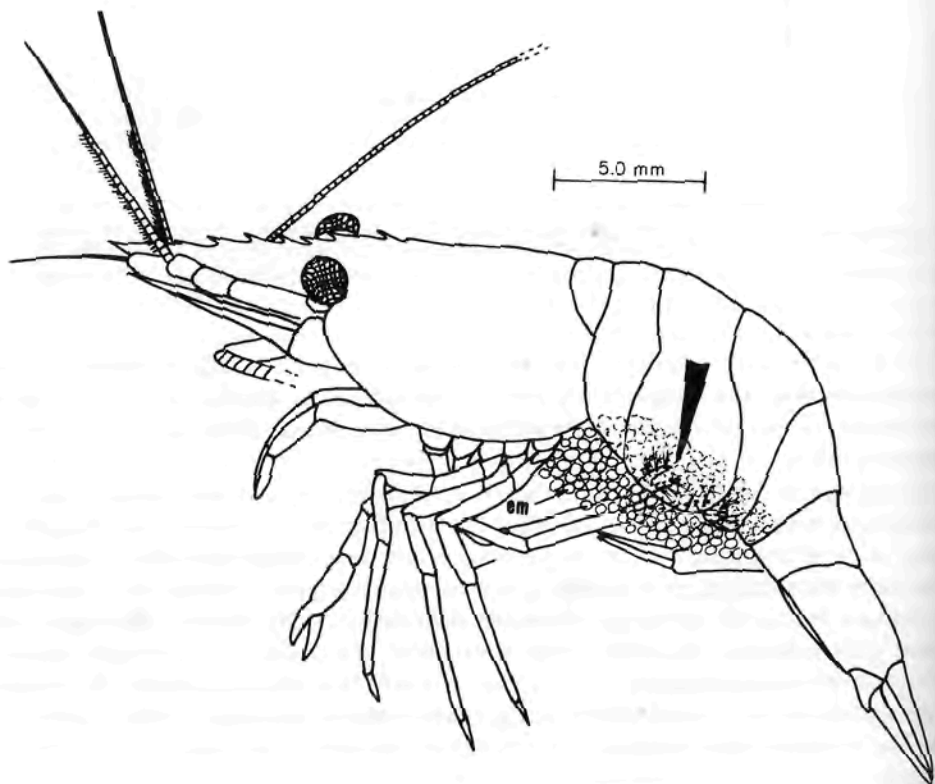

Figure 10. Embryo brushing in *Palaemon ritteri*. The first chelipeds are inserted into the egg mass beneath the abdomen (large arrow); em, embryo.

limited observation of female *P. danae* with broods. It is likely that this behaviour is widespread among carideans.

Particulate matter seems to be introduced into the egg mass by pleopod movements and by the respiratory current. Both swimming and fanning circulate water through the egg mass, but the tightly crowded embryos apparently serve as a filter which accumulates debris from this water. Also, some water being drawn toward the gills from behind the cephalothorax filters through the eggs.

Experiments on the adaptive value of cheliped brushing of eggs in Heptacarpus pictus

To test the hypothesis that cheliped brushing of eggs is necessary for successful hatching of embryos, females from the ablation experiments (gravid females, early stages of embryo development at the start of the experiment) were examined for egg death, as experimentals had the grooming chelipeds removed. Determination of whether eggs were dead or alive was made when the shrimp
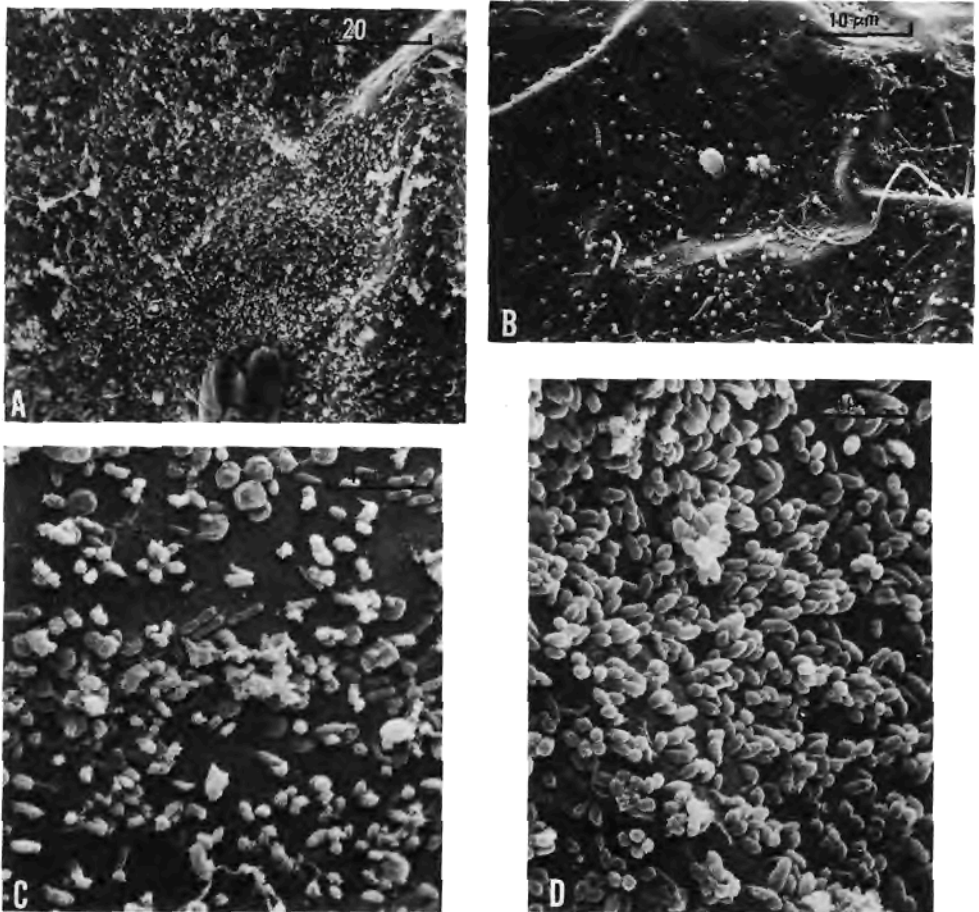

Figure 11. Fouling of the surfaces of embryos carried by females (*Heptacarpus pictus*) prevented from grooming them (by ablation): A, B, surfaces of an embryo from an experimental female and a control female, respectively, at ×1000, to show differences in fouling; C, D, view of microbial cells attached to fouled (experimental) embryos.

were still alive, i.e., on embryos stripped from living females. Beating of the embryo heart was first thought to be a good indicator of whether or not the embryo was alive, but it soon became apparent that embryos soon died under the trauma of handling (e.g., heating of the slide in a compound microscope). Only eggs that were obviously dead were counted, i.e., eggs that were discoloured due to decomposition, from light green to white or a yellowish colour. Dead eggs were also more opaque than live ones. Egg death is thus probably under-estimated.

In the April and July but not February experiments, the broods of experimental females showed macroscopic indications of egg death. Experimental egg masses always showed an accumulation of particulate debris among the embryos, whereas the controls did not. Both experimental and control embryos carried epizoites such as those found on fouled gills and antennules. Experimental embryos showed, qualitatively, a much greater growth of a microbial coat consisting of various kinds of bacterial cells (Fig. 11). Careful examination of egg masses from February (where no external signs of embryo death were seen) revealed an occasional dead embryo among otherwise healthy embryos. Numerous nematodes and hypotrichous ciliates were found among these egg masses but not among controls ($N=9$ broods for experimentals, $N=10$ for controls). Phillips (1971) reported that nematodes and ciliates were indications of death among eggs. Egg death was obvious in the April and July experimental broods, noticed first after 13 and 10 days, respectively. Dead eggs appeared in clumps in various parts of egg masses. Eggs were removed from females and the percent discoloured (obviously dead) eggs computed (Fig. 12). Control females ($N=10$) were examined in the same manner in each experiment, but broods from these females showed no signs of embryo death.
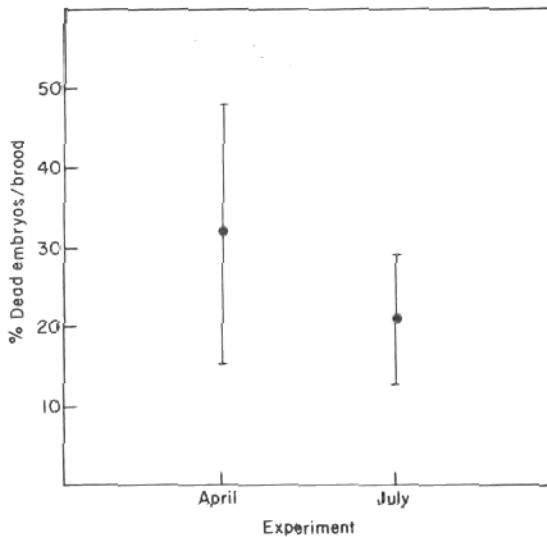

Figure 12. Per cent dead embryos/brood of gravid (experimental) females from the April and July cheliped 2 ablation experiments (*Heptacarpus pictus*); $N=9$ broods examined for the April experiment; $\bar{x}$ # embryos/brood = 249.7, standard deviation = 39.2; $N=11$ for July, $\bar{x}$ embryos = 113.9, s.d. = 63.6

DISCUSSION

Adaptive value of gill cleaning mechanisms in caridean shrimp

A wide variety of mechanisms appears to be involved in keeping the enclosed gills free of sediment and other fouling debris which enters the branchial chamber in the incurrent respiratory current in decapod Crustacea. In various decapod groups, these mechanisms include reversal of the respiratory current, setobranchs, epipods of the pereopods and/or maxillipeds which bear setobranch-type setae, and brushing of the gills by a cheliped (Vuillemin, 1967). In the caridean shrimp examined in this study, the important mechanisms were either epipod-setobranch systems or cheliped brushing, i.e., where one mechanism was present, the other was generally not. In one family in which both were found (Hippolytidae), cheliped brushing appeared to be supplanting epipod-setobranchs, in that the latter showed various degrees of reduction and complete loss. The two mechanisms are similar in that multiscaled setae appear to brush against the gills, brought about indirectly by leg movements in the epipod-setobranch system and by an active, directed effort toward the gills in the case of cheliped brushing. Simply on the basis of morphology, there is reason to believe that these mechanisms are adaptations for cleaning the gills.

Experiments with *Heptacarpus pictus* in which the cleaning chelipeds were removed showed that fouling of the gills by particulate matter and microbial fouling organisms does indeed take place in the absence of grooming. Furthermore, there are observations which indicate that this fouling had a detrimental effect on survival of the experimental animals. It thus appears that the selective pressure which is responsible for the evolution of cheliped brushing of gills is the presence of fouling material which clogs the gills.

Tidepool shrimp such as *Heptacarpus* might be sensitive to fouling interfering with gas exchange. In Southern California, where *H. pictus* occurs, lower-low tides occur at night during the spring and summer. Plant and animal respiration in tidepools decreases the oxygen content of the water in entrapped pools. Tidepool fish frequently show signs of oxygen stress (Terence Parr, personal communication). Thus, fouling might be especially hazardous for shrimps encountering such situations.

That setobranchs and their setae actually keep gills clean has only been indirectly indicated in this study. *Pandalus danae*, which possesses a well developed epipod-setobranch system, was never observed cleaning the gills with the cleaning chelipeds, and, when these were removed, no fouling took place. Excision of the setobranchs and examination for subsequent fouling are necessary to verify that the setobranchs clean the gills, and this was not done in this study. However, in the brachyuran crab *Callinectes sapidus* Rathbun, Walker (1974) found that similar setae on the epipods of the maxillipeds (which sweep over the gills) did affect the distribution of the gill barnacle *Octolasmis mulleri* (Coker). Sections of the gills over which the setae passed showed a significantly lower degree of fouling by the barnacle than sections not scraped by the setae. Again, ablation experiments are necessary to confirm the cleaning function of these setae.

Other suggestions on the nature of these setae have been made in the past. Berkeley (1928) suggested that they might be respiratory in the caridean *Pandalus danae*, but this is clearly not the case, as they show no adaptations for this

purpose, e.g., broadening to increase surface area or loss of chitinization to increase permeability, as with other respiratory surfaces. Thomas (1970) believed that the setobranch-setae helped to support the gill filaments in the crayfish *Austropotamobius pallipes*. Huxley (1880) was the first to suggest, correctly, in my opinion, that setobranch-setae "no doubt, serve to prevent intrusion of parasites and other foreign matter into the branchial chamber . . . it is obvious they must share in the movements of the basal joints of the legs; and that, when the crayfish walks, they must be more or less agitated in the branchial chamber" (observations on the crayfish *Astacus fluviatilis*).

Reversal of the branchial current has been reported to be the most universal method of keeping the branchiae clean in the Decapoda (Vuillemin, 1967). This mechanism was observed in all the species examined for this characteristic in this study (*Heptacarpus* spp., *Betaeus macginitieae*, *Pandalus danae*, *Palaemon ritteri*) and is probably present throughout the Caridea.

A setal screen filtering branchial flow can prevent particles from entering the gill chamber, but the mesh size of such screens must have a lower limit, in that the finer the mesh, the more difficult it must be to draw water through it. This mechanism, if important at all in keeping the gills clean, was always linked to other mechanisms in cleaning the gills in carideans studied. Setae fringing the lower edge of the branchiostegite (gill cover) were present in all species examined except some *Heptacarpus* species (*H. pictus, H. paludicola, H. stylus*). Wilkins & McMahon (1972), working with *Homarus*, suggested that these setae may function only in sensing large particles entering the gill chamber, triggering a reversed beat of the gill bailer. However, I have observed reversals in *Heptacarpus stylus* and *H. pictus*, species without a branchiostegite fringe; other receptors might be involved in receiving stimuli that evoke reversals.

Some representatives of certain caridean families appear to have neither cheliped brushing of gills nor epipod-setobranchs, at least on the basis of morphology (Table 2). For example, in the Crangonidea, *Crangon* spp. clean the gills with the second chelipeds, but *Paracrangon echinata* Dana has completely lost these appendages. Examination of *P. echinata* shows a possible compensatory solution to this loss. In this species, the gills are compressed together posteriorly and the branchiostegite is very tightly apposed to the coxae of the legs. The posterior edge of the scaphognathite is unusually equipped with numerous long multidenticulate setae which can sweep completely over the lateral surfaces of the gills during its beat. The very tightly compressed branchial chamber would allow a strong reversed branchial flow. Perhaps between these two mechanisms the gills are kept reasonably free of sediment. Other genera of crangonids show a similar reduction of the second pereopods and might show similar adjustments to gill cleaning if examined. *Crangon* spp. have a much more spacious branchial chamber than *P. echinata*, the branchiostegite not fitting tightly around the leg bases, and there are no long setae fringing the posterior edge of the scaphognathite. The gill bailer of *Pandalus danae* has a long setal fringe on its posterior edge; however, these setae do not appear to sweep over the gills but, instead, adhere to them, anchoring the posterior end of the bailer.

Two families from which representatives were examined have no apparent gill cleaning mechanisms (other than reversals or branchiostegal fringes) based on morphology. Pasiphaeids have no epipod-setobranchs (Thompson, 1965; personal observation on *Pasiphaea emarginata* Rathbun) and their chelipeds bear

large raptorial chelae which appear ill-adapted as cleaning limbs. Their fourth pereopod (second walking leg of other carideans) is reduced, and its propodus bears rows of serrate setae. This appendage might serve as a cleaning limb that could clean the gills. Members of another family, the Glyphocrangonidae, probably have strong reversals, due to the tightly closed branchial chamber, contrasted with that of *Crangon* spp. which are cheliped gill brushers. Glyphocrangonids appear to have a general cleaning limb (cheliped 2), but it does not bear tufts of compound setae, as do chelae of shrimps that clean the gills.

Adaptive value of embryo brushing by females

Female care of brooded embryos apparently takes two forms in caridean shrimp. One is the beating of the swimmerets without actual swimming of the female ("fanning" behaviour; Phillips, 1971). This behaviour was observed in all the species observed alive in this study. The other component of parental care is brushing the embryos by the female with cleaning appendages. This aspect was investigated in this study, using *Heptacarpus pictus* as an experimental animal.

In a manner analogous to the gills, the brooded embryos carried by the caridean *Heptacarpus pictus* act as a sediment trap. In experimental animals in which the cleaning limbs were removed, the egg masses became fouled with sediment and other particulate matter, as well as heavy microbial growth. Heavy mortality of the eggs occurred in some of the experiments, indicating that brushing of the eggs is a highly adaptive behaviour. In another decapod which engages in brooding behaviour of this type, the porcellanid *Petrolisthes cabrilloi*, the embryos suffered a similar heavy loss when the cleaning limbs (last legs) were removed (Larry Ritchie, personal communication). Cheliped cleaning of the eggs is a widespread behaviour in the carideans observed in this study and also in other decapods such as the Anomura. It is likely that heavy mortality of eggs due to fouling is the selective pressure behind this behaviour. Fisher & Wickham (1976) have described extensive mortality of embryos of the crab *Cancer magister* which is the probable result of epibiotic fouling. Experimental evidence supporting a relationship between microbial fouling and embryo mortality in *C. magister* has been given by Fisher (1976).

The only difference between control and experimental embryos that I could detect was the presence of sediment and the qualitatively heavier growth of bacteria on the experimental embryos' outer membrane. The actual cause of death could be one or a combination of the following factors: (1) direct smothering (interference with respiratory and/or excretory exchange) by sediment and bacterial growth; (2) localized pockets of anoxia, with a subsequent reducing (H_2S) environment having lethal effects on surrounding embryos due to bacterial action on the organic fraction of particulate debris; (3) pathogenic attack by microbes settling on eggs. Whatever the actual cause of death, these experiments clearly demonstrate the high selective value of cheliped brushing in *Heptacarpus pictus*.

There is the possibility that ablation of the second cheliped, with subsequent fouling of the gills in experimentals, could have injured the health of the *Heptacarpus pictus* females and reduced fanning, with this causing the observed embryo death. However, the behaviour of experimentals, which were in well aerated water, seemed qualitatively similar to that of controls. There were no

indications that other cleaning behaviours were not being carried out (e.g., no fouled antennules) which would occur if the shrimps were sluggish due to oxygen stress, as discussed under gill cleaning experiments. Thus, embryo death observed was most likely due to the absence of cheliped brushing of embryos in experimentals.

Phillips (1971) has described brooding behaviour in the caridean *Palaemon serratus* (Pennant). Embryos stripped from females were reared in an apparatus in which aerated seawater flowed past the eggs. The running water was assumed to simulate cleaning by the female while the aeration presumably served the function of fanning. Varying degrees of hatching success were obtained, with high mortality in some attempts due to microbial fouling. Artificial rearing of this sort does appear to simulate fanning, but it does not seem that the scraping of the eggs by the chelipeds would be well substituted by simple jostling of the eggs by running water, hence the high embryo mortalities suffered in Phillips's experiments. Cheliped brushing seems to be a prerequisite for successful incubation.

Cheliped cleaning of embryos may also be directed at parasites or predators of eggs which might inhabit masses of brooded embryos. It would seem unlikely that such a concentrated source of energy would not have attracted (in an evolutionary sense) predators such as copepods, polychaetes, nematodes, nemerteans, etc. Such predators do exist, e.g., the nemertean *Carcinonemertes epialti,* which attacks eggs of the brachyuran crab *Hemigrapsus oregonensis* (Kuris, 1978). Although egg masses of *Heptacarpus pictus* were examined for such predators, none were found in this study.

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