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Differential Epibiont Fouling in Relation to Grooming Behavior in *Palaemonetes kadiakensis*

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INTRODUCTION

Competition for substrates for attachment by sessile organisms is a constant process in the freshwater environment. Most unoccupied surfaces are quickly inhabited by various forms of sessile invertebrate fauna, i.e., bacteria, algae, sessile protozoans, coelenterates, bryozoans, and larval insects. These organisms which attach to the body surfaces of other animals are termed epibionts.

The nature of the crustacean exoskeleton provides a suitable substrate for attachment by epibionts. Most Crustacea are mobile, providing constant flow of water and nutrients across the exoskeleton and thereby supplying an optimal habitat for epibionts.

The harbouring of epibionts can create problems for the crustacean host, depending on the location and degree of infestation (Bauer, 1975). Suspended material in the water column caused by the constant motion of turbid water can clog and cover surfaces through which contact between the animal and the external environment must take place, i.e., gill lamellae, chemoreceptive setation, and antennae (Bauer, 1975). The physical and biological problems

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engendered by epibiont infestation has elicited the development of an elaborate system for the removal of fouling organisms and debris.

Grooming is an integral part of the activities of caridian prawns. Doflein (1910) described the brushing of gills by the first chelae of *Palaemon xiphias.* Hoglund (1943) reported the importance of cleaning prior to spawning in *Palaemon squilla.* Bauer (1975) described the relevant morphology of the grooming appendages of the caridian shrimp *Pandalus danae.*

The functional morphology of the grooming appendages was taken up by Felgenhauer and Schram (in press). *P. kadiakensis* occurs mainly in waters of the Central United States west of the Alleghenies (Holthius, 1949). The prawn is transparent in life and ranges from 30-54 mm. in length. It is too small to be of any direct commercial importance, but is of great value indirectly forming one of the important links in the food chain which supports commercial and game fish. Grooming is a constant and time-consuming process in this prawn. The process and effects of grooming had not been adequately studied in freshwater prawns. The importance of such grooming is described in this study, as is field testing which elucidates the patterns and processes of grooming in *Palaemonetes kadiakensis.*

MATERIALS AND METHODS

Collections of *Palaemonetes kadiakensis* were made by dip netting through the waterwillow *Dianthera americana* in the littoral zone of Lake Charleston, Coles County, Illinois. Field experiments were used to establish whether and how the grooming appendages prevent the prawn's exoskeleton from becoming fouled by epibionts and debris. Various combinations of amputations of grooming appendages (third maxillipeds; first, second, and fifth pereiopods) were used to establish their use and grooming effectiveness. The prawns were exposed to their natural environment for from 24 to 72 hr. periods in 4 × 6 in., one-quarter inch hardware-cloth cages. For each trial five control prawns and an equal number of amputee prawns were lowered into the environment. In addition, 1 × 3 in. glass plates were also used to establish the epizoic fauna and check for differentiation between an inanimate substrate as opposed to the prawn's body surfaces.

The pereiopods were removed at the basi-ischial joint and the third maxillipeds were cut near the base of the coxa. After amputation the prawns were then housed in aquaria for 24 hr. to monitor adjustment and mortality before beginning the field testing.

Three major field experiments were conducted in March, June, and October to establish fouling patterns and the types of epibiont settlings at various seasonal periods. Duration of the experiments ranged between 24, 48, and 72 hr. Appendages were removed in various combinations: 1) third maxillipeds, 2) first pereiopods, 3) second pereiopods, 4) first and second pereiopods, 5) fifth pereiopods, 6) third maxillipeds, first, second, and fifth pereiopods. At 24, 48, and 72 hr. intervals both the prawns and glass plates were examined for epibiont settling. Epibionts present, location, intensity of infestation, and time of year were noted.

RESULTS OF GROOMING EXPERIMENTS

Examination of experimental prawns revealed fouling by either protozoans or algae, and heavy accumulations of microscopic debris. Control prawns were found to be free of fouling in all field tests other than light aggregations of peritrichous ciliates along the crevices of the arthrodia and joints between the pereiopods.

Little seasonal differences were noted in fouling patterns between the three investigations. The March experiment expressed fouling patterns which proved to be characteristic for all three seasons when field experiments were conducted (table 1). Fouling began on experimental prawns as early as 24 hr. after being exposed to the environment. Removal of the third maxillipeds allowed light fouling of the antennae after 24 hr. with increasing numbers of sessile protozoans and debris by 72 hr. Removal of the first, second, and combinations of both first and second pereiopods permitted fouling of the antennae, gills, branchiostegites, rostrum, and eyestalks. The removal of only the first or the second pereiopod showed only slight differences between their field of grooming activity. The second pereiopod preens farther back on the margin of the carapace and grooms the antennae less frequently.

Removal of the fifth pereiopods resulted in little fouling within the first 24 hr., but significant fouling was seen on the pleopods, pleura, and telson by 72 hr. Amblations of all grooming appendages (third maxillipeds, first, second, and fifth pereiopods) afforded the

TABLE 1. Results of differential fouling experiment in March to determine the efficiency of the grooming appendages and the extent of fouling per time units as indicated. +=light fouling, ++=medium fouling, +++=heavy fouling.

Appendage amputated	antennae	branchiostegites	gills	pereiopods	pleopods	eye-rostrum	telson	pleura
24 hrs.								
3rd max.	+		+	+				+
1st. pere.	+	+	+			+		+
2nd. pere.	+		+	+		+		
1st and 2nd pere.	+	+	+	+		+		
5th pere.					+		+	
all groomers	+	+	+		+			+
48 hrs.								
3rd max.	+		+					
1st. pere.	+	++	+				+	
2nd. pere.	+	+	+					
1st and 2nd pere.	+	++	+	+	+	+		+
5th pere.		+		++			++	
all groomers	+	++	++		+		+	+
72 hrs.								
3rd max.	++	+	+			++	+	+
1st pere.	+++	++	+	+	+	++	+	+
2nd pere.	+	+++	++		++	+	+	+
1st and 2nd pere.	+++	+++	++	+	++			++
5th pere.	died							
all groomers	+++	+++	++	+	+++	+	+++	+++

TABLE 2. Survey of the epibiont types and location on the prawns during field tests during March.

appendages amputated	antennae	branchiostegite	gills	pereiopods	pleopods	eyestalk/rostrum	telson	pleura
3rd maxilliped	*Epistyla* algae debris		*Lagenophrys* *Epistyla* *Vorticella*	*Lagenophrys*	*Lagenophrys*			*Lagenophrys*
1st and 2nd pereiopods	*Epistyla* algae debris	*Epistyla* *Vorticella*	*Lagenophrys* *Vorticella* *Epistyla*	*Lagenophrys*	*Vorticella* *Epistyla* *Lagenophrys* algae debris	*Vorticella* *Epistyla*		*Epistyla* *Vorticella*
5th pereiopods			*Lagenophrys*	*Lagenophrys*	*Epistyla* *Lagenophrys* algae debris		*Epistyla* *Vorticella* algae	*Epistyla* *Lagenophrys* algae debris
All groomers	*Epistyla* algae	*Epistyla* *Vorticella*	*Lagenophrys* *Epistyla* *Vorticella*	*Lagenophrys*	*Vorticella* *Lagenophrys* *Epistyla* algae debris	*Vorticella*	*Epistyla* *Vorticella* algae debris	*Epistyla*

TABLE 3. Results of differential fouling experiment in June to determine the efficiency of the grooming appendages and the extent of fouling per time units as indicated. +=light fouling, ++=medium fouling, +++=heavy fouling.

Appendage amputated	antennae	branchiostegites	gills	pereiopods	pleopods	eye-rostrum	telson	pleura
24 hrs.								
3rd max.	+		+				+	
1st. pere.	+	+						
2nd. pere.	died							
1st and 2nd pere.	+	+	+	+		+		
5th pere.	died							
all groomers	+	+	+		+	+	+	
48 hrs.								
3rd max.	++	+	++					+
1st. pere.	++	+	++		+			+
2nd. pere.	died							
5th pere.					+		++	++
all groomers	++	+	+		+	+	++	++
72 hrs.								
3rd max.	++	+						
1st pere.	+++						+	
2nd pere.	++			+	+	+		
1st and 2nd pere.	+++	++	+			+		+
5th pere.					++		+++	++
all groomers	died							

TABLE 4. Survey of the epibiont types and location on the prawns during field tests during June.

appendages amputated	antennae	branchiostegite	gills	pereiopods	pleopods	eyestalk/rostrum	telson	pleura
3rd maxilliped	*Epistyla* *Synechocystis* *Vorticella* *Gonphonema*		*Epistyla*					
1st and 2nd pereiopods	*Epistyla* *Vorticella* *Synechocystis* debris	*Epistyla*	*Epistyla*	*Epistyla*		*Epistyla*		
5th pereiopods					*Vorticella* *Epistyla* algae debris		*Vorticella* *Epistyla* *Tokaphyra* *Squalophyra*	
All groomers	*Epistyla* *Synechocystis* debris	*Epistyla*	*Epistyla*		*Vorticella* *Epistyla* algae debris	*Vorticella* *Epistyla* *Gonphonema* *Zoothamniam*	*Vorticella* *Epistyla* *Tokaphyra* *Squalophyra* *Acineta*	*Vorticella* *Epistyla* *Zoothamniam*

TABLE 5. Results of differential fouling experiment in October to determine the efficiency of the grooming appendages and the extent of fouling per time units as indicated. +=light fouling, ++=medium fouling, +++=heavy fouling.

Appendage amputated	antennae	branchiostegites	gills	pereiopods	pleopods	eye-rostrum	telson	pleura
24 hrs.								
3rd max.		+						
1st. pere.	+	+						
2nd. pere.	+	+						
1st and 2nd pere.	+							
5th pere.			+	+	+			+
all groomers			+				+	+
48 hrs.								
3rd max.	+							
1st. pere.	+	++	+		+			
2nd. pere.	+	+				+		
1st and 2nd pere.	+	++						
5th pere.					+		+	++
all groomers	+	+	+		+	+	++	++
72 hrs.								
3rd max.	++		+					
1st pere.	++	++	+++	+	+			+
2nd pere.		++	+			+		
1st and 2nd pere.	++	+	+	+	+			
5th pere.				++	++		++	+++
all groomers	++	++	++	+	+++	+	++	+++

TABLE 6. Survey of the epibiont types and location on the prawns during field tests during October.

appendages amputated	antennae	branchiostegites	gills	pereiopods	pleopods	eyestalk/rostrum	telson	pleura
3rd maxilliped	*Vorticella* *Epistyla* algae debris		*Epistyla*					
1st and 2nd pereiopods	*Vorticella* *Epistyla* algae debris	*Epistyla* *Vorticella*	*Epistyla*	*Tokaphyra*	*Epistyla*		*Epistyla* *Tokaphyra* *Squalophyra* *Acineta*	
5th pereiopods					*Epistyla* debris		*Epistyla* *Vorticella* debris	*Epistyla*
All groomers		*Vorticella* *Epistyla* *Vaginicola* *Zoothamnium*	*Epistyla*	*Acineta* *Vorticella*	*Epistyla* *Vorticella* debris algae	*Epistyla*	*Epistyla* *Vorticella* algae	*Epistyla* *Vorticella*

prawn little protection against the settling organisms in the environment. Extensive fouling of the exoskeleton was seen by 72 hr.

A wide variety of epibiont types was noted during March on the infested areas of the experimental prawns (table 2). The most abundant protozoan seen during the March test was the peritrich *Epistyla* sp., which was observed on all portions of the exoskeleton not groomed, but was especially conspicuous on surfaces where the normal fluid flow would pass, i.e., branchiostegites, gills, antennae, and pleopods. *Vorticella* sp. was the next most prominent sessile protozoan aggregating mostly on the gill lamellae, pleopods, and eyestalk/rostrum region. Algae and organic debris were extremely common especially on the antennae and pleopods (pl. 1, figs. 1,2). *Lagenophrys* was found, however, on non-groomed portions of the exo-skeleton as well as on groomed areas and suggestions regarding this apparent enigma will be given below.

The glass settling plates collected the same fauna and debris as the prawns, except for the peritrich *Lagenophrys*, which was never seen on the settling plates. Various rotifers such as *Philodina* and *Testudinella* were observed in abundance on the settling plates, but were rarely seen attached to the prawns themselves.

Seasonal variations were noted among the protozoans and algae fouling experimental prawns and settling plates. Settling during the June investigation occured in similar locations with epibiont fauna similar to that seen in March (tables 3,4). The aesthetasc rows located upon the base of the antennae were fouled during this time of year with the blue-green algae *Synechocystis* sp. and the stalked diatom *Gonphonema* sp. (pl. 1, figs. 3,4). *Lagenophrys* was not recorded in the June field studies. Aggregations on settling plates did not differ from the epibiont infestations on the prawns, except that the plates were more densely fouled with algae and organic debris than those observed in the Spring.

The October experiment exhibited the least amount of epibiont infestations, but expressed a wider variety of epibiont types present (tables 5,6). *Vorticella* was predominant with fewer *Epistyla* than were seen in the March and June studies. The colonial peritrich *Zoothamnium* and the loricate peritrich *Vaginicola* were seen for the first time during the October investigations. During the Fall, Suctoria, including the genera *Tokaphyra* sp., *Squalophyra* sp., and *Acineta* sp., were noticed. These were located mainly upon the telson and pereiopods (pl. 2, fig. 1). Settlement on artificial substrates included various uni-celled algae, sessile protozoans, and rotifers

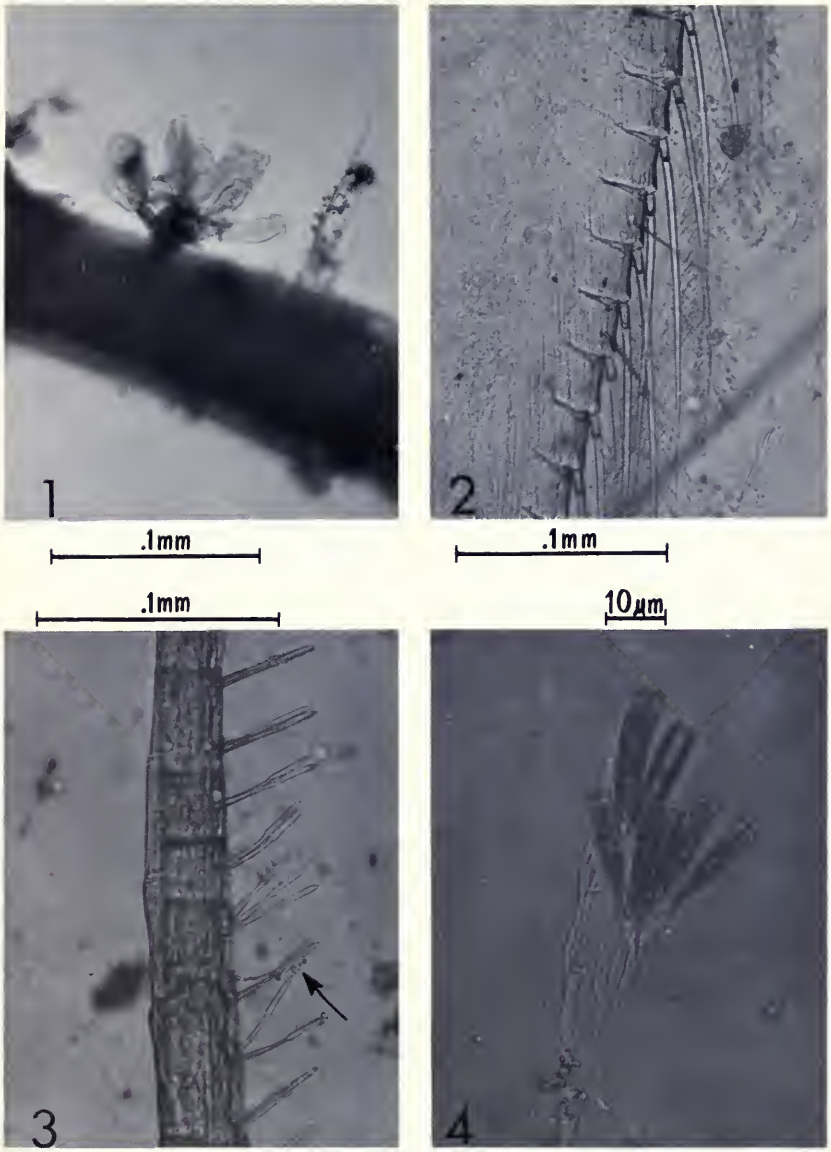


PLATE 1. 1, Fouling of antenna with debris and *Epistyla* sp.; 2, Debris fouling of pleopod; 3, Aesthetascs fouled by *Synechocystis* sp. (arrow); 4, *Gonphonema* sp.

with little differentiation between the plates and infestations on the prawns.

A fourth experiment was designed to test the effectiveness of autogrooming. (Autogrooming is defined as the mutual rubbing of paired appendages for the removal of fouling organisms and debris.) *Palaemonetes kadiakensis* was observed to autogroom frequently during grooming periods (Felgenhauer and Schram, in press). In order to test this system, one of the third maxillipeds was amputated from five prawns. Five control prawns and the amputee prawns were exposed to their natural habitat for 72 hr., with the control prawns free of any signs of third maxilliped fouling, whereas the experimental prawns exhibited heavy infestations on the maxilliped setae of algae and debris (pl. 2, figs. 2,3).

DISCUSSION

The field tests on prawns demonstrated that grooming is a functional adaptation to selective pressures which have brought about the development of elaborate morphological and behavioral changes. Experimental (amputee) prawns exhibited differential fouling by epibionts and debris on areas of the exoskeleton which were prevented from being groomed. Seasonal variations were observed between the extent and types of epibiont fouling on the prawns and the control artificial substrates. The morphology and functioning of the grooming appendages has been described by Felgenhauer and Schram (in press).

Significant differences seen between experimental and control prawns reveals that the morphology of the setal structure is effective in keeping the exoskeleton free of fouling organisms and debris. The grooming appendages are armed with varying combinations of five major types of setation: simple, serrate, multi-denticulate, plumed, and squat-hairs. The serrate and multi-denticulate setae scrape and rasp the surfaces of the exoskeleton, including the crevices of the arthrodia. The squat-hairs are mainly used for cutting and abrading. The plumed and simple setae are not morphologically designed for grooming and are not seen on the cleaning appendages.

The aesthetascs, located at the base of the antennae, become fouled with algae and debris, impairing the circulation of water which would hinder accurate olfaction. Antennular fouling impairs reproductive success as the antennae are used in conjunction with pheromones in finding a mate. Locomotion could be hindered by the

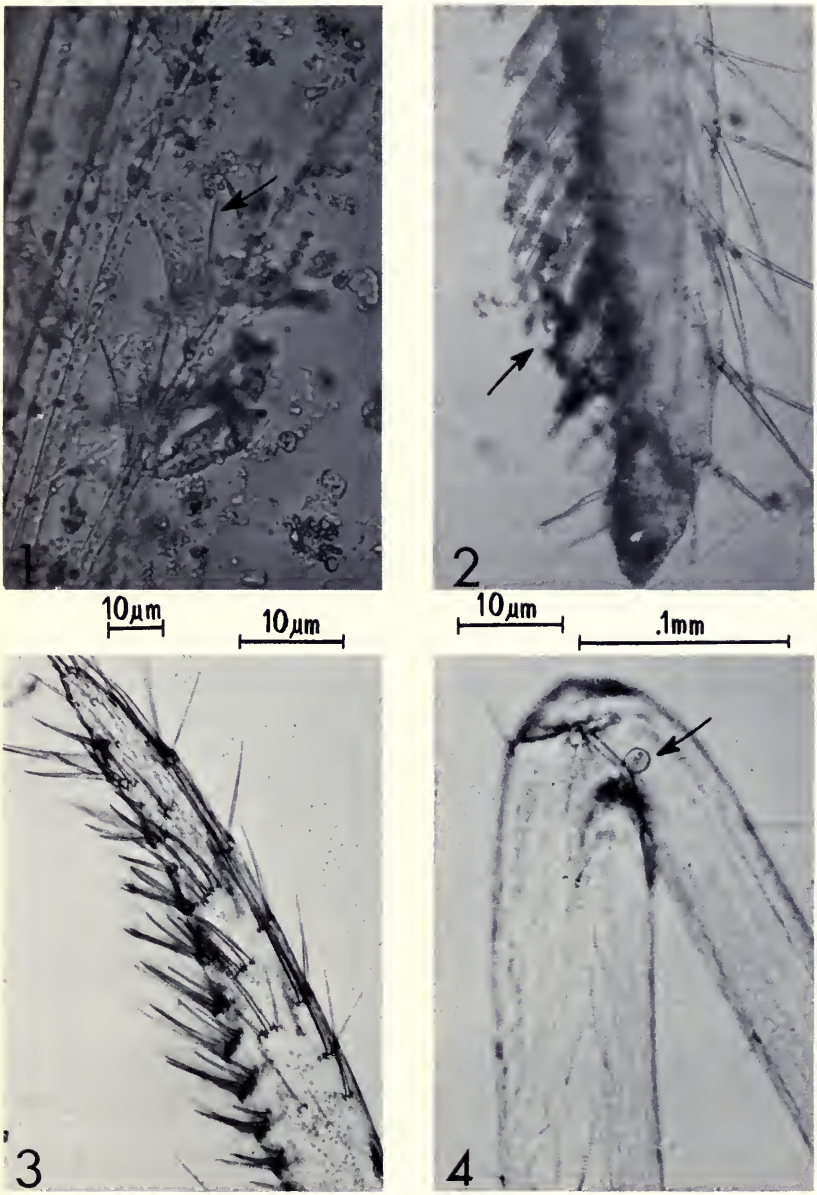
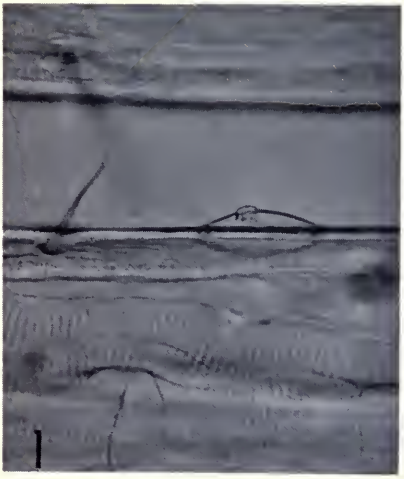


PLATE 2. 1, Fouling by suctorians (arrow) on uropod; 2, Fouling by debris on experimental non-groomed 3rd maxilliped; 3, Control on groomed maxilliped; 4, *Epistyla* sp. fouling in joint of 1st pereiopod.

settling of epibionts between the arthrodia and joints between the pereiopods (pl. 2, fig. 4). Extensive fouling of the exoskeleton creates frictional drag causing difficulties in swimming. The third maxillipeds rapidly become fouled if they are prevented from autogrooming, thereby possibly restricting the location of food sources by inhibiting the chemoreception of the serrate setation. The eyestalks of *P. kadiakensis* are constantly twitching and being preened by the first and second pereiopods so as to avert settling which would impede vision increasing the chance of predation.

The epibionts observed during the course of this study were composed mainly of various types of peritrichous ciliates. During the March investigations the peritrich *Lagenophrys* sp. was recorded. *Lagenophrys* is an epizoic protozoan found in association with crustaceans (predominantly freshwater forms). It has a limited dispersal phase and a high specificity in respect to its host (Kane, 1965). Until this investigation, *Lagenophrys* had not been reported on *Palaemonetes kadiakensis.* Descriptions of the morphology and ecology of this new protozoan-host occurrence has been discussed by Felgenhauer and Ridgeway (1977). *Lagenophrys* was mainly observed attaching to the pereiopods and gill lamellae of the prawn (pl. 3, fig. 1). This peritrich was seen in equal numbers upon experimental prawns and control prawns, suggesting that grooming is not effective in the removal of this ciliate. *Lagenophrys* is dome-like in side view and attaches directly to the exoskeleton. This smooth, spherical nature of the lorica would permit the grooming setation to pass over this peritrich without its removal, whereas the stalked peritrichs would be caught between the dense setation of the pereiopods and removed. Another plausible explanation could involve the mode of attachment seen in *Lagenophrys.* High densities of these ciliates are seen upon their crustacean hosts just after molting. The lorica of *Lagenophrys* causes a conspicuous ridge in the exoskeleton of the host (pl. 3, fig. 2). This ridge seems to be caused by the ciliate secreting the "chiton-like" lorica on the soft exoskeleton immediately after the shrimp molts, and thereby becoming firmly attached to the exoskeleton. No mechanical damage to the gill lamellae was observed by the attachment of *Lagenophrys.* The chitonous membrane surrounding the crustacean gill is much thinner than the cuticle so that respiratory exchange may take place (Burnett and Hessler, 1973). Large numbers of *Lagenophrys* covering the gill lamellae, however, would seem to decrease the functional surface area of the gill and also thicken the



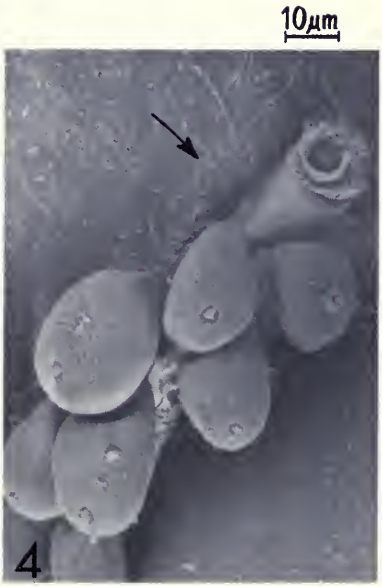


PLATE 3. 1, *Lagenophrys* sp. on first pereiopod; 2, SEM of attachment scar on prawn cuticle by *Lagenophrys* sp.; 3, SEM of bacteria covering exoskeleton; 4, SEM of *Epistyla* showing aggregation of bacteria (arrow).

gill membrane, decreasing its efficiency. The importance of an organic substrate for the attachment of *Lagenophrys* is emphasized by the fact that while it was observed on prawns, at no time was *Lagenophrys* recorded from the glass substrates.

Epistyla sp. and *Vorticella* sp. were observed through the investigations. These stalked ciliates displayed a preference for areas in which the normal fluid-flow of the prawn would pass. Aggregations of these ciliates were observed along the posterior edges of the branchiostegites, gill lamellae, abdominal pleura, and pleopods. Similar orientations of epizoic barnacles has been reported on crabs (Heath, 1976). Barnacles were found to attach in direct line with the flow of the respiratory currents across the carapace of the crab. Selection of epibionts in these areas of the prawn could prove deleterious by blocking the normal flow of water and nutrients.

Palaemonetes kadiakensis inhabits waters that range between 1 to 6 ft. in depth. Sieburth et al. (1976) found that there is a restriction of epizoic peritrichs to nearshore waters and relatively few are found in open water. It is possible, then, that since *Palaemonetes kadiakensis* inhabits shallow areas where peritrichous ciliates cohabit, the selective pressures would bring about the elaborate grooming behavior that is now seen in this prawn.

Many investigations have been conducted on the succession of organisms upon submerged substrates. O'Neil and Wilcox (1973) state the sequence of microorganisms appeared to be a very regular phase of succession, somewhat analagous to succession of land plants. The different phases observed in the formation of what is termed "primary film" are bacteria, diatoms, hydroids, algae, and, finally, higher metazoans. The phases cited refer to the predominant organisms present and do not imply the absence of other microorganisms on the surfaces. Similar results were noticed during this investigation, as very little differentiation was observed between the control glass plates and the settlings on the prawns. Various rotifers were recorded from the plates, but rarely were seen on the prawns. This occurrence is explained by the fact that most rotifers are extremely motile organisms, moving from one anchor site to another. Very few rotifers are totally sessile throughout their entire life cycle as is the case with most peritrichous ciliates. The constant motion of the prawn would deter the temporary attachment of rotifers while also not providing an appreciable food source. The rotifer *Philodina* was, however, observed in the gill chamber of *Palaemonetes kadiakensis,* and this occurrence is probably caused

by this particular rotifer being swept up by the respiratory current and becoming trapped within the branchial chamber.

Scanning electron micrographs revealed a heavy covering of bacteria (pl. 3, fig. 3). It was also noticed that the bacteria seemed to accumulate in those areas in which the peritrichs settled (pl. 3, fig. 4). Peritrichous ciliates and suctorians are known to utilize bacteria as a substantial part of their diet (Sieburth et al., 1976). It is then feasible to postulate that the peritrichs are being attracted to the crustacean exoskeleton by the bacteria as a food source. The bacteria also benefit as the number of bacteria in water adjacent to artificial substances are dramatically lower than the number attached to the artificial substance (O'Neil and Wilcox, 1973). The crustacean exoskeleton would provide a source of nutrients upon which the bacteria could feed and reproduce.

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