CHAPTER 3. COMPARISONS OF FEEDING BEHAVIORS AND MOUTHPART AND FOREGUT MORPHOLOGY OF TWO POPULATIONS OF *GAMMARUS MINUS* (AMPHIPODA)

Carol J. Haley

ABSTRACT

The purpose of this study was to determine whether habitat differences are related to mouthpart morphology or feeding behavior of *Gammarus minus*. In this study, I: 1) compared mouthpart and foregut morphology of two populations of *G. minus* that occupy different stream habitats, 2) compared mouthpart and foregut morphology of sexually immature and mature animals within each of the populations, 3) cataloged feeding behaviors of *G. minus*, and 4) compared feeding behaviors of mature and immature *G. minus*. Morphological variables were compared by correlation and regression analyses. Of 9 mouthpart and foregut structures studied, only 3 (number of cuspidate setae on outer plates of maxillipeds, length of the dactyl on maxilliped palps, and the number of hook setae on the foregut ampullae) were found to be correlated with body length. Of these, rates of increase in maxilliped setae numbers and ampullae hook setae numbers were greater for immature than mature animals, and the number of hook setae for a given sized animal was generally greater for animals at Richards Creek. When animals were presented with ground-up leaf material in the laboratory, twenty-one behaviors, which could be grouped into six broad categories, were observed. The frequency of six behaviors was found to be statistically different in immature and mature animals.

INTRODUCTION

Freshwater amphipods are believed to play a major role in the movement of nutrients and energy in the streams they inhabit (Cummins, 1979). Although freshwater gammarid amphipods are often assumed to act as shredders or collector-gatherers in streams, literature describing feeding behavior and functional morphology of feeding in freshwater amphipods by aquatic ecologists is sparse (exceptions include Willoughby 1983, Blinn and Johnson 1982).

Considerable interest in functional morphology of crustacean mouthparts and foreguts has, however, been generated by systematists who are attempting to determine evolutionary relationships and develop meaningful taxonomic systems (Barnard and Barnard 1983, Felgenhauer and Abele 1985, Felgenhauer, et al.1989). Most of this work has been conducted with marine groups, including marine amphipods. The studies of Caine (1974), Hudon (1983), Barnard et al. (1988), and Coleman (1989, 1991a, 1991b) have provided valuable information on the functional morphology of feeding in a few amphipod groups.

Mouthparts, particularly the mandibles (Fig. 1*e*) and maxillipeds (Figs. 1*b*, 6), figure in the classification of the Amphipoda and in discussions of evolution of the group (Barnard, 1969, Barnard and Barnard 1983, Barnard and Karaman 1991, Watling 1993). Watling (1993) and

Barnard and Barnard (1983) have formed tentative hypotheses regarding evolution of mouthpart differences. However, the limited knowledge of the relationship of differences in mouthpart structure to feeding function hampers the study of evolutionary significance of the differences (Barnard and Karaman 1991, Watling 1993).

Barnard and Karaman (1991) have remarked that few studies of variability of any kind of structure within taxonomic groups of amphipods have been conducted. Furthermore, studies of differences in functional morphology of feeding between populations or between sexually mature and immature animals of the same species are generally lacking.

The amphipod *Gammarus minus* (Say) inhabits hardwater springs, streams, and caves of the montane eastern United States. It occupies a variety of habitats that appear to offer different potential food sources. Additionally, sexually immature and mature *G. minus* occupy different parts of their habitats (Miller and Buikema 1977, Haley, this dissertation, chapter 2). Thus the species provides an excellent opportunity to investigate intraspecies differences in functional morphology.

The main objective of this study was to examine functional morphology of feeding of *G. minus* and determine whether there were intraspecific differences that could be related to differences in potential food sources. I hypothesized that *G. minus* found in different habitats or microhabits used different feeding strategies. I also hypothesized that feeding behavior and mouthpart morphology would provide evidence for the nature of these different strategies. For example, I hypothesized that animals living among gravels might scrape materials off the gravels. Therefore, structures that have the appearance of being used for gouging or scrubbing tools (e.g., cuspidate setae on the maxillipeds, mandibular incisors) were investigated. If scraping is a more important strategy for immature amphipods, those scraping structures should develop more rapidly in young animals than in older ones.

The specific objectives of the study were to:1) compare mouthpart and foregut morphology of two populations that occupy different stream habitats, 2) compare mouthpart and foregut morphology of sexually immature and mature animals within each of the populations, 3) catalogue feeding behaviors, and 4) compare feeding behaviors of mature and immature *G. minus*.

MATERIALS AND METHODS

Description of Habitats.— *Gammarus minus* specimens used for both the morphology and behavior studies were collected from two streams in Montgomery County, Virginia. The habitats are described in chapter 2 of this dissertation.

Collection Techniques. — For scanning electron microscopy studies, animals from both populations were relaxed at the time of collection by dousing them with seltzer water. Specimens were stored in buffered four percent formalin until they were prepared for scanning electron microscopy.

For behavior studies, animals from both streams were collected by placing substrate containing the animals into a Thermos bottle containing water taken from the stream where the amphipods were collected.

Light Microscopy.— The choice of fine mouthpart structures for further study was based on comparisons of ecological data (chapter 2) and behavioral observations (described in this chapter) and details of fine structure of mouthparts obtained with the scanning electron microscope. The following were studied: 1) length of dactyl on maxilliped palps, 2) number of cuspidate setae on inner plate of maxilliped, 3) number of cuspidate setae on outer plate of maxilliped, 4) number of teeth on the palp of the first maxillae, 5) and 6) number of teeth on the incisors of the right and left mandibles, 7) surface area of molars, 8) number of hook setae on ampullae of foreguts, and 9) number of "extra" setae on ampullae.

For light microscopy, mouthparts were removed using techniques described by Barnard (1969). Approximately 50 animals from each site were examined. Mouthparts were placed in glycerin on glass slides under coverslips.

Foreguts were dissected out of 42 specimens. Heads were gently pulled away from the body of the animals with forceps. The foregut and midgut remained attached to the head. To soften muscle attached to the foregut, the head was soaked in a solution of 10% potassium hydroxide overnight. The foreguts were then separated from the surrounding tissue and placed on a drop of glycerin on a microscope slide. Minuten pins were used to tease apart foreguts so that setae on ampullae could be observed.

Specimens were observed with a compound microscope. Mouthparts were also manipulated to gain further insight into possible functions of the mouthparts.

Scanning Electron Microscopy.— For electron microscopy, approximately 25 whole specimens were twice washed with agitation in chilled 1% sodium acetate solution, first for 5 minutes, then for 15 minutes. Some specimens were post-fixed in osmium tetroxide. Richards Creek specimens were washed a third time for 15 minutes to remove the sediment that adhered to their bodies. The specimens were then dehydrated, at room temperature, in a graded ethanol series (30, 50, 70, 85, 95, 100, 100%). Mouthparts were dissected in 70% ethanol, then were dehydrated through the rest of the series. Mouthparts were mounted on stubs with two-sided adhesive, critical-point dried in CO_2, and sputter coated with gold in preparation for examination with a JEOL JSM-35C scanning electron microscope.

Behavior. — Animals used in the behavior study were kept in the laboratory for at least two weeks prior to the study. Until 24 hours before the observations, animals from each site were kept in a culture dish containing water from the site (dechlorinated tap water was used to replenish whatever evaporated), leaves that had been collected at the Bradley Run site, and a few pebbles. The containers were kept in a growth chamber at approximately 9°C and under a photoperiod approximating that in the field at the time of the study. Twenty-four hours prior to the experiment, animals to be tested were placed in plain, cool dechlorinated water in small plastic containers which were held in the same growth chamber as the other animals.

Preliminary observations had shown that the animals were most active when given either water cress roots or ground-up leaves (rather than 1 cm squares of leaves or small pebbles). Leaves used for the experiment had been taken from the Bradley Run site and frozen. On each sampling day, before the observations were begun, the leaves were ground up with cold deionized water in a Waring blender and filtered through a 212 micron mesh sieve. The filtrate was allowed to settle, the water was decanted, and the filtrate was resuspended in cold deionized water. The suspension was kept in the growth chamber until it was used.

Thirty-two animals, ranging in size from 3 mm to 12 mm, were observed. Sixteen immature and sixteen mature amphipods were tested. Observations were made approximately between 6 and 8 pm each day (the chamber was still lit during this time). Each animal was placed in a chamber (Fig. 2) made from four plastic cover slips and a glass microscope slide held together with clear silicon caulk. Just before the animal was placed in the chamber, cool water from the holding container was added to the chamber. A small portion of the ground-up leaf suspension was added to the chamber with a Pasteur pipette, and the animal was observed with a dissecting microscope for ten minutes. Illumination was provided by a fiberoptic light source to avoid heating the water. At the end of the ten minute session, the animal's straight length was measured from the base of the antennae to the base of the telson. This measurement had an accuracy of about 0.5 mm. No attempt was made to provide a current in the chamber because field studies indicated that even though the animals live in fast moving streams, they occupy microhabitats that have very low velocities.

Behavior observations were recorded on a tape recorder during the ten minute session. The tapes were later transcribed, and a catalog of behaviors was made. A tally was made of the number of ten second intervals in which each behavior was observed to occur. For behaviors that could occur more than once during the ten second interval, such as pulling the antennae through the gnathopods, the behavior was counted as having occurred once. Frequencies of behaviors occurring in animals $\leq$ 5 mm in length were compared with those greater than 5 mm in length by means of one-way ANOVA. Previous studies (Kostalos 1979, Miller 1977, Haley this dissertation, chapter 2) have demonstrated that *G. minus* become sexually mature in the 5-6 mm size class (measured using the "straightened out" method). In the behavior study, by chance, no animals in the 6 mm size class were used and only one animal in the 5 mm size class was used. Therefore animals $< \underline{5}$ mm long were considered to be immature for the purposes of this study.

An experiment was conducted to determine whether food collected with the antennae actually reached the gut. Five animals that had been starved for forty-eight hours or more were placed in the chamber described above. A piece of nylon netting, with small enough openings to prevent the animal from reaching the bottom of the chamber, was placed in the chamber, as shown in Figure 2. Ground-up leaves were presented as above and were added repeatedly in order to maintain a suspension in the water column. After an animal stopped showing interest in the food, it was killed, its foregut was removed and mounted in glycerin on a glass slide, and the gut contents were observed under a compound microscope. Photographs were taken of both the ground up particles in suspension and the guts of the animals.

Statistics. — Regression and correlation analyses were used to study the relationship of the mouthpart variables and "straight" body length. Correlation analysis is appropriate for relationships between variables that are not dependent (Zar 1996), such as amphipod body length and the length of mouthpart dactyls. However, if body length is used as an indicator of amphipod age, the relationships could be considered dependent and therefore regression analyses would be appropriate. Regression lines were computed by means of Jandel Sigma Plot for Windows Version 2.0, and correlations were conducted using Microsoft Excel for Macintosh, version 6. ANCOVA (SAS) was used to determine whether there were differences between the populations.

Analysis of behavior was conducted by comparing frequencies of individual behaviors in the two groups with two-way ANOVA and all behaviors in the two groups with multivariate analysis of variance (SAS).

RESULTS

Scanning Electron Microscopy

Mouthparts of *G. minus* were previously described and drawn in Shoemaker (1940) (Fig. 1). Scanning electron microscopy adds detail to fine structure and arrangement of the mouthparts. The nomenclature used for setae is taken from Watling (1989), Thomas (1970), and Fish (1972). Figures 1 and 3 show the arrangement of mouthparts in side views. The arrangement of mouthparts on the head of *G. minus* is shown in Figures 4 and 5.

Maxillipeds (Figs. 4, 6, 7, 8)-- The setae are essentially as described by Shoemaker (1940). His terminology and current setal terminology differ. His "plumose spines" are now called pappose setae, and his "spine teeth" are now called cuspidate setae. He did not draw, but did describe, the row of pappose setae that are behind the three cuspidate setae on the apical margin of the inner plate (Fig. 7). The setae along the inner margin of the plate are longer, more slender, and plumose, as described by Shoemaker. The distal knob (containing the dactyl) of the last article of the maxilliped is densely packed with fine setae visible at only high magnification (1200X). The distal knob of the penultimate article also contains a pad of densely packed short setae.

Maxillae 2. (Fig. 5, 9) The inner plates of the second maxillae are located just behind the inner lobes of the maxillipeds, thus providing a setose entryway to the remainder of the mouth area.

Maxillae 1 (Fig. 10, 11) -- This structure is as described by Shoemaker. Note the scooplike appearance of the palp.

Upper lip -- The underside of the upper lip is covered with densely packed, fine setae. The upper lip is directly dorsal to the incisors of the mandible.

Mandible (Figs. 12, 13, and 14)-- Molar shape appears to vary, but no attempt was made to determine if this was correlated with size, sex, or population.

Light Microscopy

Foreguts. -- Foreguts of the *G. minus* observed in this study were much like those described by Kanneworff and Nicolaisen (1969). Excellent diagrams of typical amphipod foreguts and of the flow of materials through the amphipod digestive system can be found in Coleman (1991) Kanneworff and Nicolaisen (1969), and Martin (1964). A simplified diagram (Fig. 15) of the movement of food through the digestive system is provided in this paper.

Table 1 lists the mouthpart and foregut structures that were studied and indicates whether there was a correlation between body length of animals and number or size of the structures. Only three of the structures observed were found to be correlated with body size: a) number of cuspidate setae of the outer plates of maxillipeds, b) length of the dactyl on maxilliped palps, and c) the number of hook setae on foregut ampullae.

Relationships between body length and maxilliped palp dactyl length, number of cuspidate setae on maxilliped outer plates, and number of ampullae setae on foreguts are shown in figures 17-22.

ANCOVA demonstrated a statistically significant difference between the regressions of number of ampullae setae/body length of the two populations ($p = 0.0001$).

Behavior

Most animals, both sexually mature ($\geq$5 mm in length) and immature (< 5 mm in length), demonstrated the same set of 21 behaviors (Table 2), in the same basic sequence, when presented with food. These 21 behaviors could be lumped into six broad categories (Table 2), including the category of “eating”, the most frequently observed type of behavior observed in both groups of animals. However, there were differences, some statistically significant, between large and small animals, in the frequencies of six behaviors (Table 3).

Most of the 32 animals (16 immature, 16 mature) demonstrated a pattern of response to the addition of food to the chamber. They swam to the bottom of the container, gathered and ate whatever leaf particles floated by them, swam around the container and gathered leaf particles, and ate them. This pattern was repeated, until, eventually, the animals searched the floor of the container with their antennae, discarded a good deal of what they found, and finally, spent most of the remaining time swimming around the container.

The six categories of behaviors were searching, gathering food, pulling appendages through gnathopods, eating, handling fecal pellet, and swimming/walking.

Searching behavior patterns took two forms, best described as “passive” and “active”. Both forms of behavior were performed quickly and repeatedly. In passive searching behavior, the animals stayed in place, and, as particles were carried past the head by the current created by the beating of the pleopods, the antennae caught the particles and moved them to the mouth area, where the animals processed them. Rather than using the word "chewing" to describe what the animals did with food held near the mouthparts, I use the word "processing" because "chewing" implies grinding. Even if the molars do grind food, activity other than grinding occurs at the mouthparts. Animals often discarded particles by allowing the current created by the beating of the pleopods them to carry the particles away.

In active searching, the animals swam around the container, and stirred up particles that had settled on the bottom of the container with their antennae and gnathopods and by moving their bodies in jerky, vigorous movements,. As the particles fell, the animals gathered in some of the particles with their antennae or gnathopods, and handled the particles as in passive searching.

The current which flows over the ventral surface of the body, either from posterior to anterior or anterior to posterior, can be created while the animals are on their backs, sides, or, for short periods of time, with their dorsal surfaces up.

Active and passive searching behaviors could also be combined. After stirring up the particles, an animal might remain in one place and allow the current to carry particles to its head area. If the animal kept the particles and processed them without discarding them, the activity was considered to be a gathering activity, rather than a searching activity.

Gathering and eating behaviors were frequently observed (Table 2). Using either their antennae or gnathopods, amphipods gathered leaf particles from the bottom of the container or from the water column. Amphipods could also move food from the posterior end of the body to the mouth by passing it along from appendage to appendage. An animal usually held the food to its mouth with the gnathopods. Processing (the most frequent eating behavior) continued over several ten-second periods, unlike the processing seen in searching behaviors, which was of a much shorter duration.

Particles that floated away from the mouth or were carried away in the current were brushed back by the antennae. As the animal was eating, particles swept near the antennae were sometimes caught on the antennae. Also, when an animal remained stationary it often held its four antennae in a spread-out configuration to capture suspended particles. Periodically, the animal pulled its antennae between the dactyls and palms of the gnathopods. Less frequently, other appendages, including pereopods, pleopods, and uropods were also pulled through the gnathopods to remove particles from their surfaces.

After the antennae or other appendages were drawn over the gnathopods, the gnathopods were held up to the maxillipeds, which responded by moving vigorously. Antennae sometimes made direct contact with the maxillipeds and mandibular palps. Although the palps are normally held in the space between the bases of the second antennae, they can dip down to contact the maxillipeds and, by so doing, appear to aid in removing particles from the antennae.

DISCUSSION

Previous work (Miller and Buikema 1977, Haley this dissertation chapter 2) had shown that in two populations of *G. minus*, immature and mature animals occupied different components of the habitat. In the Richards Creek population, smaller animals tended to occupy areas containing bare gravel; larger animals were more likely to be found in areas containing aquatic vegetation.

In the Bradley Run population, smaller animals tended to be found in wood and bark detritus; larger animals are found among whole or nearly whole fallen leaves (Haley, this dissertation chapter 2). In the Bradley Run population, smaller animals were not found among leaf detritus during certain times of the year.

Presumably, *G. minus* made use of the different foodstuffs available to them in the microhabitats where they are found. By grinding up materials present in the sites studied (leaves, moss, and watercress) and comparing them with contents of foreguts of *G. minus*, I was able to determine that the animals ingested these foodstuffs. Guts also contained a great deal of unidentifiable detritus and fungal parts.

Because immature amphipods were found among potential food sources that were different from those of mature animals, I hypothesized that smaller animals used different feeding strategies than large animals. I also hypothesized that behavior or mouthpart morphology would provide evidence for the nature of the differences in strategy. At Richards Creek, the immature animals may be more likely to remove particles from the water column, to collect detritus from the interstices in the gravel, or to scrape biofilm from the gravels they inhabit. Larger animals, which tend to congregate in the watercress, may graze on watercress in addition to making use of other food sources. At Bradley Run, immature *G. minus* may ingest particles gathered from the water column, leaf detritus that has been reduced to small size, or microbes (particularly fungi) from wood and bark, and mature animals may favor whole or nearly whole leaf detritus. The first step of the study was to test the hypothesis that feeding behaviors in immature and mature *G. minus* were different.

Comparison of Behavior in Immature and Mature G. minus. —. There were no differences in the types of behaviors exhibited by immature and mature *G. minus*. Only frequencies of behaviors differed (Table 3). This is consistent with the similarities in structure usually observed in immature and mature amphipods.

Differences in the frequencies of the feeding behaviors of immature and mature animals suggest that the two groups had different feeding requirements. Mature animals were more likely to gather food with their gnathopods and pull their antennae through their gnathopods. Immature animals were more likely than adults to spend time searching, discarding particles, rolling the posterior parts of the body toward the mouth, removing fecal pellets from the body (perhaps as a means of testing the quality of food ingested), and, possibly, processing food at their mouthparts.

One explanation for the differences in frequencies is that ground whole leaves were not a type a food acceptable to the the younger animals or that the offered food was of an inappropriate size. Young amphipods at Bradley Run are not present when leaves have recently fallen and therefore may be adapted to making use of some other food source, perhaps biofilms on wood and bark (Haley, this dissertation, chapter 2). Richards Creek young typically are found among gravels and do not have access to fallen leaves.

Comparison of Behavior of G. minus with Behavior of other Amphipods. —.Detailed observations reported in the limited literature of amphipod feeding behaviors allow comparison of behaviors among different species of amphipods. One of the most striking aspects of this comparison is the similarity among amphipods, even among those in different suborders. While individual behaviors may not be carried out in exactly the same ways or by the same structures, there appears to be a small suite of behaviors common to amphipods that use detritus or material filtered from the water column as food sources. Standardization of amphipod behaviors and development of behavior catalogs for other species will permit more thorough analyses of similarities and differences (Li and Gregory 1989). These analyses, coupled with information regarding functional morphology of feeding structures, should provide insight into phylogeny and evolution of amphipods.

An example of the similarity in feeding behaviors between amphipod species is recorded in the work of Hudon (1983). The marine amphipod *G. oceanicus* resuspended detritus from sediment in the same manner (“strong body flexions”) as *G. minus* and, like *G. minus*, used currents caused by the rapid beating of pleopods to carry particles toward the mouth area, where "periodic cleaning and tearing movements take place" (Hudon 1983). In a similar manner, *Calliopus laeviusculus*, another marine amphipod, used the current caused by the beating of the pleopods to resuspend particles and carry them to the mouth (Hudon 1983).

Also common to *G. minus* and other amphipods are feeding behaviors that involve the removal of particles from body parts and the transfer of particles to the mouthparts. These are behaviors that may also be classified as grooming behaviors. Living among small, loose detritus requires amphipods to remove from their body parts particulate matter that may hinder functions such as respiration. Although amphipods are thought to groom themselves to remove particles from their bodies, they also purposely suspend particles, apparently in order to catch them. This suggests that both the behaviors associated with this resuspension and the “grooming” behavior that occurs immediately afterward should be considered feeding behaviors. Caine (1974) suggested that the grooming and feeding are closely linked in amphipods and that filter-feeding is a modification of antennae cleaning.

The feeding/grooming behavior observed in *G. minus* is similar in some aspects to that seen in *Paraceradocus,* a detritus-feeding, burrowing amphipod found near the Antarctic Peninsula (Coleman 1989). Like *G. minus*, *Paraceradocus* cleaned particles from the antennae, uropods and pereopods with the gnathopods and cleaned pereopods less frequently than the antennae and only for a short time. As in *G. minus,* particles removed from the body are transferred to the maxillipeds.

The behavior of *Paraceradocus* and *G. minus* differed in subtle ways: the first antennae are cleaned more frequently in *Paraceradocus*, whereas in G. *minus*, the second antennae are more frequently cleaned. Also, in *Paraceradocus* the antennae were pulled between the medial surfaces of the 5th segment (the "wrist") of the gnathopod, whereas *G. minus* pulls the antennae between the dactyl and the propod. *Paraceradocus* uses a dense brush of setae on the medial surface of the carpi of the gnathopod to clean particles from the antennae. These differences would be of use in phylogenetic studies.

Suggestions for additional studies.— One must take care in basing generalizations concerning feeding behavior on studies conducted in the laboratory in small chambers. In order to determine whether the behaviors observed in this study are those used by the animal in its habitat, it will be necessary extend the observations to animals in large tanks or in the field.

Further work also needs to be conducted to determine the food preferences of *G. minus*. Determining whether biofilms on structures such as wood, bark and gravels are a food choice for immatures would provide particularly useful information.

Mouthpart and foregut morphology. —. The results of the behavior study provided evidence that immature and mature *G. minus* have different approaches to feeding. Earlier studies of the spatial and temporal distribution of immatures and matures in the habitats of the two study sites indicated that the two groups ate different foods.

It appears that mature *G. minus* might cut leaves and watercress and other large plant material, collect particles in the sediment, or remove particles from the water column, perhaps after resuspending the sediment. Immature animals appear more likely to scrape biofilm from gravels, wood, or bark, collect particles in the sediment, or remove particles from the water column.

A useful approach to determining the relationship of the structure of mouthparts and other appendages to feeding strategy is to think of the behaviors and structures as tools that have evolved to solve problems encountered in feeding (Arens 1989). The problems *G. minus* may have to solve include: 1) breaking up loose aggregates (clumps) of particulate detritus, 2) reducing the size of particles in order to be able to move then through the mouthparts, esophagus, and foregut, 3) scraping food from surfaces such as wood, gravels, or bark, 4) cutting pieces off very large (relative to their body size) leaves or aquatic plants, 5) capturing particles of detritus that are suspended in the water column, 6) transporting food to the mouth without losing food to the current, and 7) not losing food while it is being moved from the outer mouthparts to the inner ones.

ReducingTools: Maxilliped dactyls and mandibular molars. I assumed that immature animals would be more likely than mature animals to have to reduce the size of detritus particles or aggregates and to remove biofilm from other surfaces. Hypotheses were established to study the possible relationship of mouthpart structures to feeding. I have observed *G. minus* using the maxilliped dactyls to break up loose aggregates of particles that are held in the gnathopods.

Therefore, one hypothesis was that the dactyls of maxilliped palps would be relatively more developed in immature than in mature animals. Another hypothesis related to reducing the size of particulate matter was that molar surface area would be relatively greater in immatures than in matures.

Maxilliped dactyl length increased at the same rate throughout the life of the animals (Figs. 19 and 20). Perhaps a picking tool is needed at all stages of the life of *G. minus* or length may not be the best indicator of development of the dactyl. No major differences were observed in rate between the two populations.

Although molar areas also appeared to be correlated with body length, the error introduced by the inability to quantify the 3-dimensional structure of the molar surface was considered to be too great for meaningful interpretation at this time. However, this relationship should be examined further, perhaps using confocal scanning electron microscopy to quantify the 3-dimensional structure, when the methodology becomes more readily available.

Scraping tools. If immatures were more likely to scrape biofilms from objects, mouthpart structures such as cuspidate setae on the inner and outer plates of the maxilliped or the number of teeth on the palp of the right maxillae 1 might be relatively greater in number in immatures than in mature animals.

Setae on maxillipeds and maxillae 1 may be involved in scraping activities of other amphipods. Setae on the inner and outer plates of the maxillipeds were considered to be important structures in scraping habits of caprellids (Caine 1974). In some caprellid amphipods (Caine 1974), the palp and endite (there is only one plate) of maxillae 1 and the incisor are used to scrape organisms from objects. Maxillary setae, such as those found on the inner plate of maxillae 1 in the amphipod *Anchiphimedia dorsalis,* are used to scrape food from sand grains or other structures (Coleman 1991b).

The absence or reduction of these setae in amphipods whose habit does not include scraping adds further support for the hypothesis that setae on the maxillae and maxillipeds can be used for scraping. For example, in *Anonyx*, a scavenger amphipod that is adapted to cutting tissue, the number of setae on the outer plate of the maxilliped and the inner plate of maxillae 1 have been reduced (Steele and Steele 1993).

The results of my study support the possibility of the use of cuspidate setae on maxillipeds as scraping tools in *G. minus*. When animals are feeding, the flexible maxillipeds are very active. Just as aggregates can be held to the maxillipeds by gnathopods and antennae, so could small objects, such as gravels. Maxillipeds contain many plumose and pappose setae, structures that may capture particles that fall from other parts of the mouth.

Examination of the arrangement of the cuspidate setae on the outer plate of the maxillipeds and manipulation of the maxillipeds. indicated that while an object is held to the mouth area, a movement of the right and left maxilliped toward one another would permit the cuspidate setae to scrape off materials in *G. minus*. Another movement, the extension of the maxillipeds along a flat surface and then pushing along the surface and then closing the maxillipeds, would also permit scraping. (See Fig. 16 for the possible roles of mouthparts in feeding.)

In this study, the rate of increase in numbers of cuspidate setae on the outer plate of the maxilliped appears to decline as the animals get larger (Figs. 17 and 18). This may indicate the greater importance of scraping as a feeding strategy in smaller animals. (In Bradley Run animals, there may be an increase in rate as the animals get beyond 10 mm.)

Cutting tools: Incisors. Newly fallen leaves at the Bradley Run site and watercress plants at the Richards Creek site were very large relative to the size of the amphipods. It is generally assumed that incisors of the mandibles are used to cut materials. In order to do that, the incisors must be sturdy and sharp enough to cut through the material. The incisors must also be positioned in such a way that they can easily access the material. The incisors of *G. minus* are blunt structures, and, in death, are held well within the field of the mouthparts (Fig. 4). They appear to be protected under the upper lip and behind the palps of the first maxillae. It may be that only small items brought into the protected area are cut by the incisors or that the incisors are used to shear layers off the surface of the leaf or plant rather than taking “bites” all the way through the leaf. The advantage to the location of the incisors is that any pieces that fall off are readily captured by the upper lip, the palps of the first maxillae, and the apical setae of the inner plates of the maxillipeds.

Watling (1993) suggested that in families of amphipods that must cut tissue from dead animals or are predators, the mandible has become elongated and the orientation of the incisor has moved so that it is in the oblique or vertical, rather than a more horizontal, plane of the animal body of the animal. A longer structure provides more leverage. Another adaptation for cutting in amphipod incisors is seen in the lyssianassoid amphipod *Anonyx* where the incisors are widened and toothless and are precisely guided together while cutting. They are thus capable of shearing the tissues of the dead animals which are their food (Steele and Steele 1993). *Gammarus minus* demonstrate no tendency toward a highly evolved cutting structure.

Capturing tools. Collecting particles from the sediment or the water column by *G. minus* is achieved through the use of the antennae and other appendages, as demonstrated in the behavior studies. The greatest problem faced in feeding, however, is one associated with feeding in running water: ensuring that the food particles that have been dislodged from the environment or reduced in size are kept from floating away or being swept away by the currents.

Fields of hairs (setae) capture the particles, and mouthpart structures that Arens (1989) categorizes as brooms and combs clean the setae. *Gammarus minus* mouthparts and gnathopods possess a variety of structures that appear to be capable of trapping particles. The position of the hair (setal) pad on the penultimate article of the maxilliped palps and on the upper lip probably enable them to trap particles that are loosened by the dactyls and incisors (Figs. 3 and 9). The orientation of the setae on the palps is suitable for capturing particles that released by the dactyls, and setae on the upper lip would keep particles in the vicinity of the mouth. Numerous setae on the gnathopods and antennae of *G. minus* allow the capture of particles that are swept away from the mouth area.

The solutions to the problem of keeping food in place often have the appearance of adaptations to filtering. Marine amphipods that filter-feed use the antennae for this purpose and wipe the antennae with their gnathopods (Barnes 1980) in ways similar to those of *G. minus*.

Maxillae 2 are also important in filtration. For example, a comparison of the size of maxillae 1 and 2 in two species of *Hyalella* indicated that an increase in size of the maxillae and in the area of maxillae covered by filtering setae allows one species to filter plankton (Wagner and Blinn 1987).

Particles trapped on setae are removed by comblike structures on maxillae 1 (Wagner and Blinn 1987). The outer plates of maxillae 1 in *G. minus* contain structures that Arens (1989) would describe as rakes (these look like combs) (Fig. 11). They are in a position to remove food particles from plentiful setae (Figures 4 and 5) of maxillae 2. The rakes of *G. minus* may also remove food from the setose parts of the maxillipeds and appear to be in a position to clean the fine setae of the upper lip.

Because of the nature of feeding in an aquatic environment, particularly at the interface of a relatively loose bottom substrate and the water column, where particles tend to be suspended by a number of forces, the blurring of gathering and filtering behaviors is to be expected. *Gammarus minus* obviously spends a considerable amount of energy during feeding holding particles in place, so it is not surprising that it has developed adaptations such as plentiful setae, setal pads, and body positions to make feeding more effective.

Foregut morphology. The foregut of *G. minus* is similar to that seen in other amphipods (Coleman, 1991, Kanneworff and Nicolaisen). Recently, Sampson (personal communication, Maine 1995) studied the relationship of foregut structures to food sources of different marine amphipod species. He discovered that species that ate loose particulate matter tended to have more rows of ampullae setae. In my study, a second row of ampullae setae, containing one or two setae, was observed in both populations, but no differences were noted between the populations.

Analysis of covariance indicates a trend toward a greater number of ampullae hook setae for a given body length in Richards Creek animals compared to Bradley Run animals (Figs. 21 and 22). The correlation is also stronger in the Richards Creek animals (Figs. 21 and 22). The Richards Creek animals do not have access to large leaf particles. This greater number of setae appears to indicate a greater density of setae, suggesting that more closely spaced setae ensure the more efficient transfer of what are probably smaller or softer particles to to the cardiac region of the foregut. The rate of increase in number also decreases with body length, perhaps reflecting the smaller particle size entering the guts of smaller animals.

A proposed scheme for the movement of food through *G. minus* mouthparts based on the results of this study and others is provided in Figure 16. It is offered to serve as a starting point for further studies of the functional morphology of amphipod feeding. This is the first time behaviors of *G. minus* have been catalogued. Comparing these observations of amphipod behaviors with those of other investigators has been difficult because amphipod behaviors have not yet been standardized. As Li and Gregory (1989) have explained, if behavioral data are to be used in ecological, systematics, or ecotoxicological studies, it will be necessary to identify and describe as many specific behaviors as possible so that they may be compared.

McShaffrey and McCafferty (1988) developed a scheme for delineating functional feeding groups for invertebrates that feed on particles ("relatively small materials"). They propose that the material be viewed in terms of a continuum "ranging from material suspended in the water, to material bound or growing attached to the substrate." Origin of the material is not considered. Feeding strategies are assigned on the basis of adaptations to the parts of the continuum. A species may fit into different groups in different places and times, and certain adaptations may be used in more than one way.

According to this scheme and based on the evidence gathered in the current study, *G. minus* may act as deposit filterers (filterers that resuspend deposits to filter them), scrapers, shredders, and gatherers (organisms that obtain loose or lightly attached material with structures other than setae). Thus, organisms use more than one mode of feeding by varying behavior and using the same structures to carry out different feeding strategies (e.g., *G. minus* uses antennae to gather loose food from the bottom of a container and to filter food out of the water column). For example, it explains how *G. minus* can successfully occupy habitats containing a wide array of possible food types. This concept has, in addition to advantages mentioned by the authors, the advantage of determining the functional feeding groups by considering the habitat of the organism as well as its structure, because many of these organisms have feeding structures that are adapted to more than one feeding strategy.

LIST OF REFERENCES

Arens, W. 1989. Comparative functional morphology of the mouthparts of stream animals feeding on epitlithic algae. Arch. Hydrobiol. Suppl. 83: 253-354.

Barnard, J. L. 1969. The families and genera of marine gammaridean amphipoda. United States National Museum Bulletin 271. 535 pp.

Barnard, J.L. and C.M. Barnard. 1983. Freshwater amphipoda of the world. I. evolutionary patterns. Hayfield Associates, Mt. Vernon, VA.

Barnard, J.L. and G.S. Karaman. 1991. The families and genera of marine gammaridean Amphipoda (except marine gammaroids). Records of the Australian Museum Supplement 13.

Barnard, J.L., J.D. Thomas, and K.B. Sandved. 1988. Behavior of gammaridean amphipoda: *Corophium, Grandidierella*, *Podocerus,* and *Gibberosus* (American *Megaluropus*) in Florida. Crustacean Suppl. 13: 234-244.

Blinn, D.W. and D.B.Johnson. 1982. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. Freshwat. Invertebr. Biol. 1(2): 48-52.

Caine, E. A. 1974. Comparative functional morphology of feeding in three species of caprellids (Crustacea, Amphipoda) from the northwestern Florida Gulf Coast. J. Exp. Mar. Biol. Ecol. 15: 81-96.

Coleman, Ch. O. 1989. Burrowing, grooming, and feeding behavior of *Paraceradocus*, an Antarctic amphipod genus (Crustacea). Polar Biology 10: 43-48.

Coleman, Ch. O. 1991a. Comparative fore-gut morphology of Antarctic amphipoda (Crustacea) adapted to different food sources. Hydrobiologia 223: 1-9.

Coleman, Ch. O. 1991b. Redescription of *Anchiphimedia dorsalis* (Crustacea, Amphipoda, Iphimediidae) from the Antarctic, and functional morphology of mouthparts. Zoologica Scripta 20: 367-374.

Cummins, Kenneth W. and Michael J. Klug. 1979. Feeding ecology of stream invertebrates. Ann. Rev. Ecol. Syst. 10:147-172.

Felgenhauer, B.E. and L.G. Abele. 1985. Feeding structures of two atyid shrimps, with comments on caridean phylogeny. Journal of Crustacean Biology 5(3): 397-419.

Felgenhauer, B.E., L. Watling, and A. B. Thistle, eds. 1989. Functional morphology of feeding and grooming in Crustacea. A.A. Balkema. Rotterdam.

Fish, S. 1972. The setae of *Eurydice pulchra* (Crustacea: Isopoda). J. Zool., London. 166: 163-177.

Hudon, C. 1983. Selection of unicellular algae by the littoral amphipods *Gammarus oceanicus* and *Calliopius laeviusculus* (Crustacea). Marine Biology 78: 59-67.

Kanneworff, E. and W. Nicolaisen. 1969. The stomach (foregut) of the amphipod *Bathyporeia sarsi* Watkin. Ophelia 6: 211-229.

Kostalos, Mary S. 1979. Life history and ecology of *G. minus* Say (Amphipoda, Gammaridae). Crustaceana 37: 113-122.

Martin, A.L. 1964. The alimentary canal of *Marinogammarus obtusatus* (Crustacea, Amphipoda). Proc. Zool. Soc. Lond. 143: 525-544.

Miller, J.D. 1977. Factors influencing the abundance and life history of the spring form of *Gammarus minus* Say (Amphipoda, Gammaridae). Unpublished Master thesis. Virginia Polytechnic Institute and State University. Blacksburg, VA.

Miller, J. D. and A. L. Buikema, Jr. 1977. The effect of substrate on the distribution of the spring form (form III) of *Gammarus minus* Say, 1818. Crustaceana Suppl. 4: 153-163.

Shoemaker, C.R. 1940. Notes on the amphipod *Gammarus minus* Say and description of a new variety, *Gammarus minus* var. *tenuipes*. Journal of the Washington Academy of Sciences 30: 388-394.

Sokal, R.R. and F. J. Rohlf. 1981. Biometry: The principles and practice of statistics in biological research. New York: W.H. Freeman and Company. 859 p.

Steele, D.H. and V.J. Steele. 1993. Biting mechanism of the amphipod *Anonyx* (Crustacea:Amphipoda: Lysianassoidea). Journal of Natural History 27: 851-860.

Thomas, W.J. 1970. The setae of *Austropotamobius pallipes* (Crustacea: Astacidae). J. Zool., London 160: 91-142.

Wagner, V.T. and D.W. Blinn. 1987. A comparative study of the maxillary setae for two coexisting species of *Hyalella* (Amphipoda), a filter feeder and a detritis feeder. Arch. Hydrobiol. 109: 409-419.

Watling, L. 1989. A classification system for crustacean setae based on the homology concept. Pages 15-26 in B.E. Felgenhauer, L. Watling, and A.B. Thistle. Functional morphology of feeding and grooming in crustacea. A.A. Balkema, Rotterdam.

Watling, L. 1993. Functional morphology of the amphipod mandible. Journal of Natural History 27: 837-849.

Willoughby, L. G. 1983. Feeding behaviour of *Gammarus pulex* (L.) (Amphipoda) on *Nitella*. Crustaceana 44: 245-250.

Zar, J.H. 1996. Biostatistical analysis. Upper Saddle River (NJ): Prentice Hall. 899 p.

LIST OF TABLES FOR CHAPTER 3

Table 1. Mouthpart and foregut structures studied.

Table 2. Percent of each catalogued behavior/total number of activities observed in *G. minus* adults and immatures during feeding experiment.

Table 3. Comparisons of frequencies of behaviors observed in immature and adult animals during laboratory feeding experiment

LIST OF FIGURES FOR CHAPTER 3

Fig. 1. *G. minus* mouthparts, antennae, and gnathopod 1.

Fig. 2. Chamber used for studying behavior. Netting was removed for most studies.

Fig. 3. Lateral views of arrangement of mouthparts on head of *G. minus*.

Fig. 4. SEM of mouthparts.

Fig. 5. Same view as 4 with maxillipeds removed.

Fig. 6. Maxillipeds.

Fig. 7. Enlargement of one maxilliped.

Fig. 8. Inner plate of maxilliped.

Fig. 9. Second maxilla.

Fig. 10. Right maxillae 1.

Fig. 11. Close-up of comb setae on maxilla 1

Fig. 12-14. Molars

Fig. 15. Schema of food movement through amphipod digestive system.

Fig. 16. Flow-chart depicting hypothesized movement of food through mouthparts of *G. minus*. (2 pages)

Fig. 17. Relationship of number of cuspidate setae on outer plates of maxillipeds and body length of Bradley Run animals.

Fig. 18. Relationship of number of cuspidate setae on outer plates of maxillipeds and body length of Richards Creek animals.

Fig. 19. Relationship of length of maxilliped palp dactyl and body length of Bradley Run animals.

Fig. 20. Relationship of length of maxilliped palp dactyl and body length of Richards Creek animals.

Fig. 21. Relationship of number of hook setae on foregut ampullae and body length of Bradley Run animals.
Fig. 22. Relationship of number of hook setae on foregut ampullae and body length of Richards Creek animals.

Table 1. Mouthpart and foregut structures studied.

Mouthpart or part of foregut	Structure	Correlation with body length?
Maxilliped: Inner Plate	No. of cuspidate setae	No, number of setae rarely more or less than 3
Maxilliped: Outer Plate	No. of cuspidate setae	Yes
Maxilliped: Palp	Length of dactyl	Yes
Maxilla 1 (right): Palp	No. of teeth	No, number of teeth ranged from 3-5
Mandible: Molar	Surface area	Perhaps. See text.
Mandible (right): Incisor	No. of teeth	No, constant at approx. 5
Mandible (left): Incisor:	Number of teeth	No, constant at approx. 4
Foregut: ampullae	No. of hook setae	Yes
Foregut: ampullae	No. of “extra” setae next to row of hook setae	No, animals had either 1 or 2 “extra setae”. No trends.

Table 2. Percent of each catalogued behavior/total number of activities observed in *G. minus* adults and immatures during feeding experiment. Total number of activities observed: 3480. Number of animals = 32

Behaviors by category (percent for category)	Percent frequency of behavior/total activities
Eating (44.9%)	
Processing	29.1
Holding food in place with gnathopods	13.9
Keeping food in place with antennae	1.9
Holding clump of particles in place with larger piece of food	0.01
Swimming/walking (17.5%)	17.5
Gathering food (16.1%)	
Gathering food with antennae	14.5
Gathering food with gnathopods	1.4
Holding gnathopods out	0.2
Searching (10.2%)	
Searching bottom of container with antennae or gnathopods	7.0
Kicking up particles	0.7
Discarding particles	2.5
Pulling appendages through gnathopods (8.8%)	
Pereopods pulled through gnathopods	0.1
Pleopods pulled through gnathopods	0.6
Uropods pulled through gnathopods	0.5
Antennae pulled through gnathopods	7.6
Handling fecal pellet (1.9%)	
Removing fecal pellet from body with gnathopods	0.3
Eating fecal pellet	0.1
Discarding fecal pellet	0.2
Rolling posterior part of body toward mouth	0.9
Pulling gnathopods over the dorsal, posterior portion of body	0.4

Table 3. Comparisons of frequencies of behaviors observed in immature and mature animals during laboratory feeding experiment. Values that differed significantly ($p < 0.05$) are denoted by an *. Numbers in () = number of animals exhibiting behavior.

	Total frequencies		Average frequencies	
Behavior	Immature	Mature	Immature	Mature
Processing	554 (16)	460 (16)	35	29
Swimming/walking	242 (14)	368 (15)	17	25
Gathering food with antennae	270 (16)	233 (15)	17	16
Holding food in place with gnathopods	278 (16)	209 (15)	17	14
*Antennae pulled through gnathopods	67 (13)	198 (14)	5.2	14
*Searching bottom of container with antennae or gnathopods	153 (14)	91 (15)	11	6.1
*Discarding particles	71 (14)	15 (8)	5.1	2
Holding food in place with antennae	60 (7)	6 (3)	9	2
*Gathering food with gnathopods	7 (5)	40 (12)	1	3.3
*Rolling posterior part of body toward mouth	25 (10)	7 (3)	2.5	2
Kicking up particles	5 (4)	19 (6)	1	3
Pleopods pulled through gnathopods	17 (1)	4 (3)	17	1
Uropods pulled through gnathopods	9 (2)	8 (3)	5	3
Using posterior part of body to push food to mouth or to hold it in place	16 (3)	0 (0)	5	0
Pulling gnathopods over the dorsal, posterior portion of body	9 (4)	6 (4)	2	2
*Removing fecal pellet from body with gnathopods	9 (7)	2 (2)	1	1
Discarding fecal pellet	6 (5)	2 (2)	1	1
Holding gnathopods out	1 (1)	5 (3)	1	2
Eating fecal pellet	3 (2)	1 (1)	1	1
Pereopods pulled through gnathopods	0 (0)	3 (2)	0	2
Holding food in place with appendage other than gnathopods or antennae or with another piece of food	1 (1)	0 (0)	1	0
Total number of activities observed	1803	1677		
Range of numbers of frequencies for a given behavior	67-153	65-142		
Average number of activities/animal (16 animals per group)	113	99		

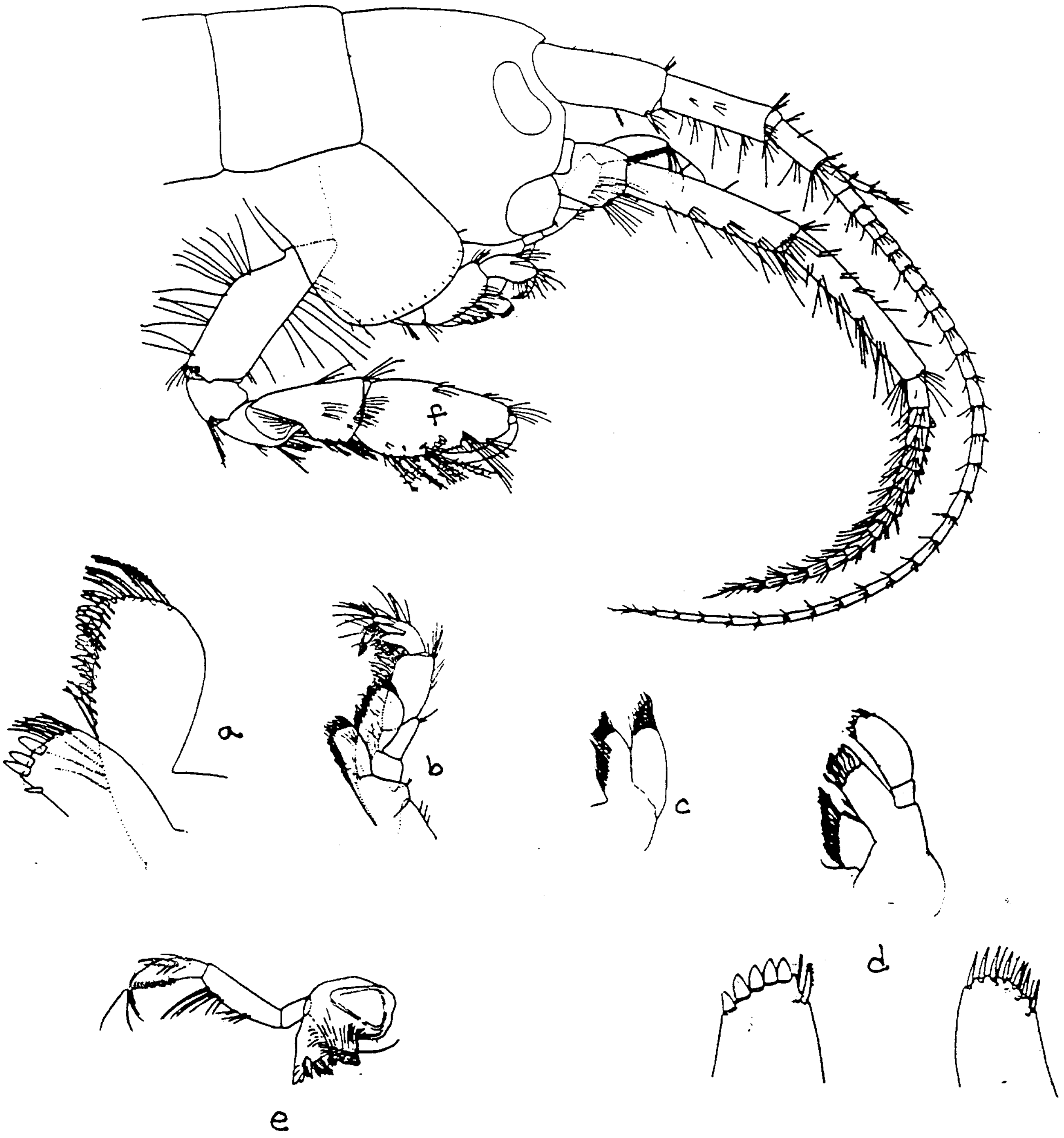


Fig. 1. *Gammarus minus* mouthparts, antennae, and gnathopod 1 (modified from Shoemaker 1940). *a*, inner and outer plates of maxilliped (MP), enlarged; *b*, MP; *c*, inner and outer plates of maxilla 2; *d*, maxilla 1, with details of right and left palps; *e*, mandible; *f*, gnathopod 1.

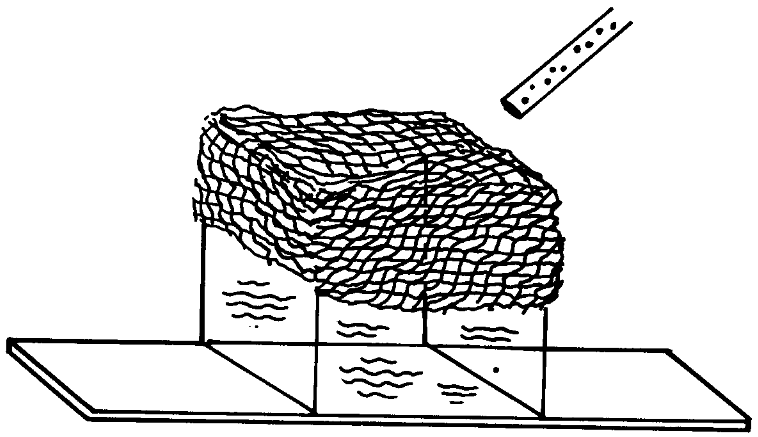


Fig. 2. Chamber used for studying behavior. Netting was removed for most studies.

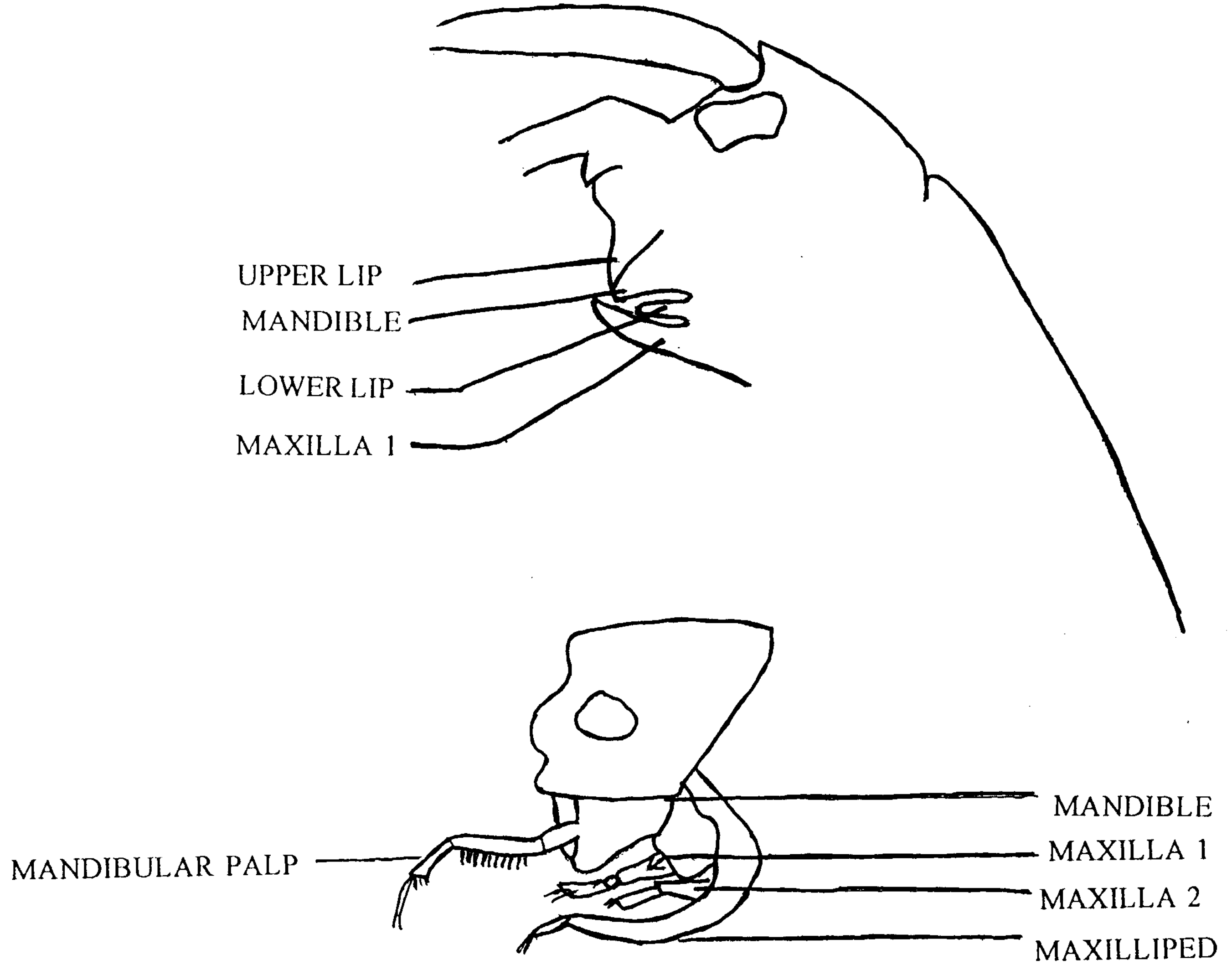
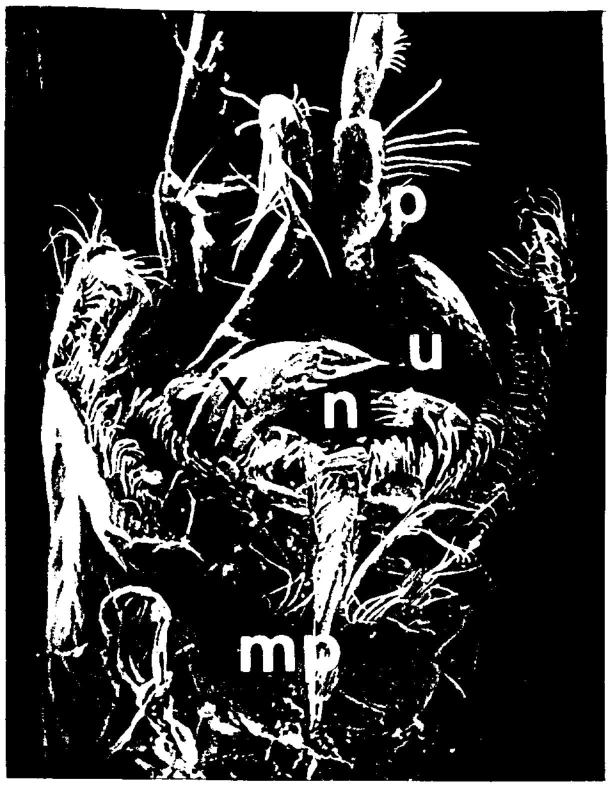
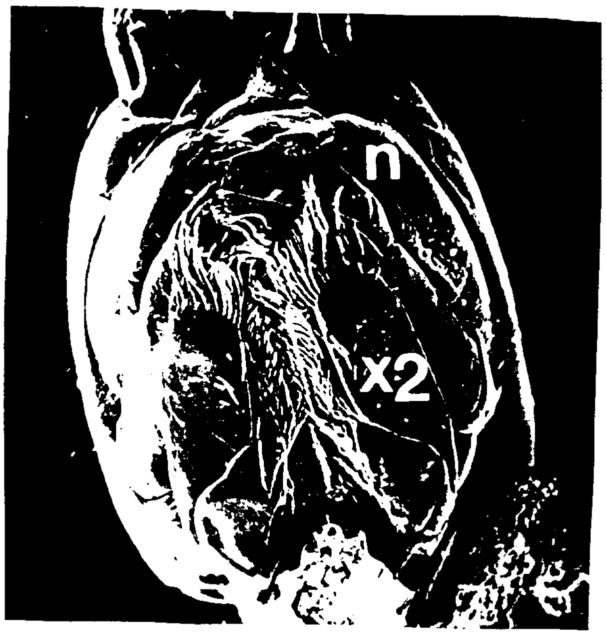


Fig. 3. Lateral views of arrangement of mouthparts on head of *G. minus*.

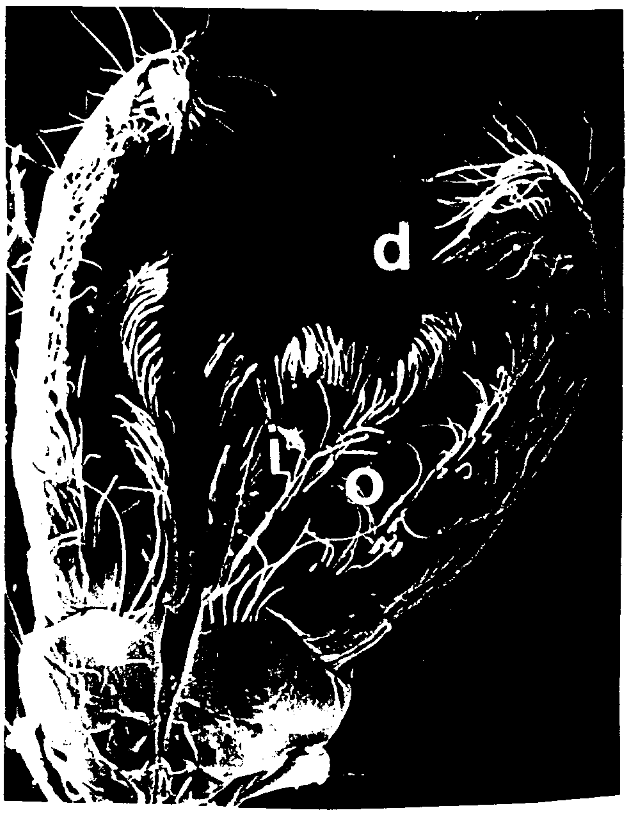
4



5



6



Figs. 4-6. Fig. 4. SEM of mouthparts. Fig. 5. Same view as 4 with maxillipeds removed. Fig. 6. Maxillipeds. *d*: dactyl, *i*: inner plate, *mp*: maxillipeds, *n*: mandibular incisor, *o*: outer plate, *p*: palp of mandible, *u*: upper lip (to left of letter), *x*: maxilla 1 palp, *x2*: outer plate of maxilla 2.

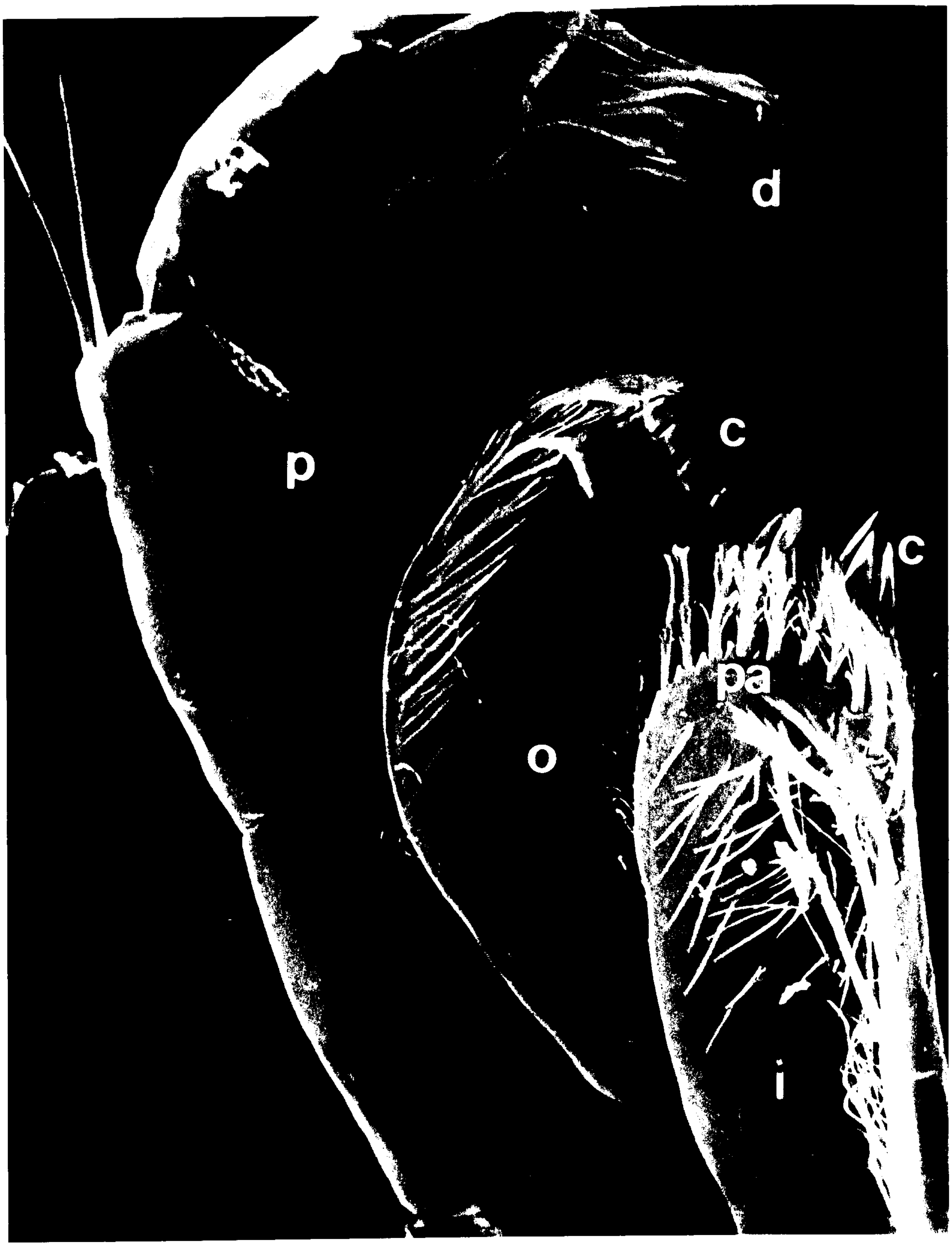
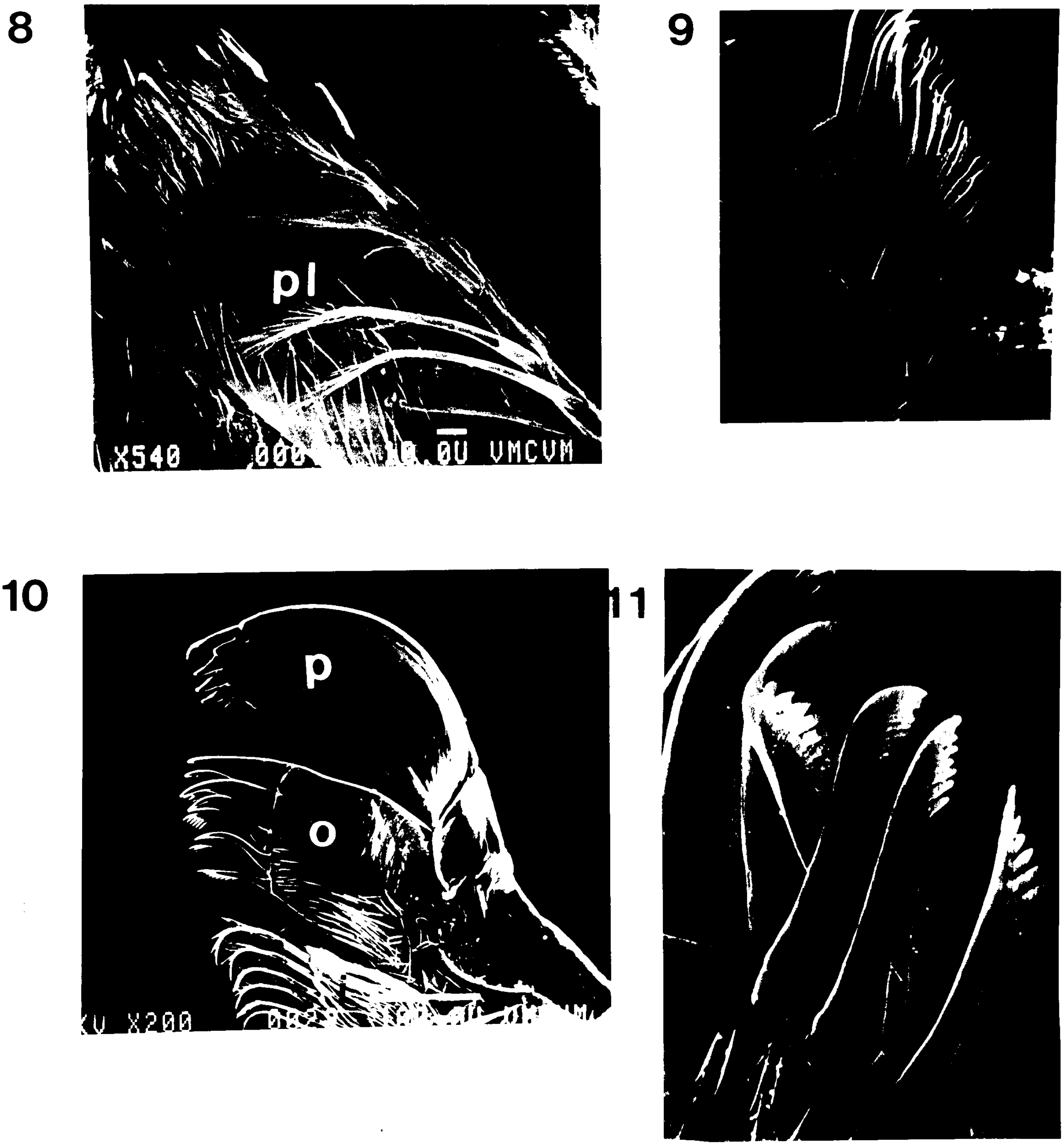
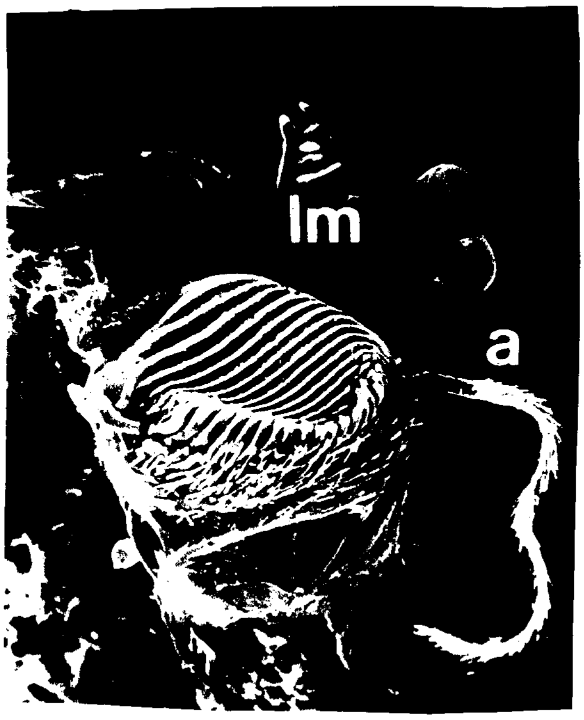


Fig. 7. Enlargement of one maxilliped. *d*: dactyl, *c*: cuspidate setae, i: inner plate, *o*, outer plate, *p*: palp, *pa*: pappose setae.



Figs. 8-11. Fig. 8. Inner plate of maxilliped. Fig. 9. Second maxilla. Fig. 10. Right maxillae 1. Fig. 11. Close-up of comb setae on maxilla 1. *pl*: plumose setae, *p*: palp, *i*: inner plate, *o*: outer plate.

12



13

14

Figs. 12-14. Molars. *i*: incisor, *u*: upper lip, *a*: accessory flagellum, *f*: flake, *lm*: lacinia mobilis

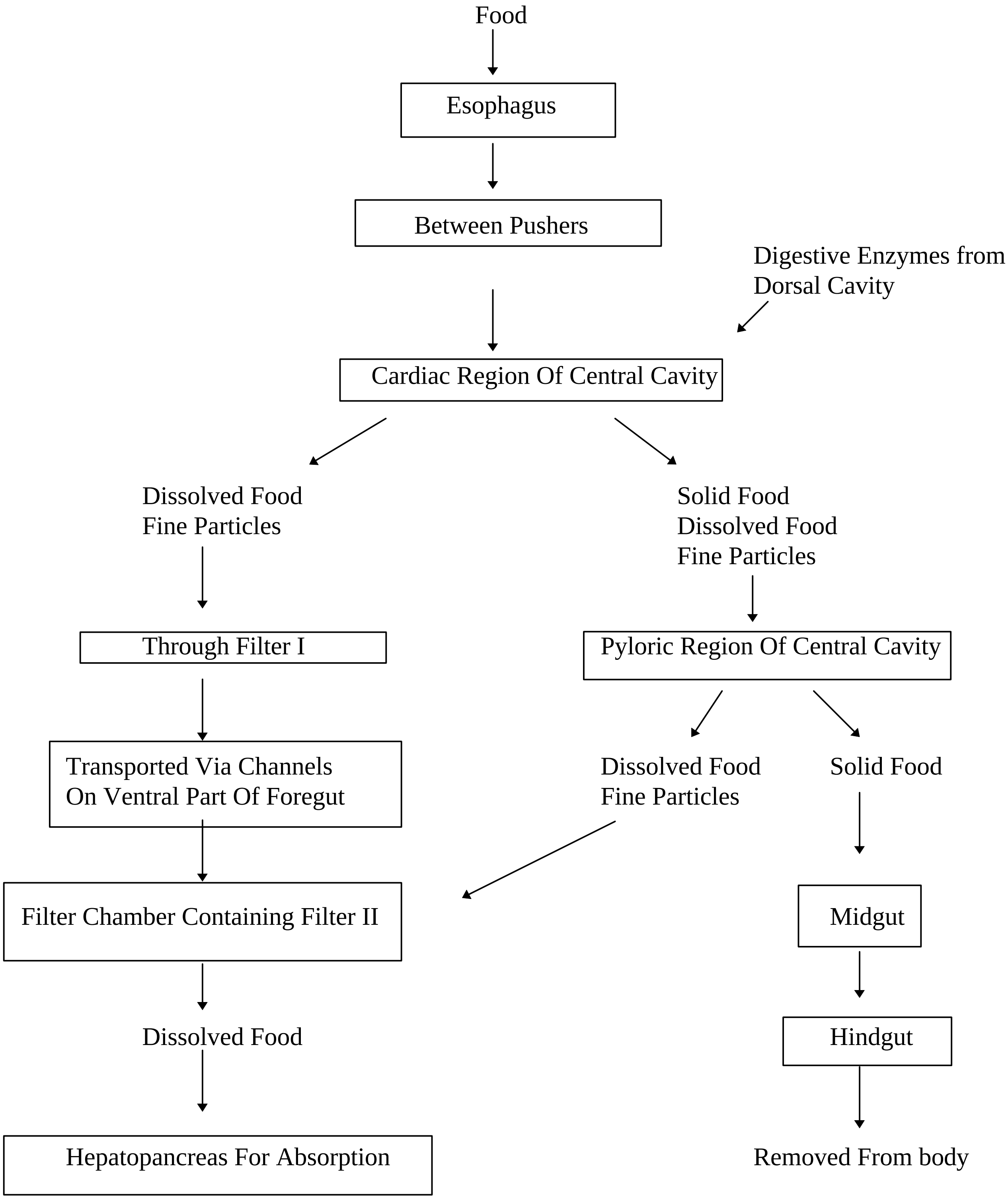


Fig. 15. Schema of food movement through amphipod digestive system.

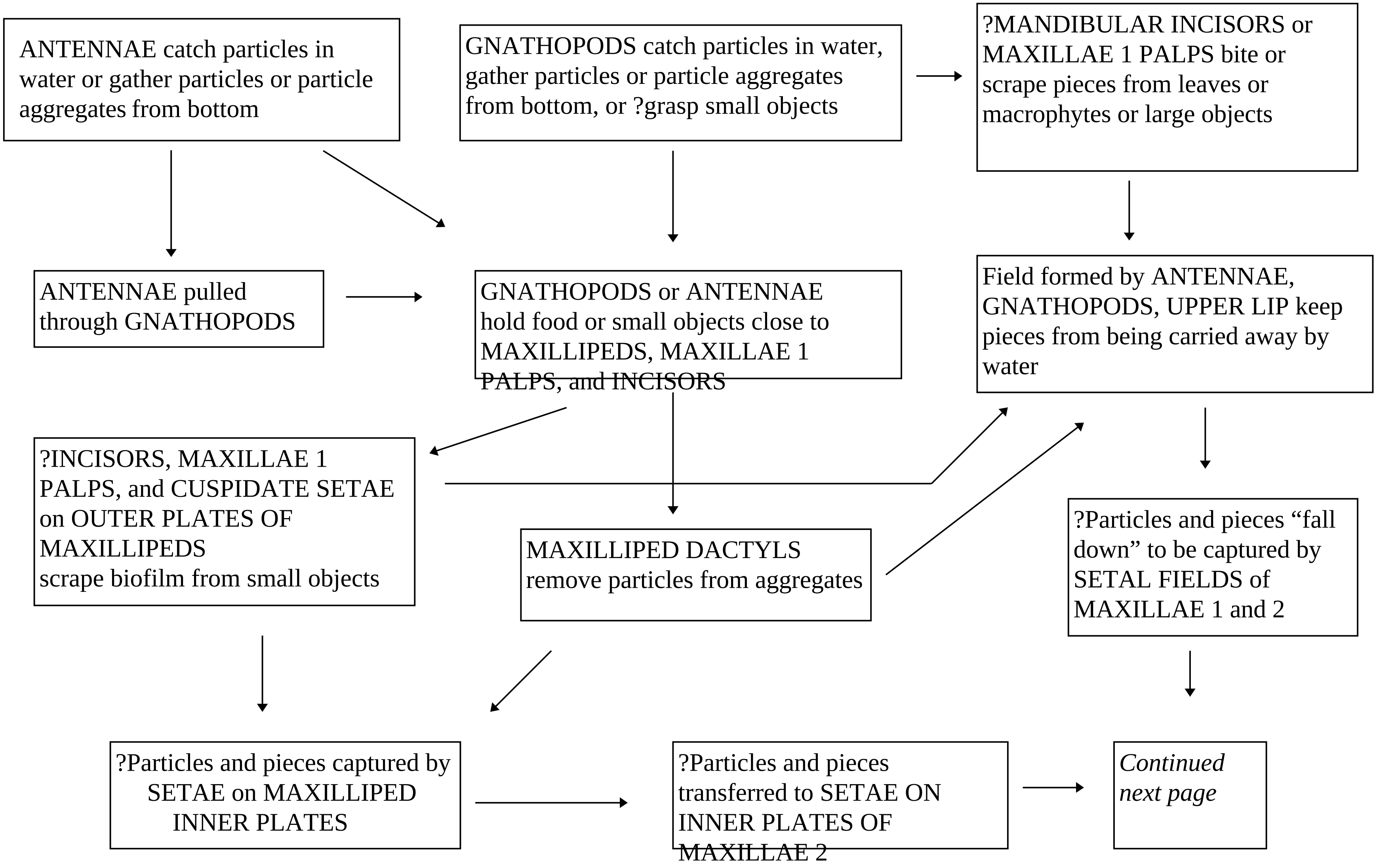
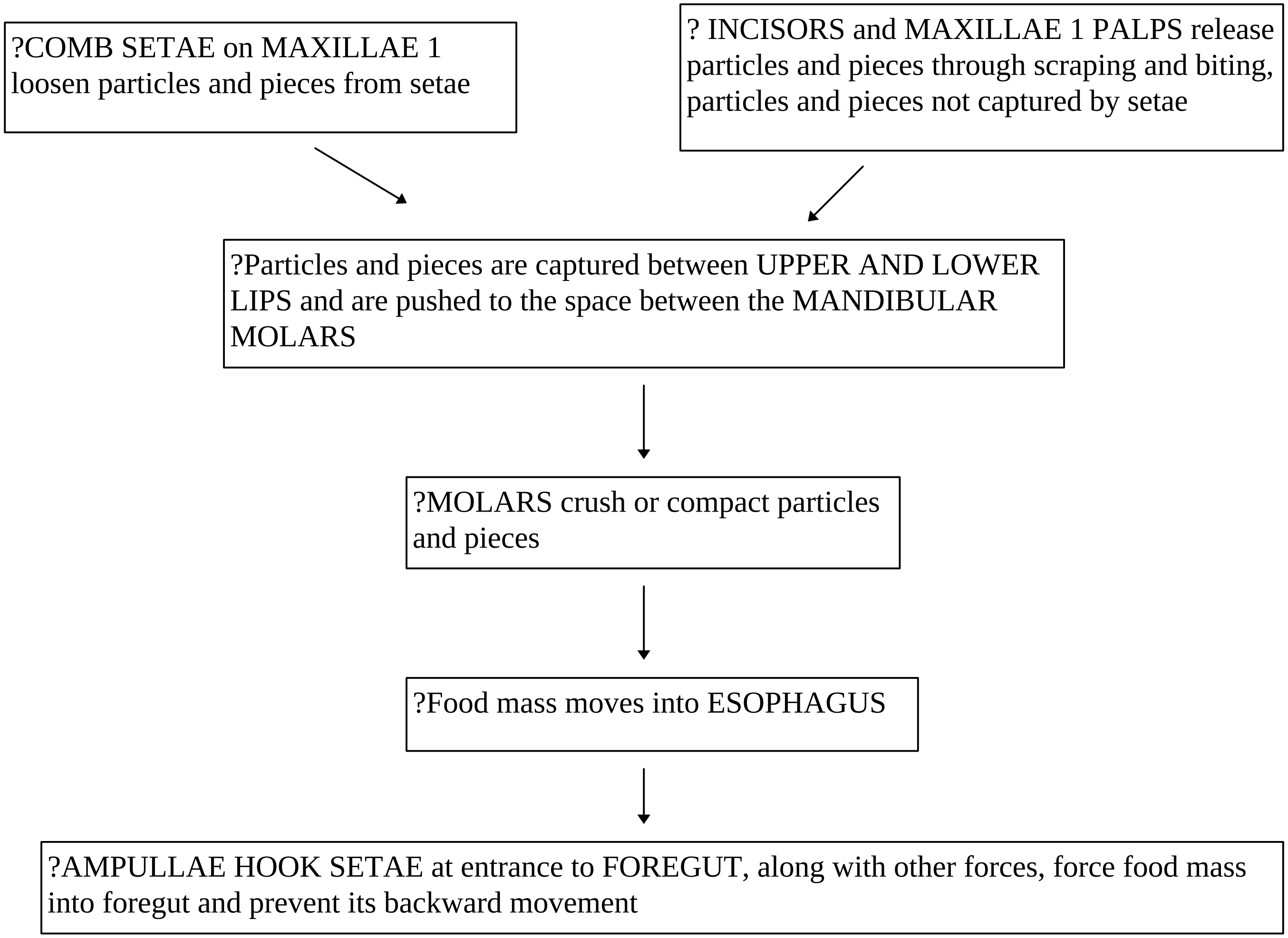


FIG. 16. Flow-chart depicting hypothesized movement of food through mouthparts of *Gammarus minus*. Question marks indicate that the function has not been observed, but only assumed from structure and other indirect information.



?COMB SETAE on MAXILLAE 1 loosen particles and pieces from setae

? INCISORS and MAXILLAE 1 PALPS release particles and pieces through scraping and biting, particles and pieces not captured by setae

?Particles and pieces are captured between UPPER AND LOWER LIPS and are pushed to the space between the MANDIBULAR MOLARS

?MOLARS crush or compact particles and pieces

?Food mass moves into ESOPHAGUS

?AMPULLAE HOOK SETAE at entrance to FOREGUT, along with other forces, force food mass into foregut and prevent its backward movement

FIG. 16 Continued

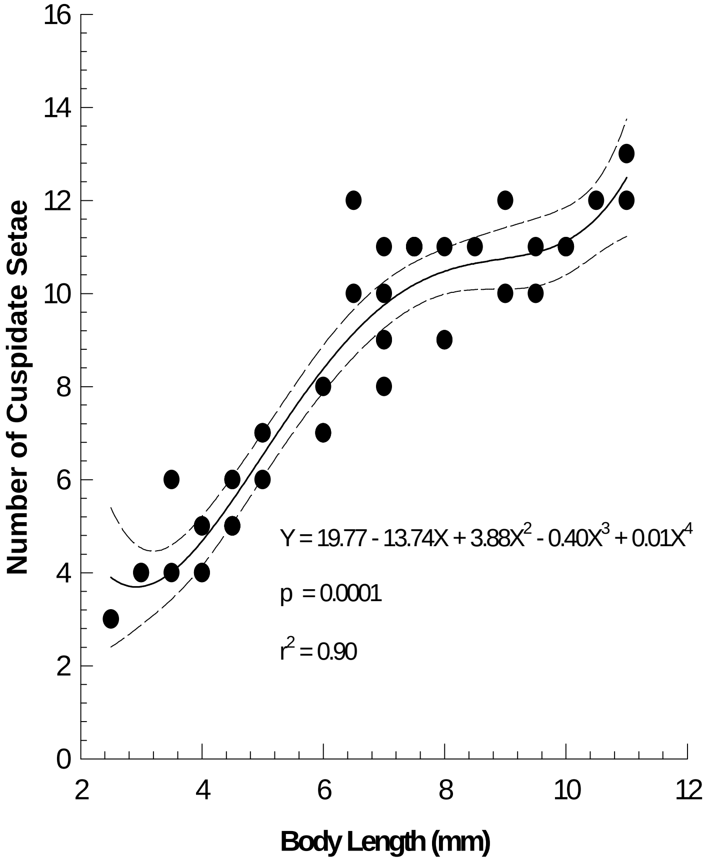


Fig. 17. Relationship of number of cuspidate setae on outer plates of maxillipeds and body length of Bradley Run animals. Solid lines : regression. Dashed lines : 95% confidence intervals.

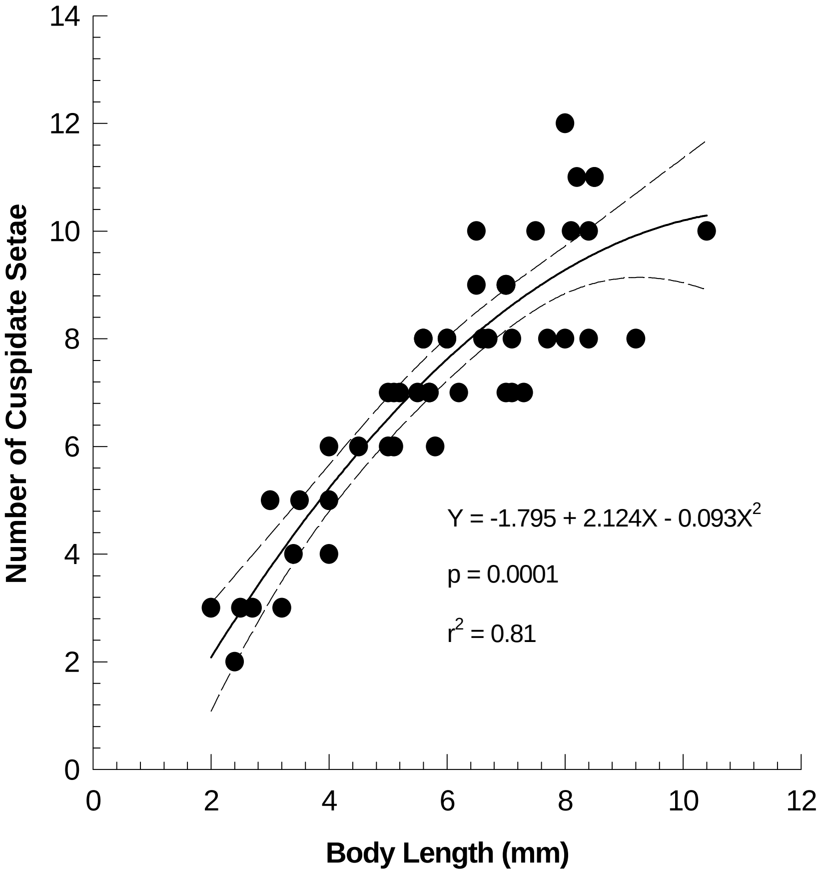


Fig. 18. Relationship of number of cuspidate setae on outer plates of maxillipeds and body length of Richards Creek animals. Solid line:regression. Dotted lines: 95% confidence interval

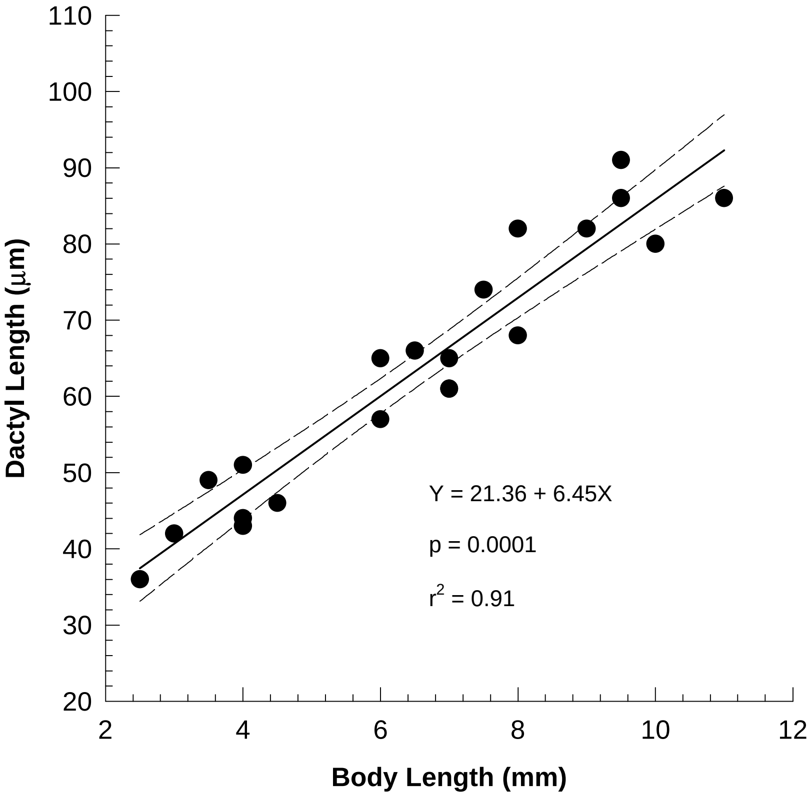


Fig. 19. Relationship of length of dactyl of maxilliped palp and body length of Bradley Run animals. Solid lines: regression. Dashed lines : 95% confidence intervals.

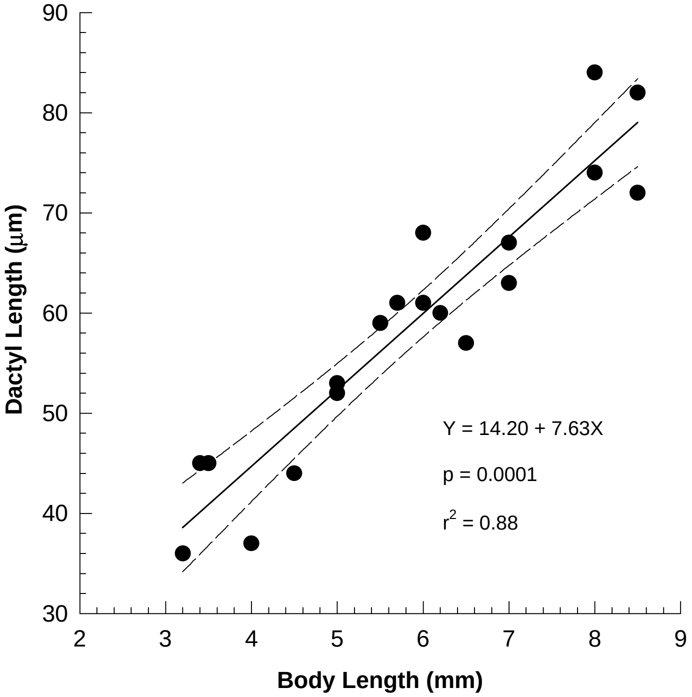


Fig. 20. Relationship of length of maxilliped palp dactyl and body length of Richards Creek animals. Solid line: regression. Dashed lines: 95% confidence intervals.

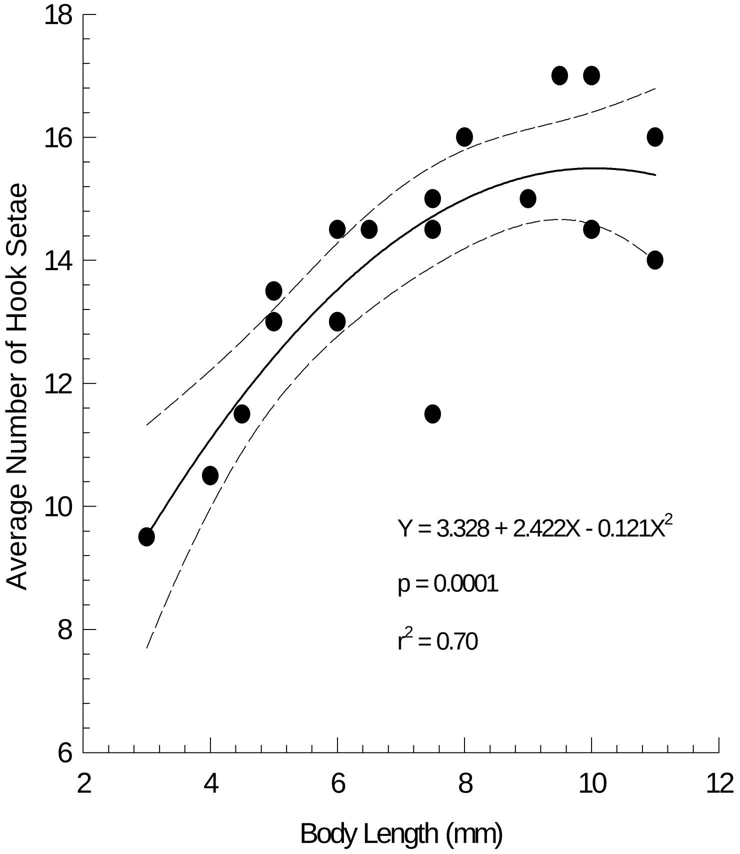


Fig. 21. Relationship of number of hook setae on foregut ampullae and body length of Bradley Run animals. Solid line: regression. Dashed line: 95% confidence interval.

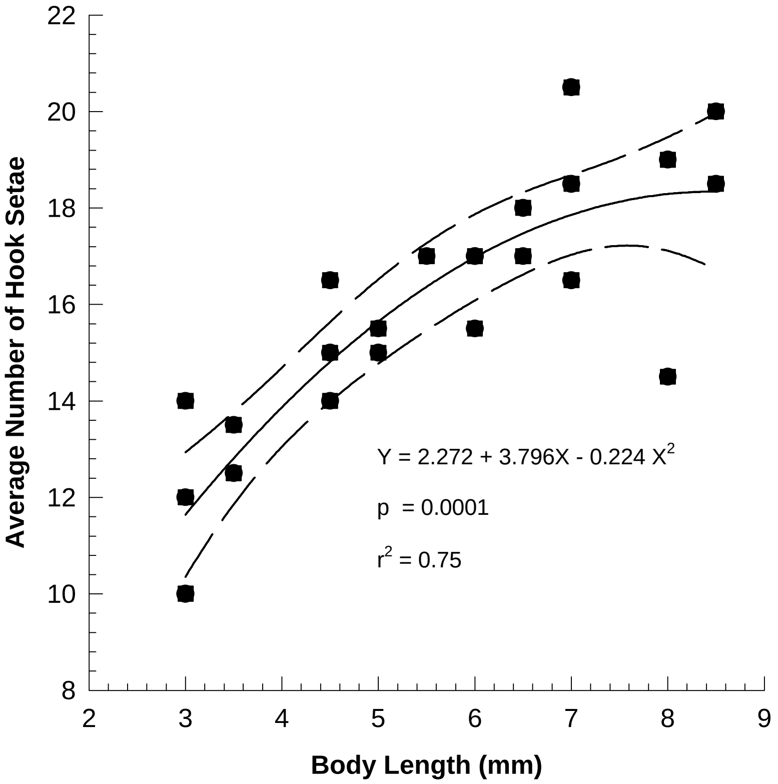


Fig. 22. Relationship of number of hook setae on foregut ampullae and body length of Richards Creek animals. Solid lines: regression. Dashed lines: 95% confidence intervals. Probability is for the regression.