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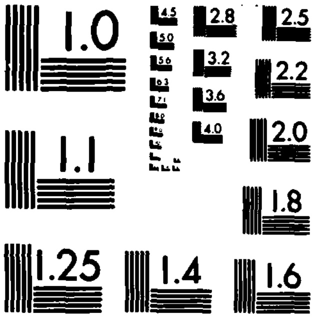
MICRODEUTOPUS GRYLLOTALPA A MODEL FOR MARINE FOULING
PROCESSES(U) NEW YORK ZOOLOGICAL SOCIETY BROOKLYN 1984
UNCLASSIFIED N00014-82-C-0482 F/G 8/1 NL



MICROCOPY RESOLUTION TEST CHART
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New York Zoological Society
Brooklyn, NY

FINAL REPORT

CONTRACT N00014-82-C-0482

STUDIES ON THE BIOLOGY OF THE TUBE-BUILDING AMPHIPOD *MICRODEUTOPUS GRYLLOTALPA* . A MODEL FOR MARINE FOULING PROCESSES

RESUMÉ

The principal objective of the proposed studies was to observe the reproductive biology of *Microdeutopus gryllotalpa* , with an eye toward devising an effective biological method for controlling fouling. Observations on this and seven other species were conducted. The information obtained covers a wide area, and has provided data for at least nine papers; three published, two in press, and four in preparation. The most important contribution of these studies has been the development of a reliable, objective bioassay for water-borne pheromones, and its use to establish the existence of these substances in two species. It is now possible to conduct studies directed toward determining the chemistry of these substances. Once determined, it may become feasible to synthesize it in sufficient quantities to be employed to lure males to an underwater trap.

THE TUBE-BUILDING SPECIES

Jassa falcata

This species is ubiquitous in fouling communities world-wide. It is abundant on the docks in the waters of Jamaica Bay, New York, and it is also abundant in the excurrent pipes of the cold water tanks in the New York Aquarium. I decided to study this species because it is a fouling organism, and because it was readily available nearby.

Several important observations were made on this species. First, it exhibits the cruising male pattern of reproductive behavior (Reprint I). While adults of both sexes build tubes on the substratum, females tend to remain in their tubes while males travel from tube to tube seeking receptive females. (This pattern of behavior was also demonstrated for another tube-building amphipod *Ampithoe valida* and a tanaid *Tanais cavolinii* (Reprint I). It appears that this behavior is a common solution when a species has the following ecological and physiological constraints: 1) individuals reside at fixed sites; 2) females have multiple sequential broods; and 3) there is no sperm storage.)

The second important observation was that unlike most other amphipods, male *Jassa falcata* do not have indeterminate growth; they exhibit a terminal molt. The terminal molt is accompanied by a change in the morphology of the male's chelae (Reprint I), and with a significant change in its behavior (In Preparation I). Before the terminal molt, males are fully fertile, but rarely reproduce because they remain in their tubes. After their terminal molts, however, they begin to travel over the substratum seeking receptive females (In Preparation I). I believe that the sudden change in the male's behavior, coincident with its terminal molt, is an adaptation which permits males to grow to the largest size possible in the relative safety of their tubes before they actively attempt to reproduce. Males outside their tubes are subject to predation and to being washed away by the strong currents to which they are exposed in their preferred habitat. The larger they are, the better they may be able to cling to the substratum, and the better they may be able to defend a receptive female. An important difference between *J. falcata* and the other tube-builders is that males do not move into the shelter of receptive females' tubes; they remain outside and on top of them. Thus, reproductive males of this species are exposed to predation and the shearing forces of currents for longer periods of time than are the other tube-building species, and must be subject to more intense adverse selection.

Microdeutopus gryllotalpa

This species also exhibits cruising males (Reprint II). In contrast to *Jassa falcata* , however, it thrives under laboratory conditions, and withstands a relatively great deal of experimental manipulation. Therefore the behavior of this species could be studied in greater detail than could *J. falcata* 's.

The major objective of studies on this species has been the determination of how the female conveys its reproductive condition to the male. To simplify the analysis, the males' reproductive behavior was divided into three sequential stages. In the first stage, males travel to receptive females' tubes. In the second stage, they exhibit courtship behaviors and move into the female's tube. The pair remains together inside the tube until the third stage occurs, which involves the female's molt, followed within minutes by copulation.

The first hypothetical stimulus that was tested was the existence of some specific behavior that the female expresses only in a reproductive context. But no distinct female reproductive behavior was detected (Reprint II).

In light of the number of crustacea which have been shown to have pheromones, the next hypothetical stimulus tested was water-borne pheromones. An apparatus was designed which combined the two-choice test paradigm with ambient conditions similar to the animals' typical habitat. It was shown that males respond to the water-borne secretions of receptive females, but not to non-receptive females or males (Preprint I). Thus, pheromones influence the first stage of the males' behavior.

Water-borne pheromones have been invoked as the stimuli for the panoply of reproductive behaviors observed in aquatic crustacea. Therefore, the next step was to determine whether the water-borne secretions which had been shown to have attractant properties would stimulate the rest of male _M. gryllotlpa'_ s behaviors. Surprisingly, receptive female secretions had no discernible effect on any other of the male's behaviors. Thus, in _M. gryllotalpa_ the water-borne pheromone is merely a guide which directs the males' movements toward the tube of a receptive female.

It should be noted that this is a significant contribution to the understanding of the control of reproductive behaviors in aquatic crustacea. In contrast to the general apprehension about the function of pheromones in crustacea, it appears that they may not release all reproductive behaviors, but may merely alert the male that a receptive female is in the neighborhood.

The next step was to try to learn what the stimuli for courtship and mating behavior could be, given that it wasn't the two most obvious possibilities. The results suggest that at least four different stimuli elicit the male's courtship behavior: the female's body, her general movements, the receptive female's tube and the female's exoskeleton. A fifth, as yet unidentified factor, is produced by live receptive females (In Preparation II). The effects of these stimuli are additive. The absence of one, or even several, stimuli, does not automatically ensure the failure to reproduce.

This is the first demonstration of site-marking to indicate reproductive stage in a crustacean. The determination of the nature of this marking may also lead to a method of controlling fouling. If it is a chemical marker, this substance combined with the water-borne attractant would make an effective trap; the latter substance would lure males to the trap, and the former substance would induce them to remain there.

Finally, data which has not yet been written up, show that copulation is stimulated by the female's exoskeleton. The results are best explained by the existence of contact pheromones on the exoskeleta of females. The exoskeleton may be the source of one kind of pheromone when the female is receptive, and another kind when she has just molted and can copulate. Males rarely confuse receptive and newly molted females.

THE EPIBENTHIC SPECIES

The use of the males' chelae during precopulation

Like the tube-builders, the pattern of reproduction among the epibenthic species may also be divided into three sequential stages. First the male and female find each other. Second, they remain together, not in the same tube (these species do not build tubes) but via the male's attaching its chelae to the female and carrying her about. The third stage involves the female's molt, followed immediately by copulation.

Gammarid amphipods typically have sexually dimorphic chelae. Males generally have one of the two pairs greatly enlarged and with many differences in their morphologies. The consensus had been that the function of the males' enlarged chelae was to carry the female about. I became suspicious of this belief during observations on *Jassa falcata* , the species which exhibits about the greatest degree of dimorphism of any other, but which does not precopulate. Therefore I examined the ways males employed their chelae during precopulation in five epibenthic species. The observations showed that in fact males employ the smaller of the two pairs of chelae to carry the females. But, in addition, males of different genera carry their females in different, but highly stereotyped ways. In some species, females have distinctive processes on their exoskeleta to which the males attach their chelae, and which are absent in males (Preprint II). The males probably use their larger chelae to defend the female from other males. Larger males have been observed to dislodge smaller males from their females using their larger chelae.

The females' replacement of dislodged eggs into their brood pouches

The brood pouches of gammarid amphipods are formed of four pairs of overlapping plates. The plates are thin and flexible and there are spaces between them. It had been observed that some females, in particular the larger ones, had such large broods that eggs became dislodged in the course of the females' general movements. It seemed highly non-adaptive for a female to produce so many eggs at one time that they had a strong probability of not developing to hatching. (Once outside the brood pouch development does not continue, and such eggs are subject to predation.) Therefore, I observed how different types of individuals of two closely related species treat eggs outside the brood pouch (Reprint III).

Males ate every egg they encountered, but females did not. Females picked up eggs, palpated them with their mouthparts, and then either ate them, dropped them, or replaced them in their brood pouches. It was found that female Gammarus palustris ate Gammarus mucronatus 'eggs and replaced their own eggs most often. In contrast, female G. mucronatus treated all classes of eggs about the same way. These findings were consistent with earlier studies which had shown that G. palustris exhibits a greater degree of maternal care than does G. mucronatus . It also suggested that dislodged eggs are probably not lost or eaten, but are generally replaced by the female and therefore have the chance to develop to hatching.

Stimuli for the males' reproductive behaviors in Gammarus palustris

Individuals of Gammarus palustris are about three times larger than Microdeutopus gryllotalpa , are much less fragile, and can be collected in the field in great numbers through most of the year. It was felt that water-borne pheromones would be easier to analyze in the former than in the latter species, if they were shown to exist. Therefore, the apparatus employed for M. gryllotalpa was modified to permit the testing of G.palustris individuals.

Water-borne pheromones were demonstrated in this species as well (In Preparation III). But there are several interesting differences between the two species. First, males only responded to secretions when the tests were conducted in the dark. Male M. gryllotalpa responded in the light. Second, there is a species-specific substance to which both sexes are attracted. This makes sense, considering that individuals of both sexes travel freely in their preferred habitat. But, third, males were attracted to receptive females' secretions and females were attracted to males' secretions more often than to the secretions of other types of individuals. Again, this makes sense, because although it is adaptive to seek individuals of your own species in general (they are probably already in your preferred habitat), it is particularly useful to find an individual of the opposite sex who is receptive.

Further tests on the receptive female attractant showed that, as in M. gryllotalpa , these substances had no discernible effect on the other male reproductive behaviors. Instead, the female's behavior and its exoskeleton were shown to be important not only for the onset of precopulation (the second stage of reproductive behavior) but also for copulation (the third stage) (In Preparation IV). Again, the data point to the existence of contact pheromones on the female which convey her reproductive stage to the male. But, in addition, there are several subtle, yet important female-specific behaviors which may stimulate the male's appropriate behaviors (In Preparation IV). In contrast, no distinct reproductive behaviors were observed in M. gryllotalpa .

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Marine Biology 77, 257–263 (1983)

Marine Biology
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Reprint I

Reproductive behavior of three tube-building peracarid crustaceans: the amphipods *Jassa falcata* and *Ampithoe valida* and the tanaid *Tanais cavolinii*

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Abstract

Laboratory observations indicate that *Jassa falcata*, *Ampithoe valida*, and *Tanais cavolinii* have quite similar patterns of behavior: males attend females until ovulation and copulation occur, then the male and female separate. Females tend to remain inside their own tubes, and males tend to move between the tubes of receptive females. This pattern of behavior (called "cruising males") may be common in crustaceans that exhibit some fidelity to a specific site, especially if the females produce several broods in succession, do not store sperm, and reproduce asynchronously relative to each other. Male *J. falcata* have a terminal molt which is marked by the presence of a specific sexually dimorphic characteristic, the "thumb" on the second gnathopod. Alternating ovulatory and anovulatory molts occur in *T. cavolinii*.

Introduction

The amphipods *Jassa falcata* and *Ampithoe valida*, and the tanaid *Tanais cavolinii*, are benthic, tube-building peracarid crustaceans. Males and females of these three species construct individual residential tubes by gluing debris together with secretions from specialized glands. Females have several broods in succession during the reproductive season but do not store sperm. Thus, for a viable brood, copulation must occur each time the female ovulates. Another common characteristic of these species is that females of a given species reproduce asynchronously relative to each other.

Related species subjected to similar environmental factors often exhibit convergent adaptations. Convergence is most easily recognized in morphology but can occur in behavior patterns as well. The purpose of this study was to test the hypothesis that species having similar reproductive physiologies and subjected to similar ecological factors have similar reproductive behaviors.

Material and methods

Source of animals

Adult *Ampithoe valida* were taken from laboratory stock cultures, founded by individuals collected from the intertidal zone at Jamaica Bay, Gateway National Park, Brooklyn, New York, USA. Stocks were maintained in 20-cm diameter glass culture dishes, with a glass cover plate to reduce evaporation. They were held at room temperature (20.6 °C ± 2.3 C°) at 15 hL:9 hD, in Instant Ocean Sea Salts (Eastlake, Ohio) diluted with tap water to 24‰ S (the average salinity of the collection site).

Individuals of *Jassa falcata* and *Tanais cavolinii* were taken from the cold-water exhibit tanks at the New York Aquarium, Brooklyn, New York. These tanks are continuously supplied with sea water from a well whose average temperature and salinity is 14.5 °C and 29‰ S, respectively.

Observations of individuals in pairs

Individuals were arranged in single heterosexual or isosexual pairs in individual 10-cm culture dishes in water of the appropriate salinity. Individuals were provided with *Ulva* sp. thalli *ad libitum* and occasional bits of mussel adductor muscle, which they used for food and for tube-building materials. Dishes were kept at room temperature, 15 hL:9 hD, and observed twice daily, at 7.00 hrs and 19.00 hrs during the light part of the cycle. The presence of casts, the occurrence of tube-sharing, ovulation and hatching, and any other pertinent reproductive activities were noted.

About 20 heterosexual pairs of each species were originally set up, and the data on lengths of intermolt periods were used from all pairs possible. The data from four male and four female isosexual, and eight heterosexual pairs that survived through several female ovulatory molts were used in behavioral analyses (21, 42, and 14 d for *Ampithoe valida*, *Tanais cavolinii*, and *Jassa falcata*, respectively). This was done to maximize the probability that the individuals were healthy and their behavior normal. Isosexual pairs of *T. cavolinii* were only observed once a day.

Observations of isolated male *Jassa falcata*

Fifty male *Jassa falcata* were isolated in individual 10-cm glass culture dishes and the occurrence of molts observed daily until the males died. In males of this species, the propodus of the fully formed posterior gnathopod has a nonprehensile posterio-ventral process resembling a thumb. After each individual died, its body, second gnathopod, and "thumb" lengths were measured. Body lengths were measured from the dorso-anterior tip of the rostrum to the dorso-posterior tip of the telson. Both the gnathopod and "thumb" lengths were measured from their dorso-anterior to their dorso-posterior tips.

Observations of *Ampithoe valida* in mass culture

Mass cultures were established by placing ten males and ten females in a 30×20×5.5 cm glass dish. The behavior of individuals outside tubes was observed daily for a five-minute period. These individuals were removed from the dish and replaced with an equal number of new individuals of the same sexes. The numbers and behaviors of each sex observed were recorded. This procedure maintained the number and sex ratio of adults in the dishes while guaranteeing that individuals outside tubes would only be scored once. Observations were continued until 50 individuals had been scored.

Results

Molt and reproductive cycles

The average lengths of intermolt periods differed among the species and between the sexes of each species (Table 1). Female *Jassa falcata* molted regularly, but males stopped molting when the "thumbed" stage appeared. When isolated, 27 males had "thumbs" and 23 did not. "Thumbed" and "thumbless" males survived about the same length of time (20.4±8.5 d, range 1–36 d; 17.0±10.5 d, range 1–36 d, respectively; $t = 1.299$, $P > 0.05$).

Eighteen "thumbless" male *Jassa falcata* molted: fifteen molted one time and three, twice. The former group molted directly into a "thumbed" gnathopod stage, while the latter group's "thumbed" gnathopod appeared after the second molt. The intervals between the two successive molts of the three individuals were 6, 7, and 7 d. Five of the original 23 "thumbless" males did not molt. Since they only survived from one to four days and the average intermolt period was 6.7 d, it is possible that these five simply did not survive long enough to molt.

No "thumbed" male *Jassa falcata* ever molted: neither the 27 males that had "thumbs" at the beginning of the experiment nor the 18 males that molted into "thumbed" stages during the course of the observations. Thus male *J. falcata* have terminal molts marked by the appearance of a "thumb" on their second gnathopods. In contrast, females have indeterminate growth.

The size distribution for "thumbed" and "thumbless" male *Jassa falcata* overlapped broadly (Fig. 1). Thus the occurrence of the terminal molt was not triggered by the males' absolute body length. In addition, the size of the "thumb" increased allometrically with male size even though the "thumb" exhibited no growth per se.

Female *Ampithoe valida* and *Tanais cavolinii* molted more frequently than males of the same species when maintained in heterosexual pairs (Table 1). No male *Jassa falcata* molted, because only "thumbed", terminal molt stage males had been used in observations on pairs.

In the amphipods, every female molt was accompanied by ovulation when a male was present. But in *Tanais cavolinii*, ovulatory molts alternated regularly with anovulatory molts (44 molts observed: only two molts in which two anovulatory molts were observed in succession). Ovulatory intermolt periods were significantly longer than anovulatory intermolt periods in the same female (paired Student's t-test: $t = 2.88$, $df = 10$, $P < 0.05$).

Anovulatory intermolt periods of *Tanais cavolinii* were readily distinguishable from ovulatory intermolt periods on the basis of brood sac morphology. In this species there are two brood sacs suspended from the ventral portion of the coxae of the fifth pair of peraeopods (Lang, 1961). The brood sacs of females in anovulatory intermolt periods were about ¼ the volume of ovulatory period brood sacs, and also lacked a brood. Ovulatory females cast off their brood sacs, not their entire exoskeletons, toward the end of this intermolt period, leaving either ragged ends, or

Fig. 1. *Jassa falcata*. The relationship between body length and length of the thumb of the second gnathopod of male *J. falcata*. Males with thumbs are indicated by dots; males without thumbs are indicated with "X"s

Table 1. Number of molts and lengths of intermolt periods observed in individuals maintained in heterosexual pairs

	Number of molts observed	Number intermolt periods observed[a]	Average intermolt period (days)	Range (days)
Jassa falcata				
Female	18	14	5.9 ± 1.69	4 – 9
Male	0	0	NI[b]	NI
Ampithoe valida				
Female	17	10	11.8 ± 2.53	10 – 18
Male	6	0	NI	NI
Tanais cavolinii				
Female anovulatory	12	17	13.1 ± 3.46	9 – 20
Female ovulatory	13	14	15.6 ± 3.64	11 – 22
Male	8	1	61	NI

[a] Two successive molts of the same individuals observed
[b] NI = no information available

a distinct scar, at the brood sac's point of attachment. Thus, females in ovulatory intermolt periods either had large brood sacs, or had none. On six occasions a female molted into the large brood-sac form, but ovulation did not occur. All of these molts followed typical anovulatory intermolt periods. Thus, the females underwent alternation of brood-sac forms whether or not ovulation occurred.

The length of the females' intermolt periods coincided with the length of time necessary for the amphipods' broods to complete development and leave the marsupium. Amphipods exhibited direct development and hatched into miniature adults (with a few juvenile characteristics). Juveniles left the mother's tube and built individual tubes within a day of emergence from the marsupium. Thus, amphipod offspring passed from the zygote to independent existence within the time span of a single female intermolt period.

In contrast, the development of independent existence for offspring of *Tanais cavolinii* coincided with two consecutive intermolt periods of the mother: the ovulatory period plus about ½ the anovulatory period. These offspring hatched and emerged from the brood sac as larvae (called "mancas", Kaestner, 1970). Hatching and emergence coincided with the brood sac's detachment from the female. Mancas remained inside the female's tube until they molted into miniature adults, about midway through the female's anovulatory period. They then left the maternal tube and built their own.

Occurrence of heterosexual proximity

Males of all three species attended females before the females' molt. In *Jassa falcata*, as the time for the female's molt approached, the male was generally found on the outside of the female's tube. Twelve hours before their molt, 12 of 18 females were attended by males, but 12 h after their molt only 3 of 15 were so attended (Fisher exact probability test, $P = 0.009$).

In *Ampithoe valida*, tube-sharing generally occurred before the female's molt. After the female molted, the male and female tended to separate. Seventeen female molts were observed. Twelve hours before these molts, tube-sharing was observed 16 times, but 12 h after the molts, tube-sharing was observed three times (Fisher exact probability test, $P < 0.001$). In contrast, tube-sharing was never observed either 12 h before or 12 h after the six male molts that occurred.

Tube-sharing also occurred in *Tanais cavolinii*. However, this behavior was generally observed at ovulatory, not anovulatory molts (Table 2). Tube-sharing was observed significantly more often 12 h before than 12 h after ovulatory molts (Fisher exact probability test, $P = 0.001$), while individuals generally did not tube-share when anovulatory molts occurred (Fisher exact probability test, $P = 0.891$). Tube-sharing occurred significantly more often 12 h before ovulatory than 12 h before anovulatory molts (Fisher exact probability test, $P = 0.004$). No tube-sharing was observed either 12 h before or 12 h after the eight male molts that occurred.

Table 2. *Tanais cavolinii.* The occurrence of tube-sharing before and after female molts

	Tube-sharing pairs	Non-tube scharing pairs
Ovulatory molts		
During the 12 h before the molt	10	3
During the 12 h after the molt	1	12
Anovulatory molts		
During the 12 h before the molt	2	10
During the 12 h after the molt	1	11

Thus, in all three species, a male and female were generally in close proximity shortly before the female molted and ovulated, and separated shortly afterward.

In contrast, no relationship between the occurrence of male molts and heterosexual proximity was detected. Isosexual proximity was never observed. Precopulation behavior, in which a male carries a female about before the female's molt, was never observed although it is common in nontube-building amphipods (Borowsky, in press).

Evidence for cruising males

Most individual *Jassa falcata* built their tubes perpendicularly to the substratum, so one end was closed off and the other end opened into the water column. The resident was most often found with its antennae extending from the tube's entrance.

Male *Jassa falcata* were outside the tube significantly more often than inside, both in heterosexual and isosexual pairs (Table 3: $X_1^2=54.76$, $P<0.001$; $X_1^2=111.6$, $P<0.001$, respectively). In contrast, females were outside the tube significantly less often than inside ($X_1^2=38.44$, $P<0.005$; $X_1^2=75.30$, $P<0.001$). When a male attended a female before the female's molt, females were always inside the tube, and males outside the tube.

The tubes of *Ampithoe valida* and *Tanais cavolinii* were oriented horizontally on the surface of the substratum and had two open ends. When tube-sharing occurred, the heads of the two individuals of the pair generally faced the two opposite openings of the tube.

Table 3. Presence of individuals inside vs outside the tubes

	Number inside	Number outside	Total observations	Percent outside
Jassa falcata				
Isosexual pairs				
Males	31	187	218	85.8
Females	175	46	221	20.8
Heterosexual pairs				
Males	57	168	225	74.7
Females	159	66	225	29.3
Ampithoe valida				
Isosexual pairs				
Males	264	56	320	17.5
Females	320	2	322	0.6
Heterosexual pairs				
Males	300	31	331	9.4
Females	314	18	332	5.4
Tanais cavolinii				
Isosexual pairs				
Males	224	103	327	31.5
Females	265	81	346	23.4
Heterosexual pairs				
Males	387	147	534	27.5
Females	368	166	534	31.1

Male *Ampithoe valida* generally travel between females' tubes and females generally remain in place. Both male and female *A. valida* were usually inside tubes in the pair experiments (Table 3). Of those outside the tubes, males predominated (Fisher exact probability test, $P<0.001$; $X_1^2=3.7248$, $P<0.05$, one-tailed, isosexual and heterosexual pairs, respectively). Forty-four of the 50 individuals observed outside the tubes in mass culture dishes were males. Males were more mobile than females. Four of the six females never moved during the five-minute observation period while only four of the 44 males never moved (Fisher exact probability test, $P=0.004$). The remaining males travelled to one to ten different tubes ($\bar{x}=3.20\pm2.4$ tubes touched per male).

Male *Ampithoe valida* travelled to tubes at random in mass culture dishes. Of the 128 tubes touched by males, 74 were empty, 23 contained males, 25 contained females, and 6 contained juveniles. Tube-sharing with a female occurred once. The number of empty tubes was not significantly different from the number of occupied tubes visited (74 vs 52, respectively; $X_1^2=3.13$, $P>0.05$), and the number of male and female tubes visited were also about equal (23 vs 25, respectively). Observations of *Tanais cavolinii* did not reveal whether just one or both sexes travel between tubes. Males were outside the tubes more often than females in isosexual pairs ($X_1^2=5.536$, $P<0.01$, one-tailed), but there was no significant difference between the sexes in heterosexual pairs ($X_1^2=1.632$, $P>0.05$).

Discussion

Molt and reproductive cycles

Females molted more frequently than males in *Ampithoe valida* and *Tanais cavolinii*. This is because the molt cycles of female peracarids are tied to their reproductive cycle while the males' cycles are not (Barnard, 1969).

Male *Jassa falcata* have terminal molts accompanied by the appearance of a distinct morphological characteristic, a previously unreported phenomenon in amphipods. The characteristic is the "thumb" on the males' second gnathopods. The sizes of the "thumbs" were directly correlated with the males' sizes. This suggests that the morphology of the gnathopods depends upon the size of the male at its terminal molt. Whether the number of molts is fixed in males, or whether the terminal molt is triggered by a specific stimulus is not known.

Amphipod females ovulated after each molt. Their broods exhibited direct development, and hatched into miniature adults which built tubes shortly after leaving the females' marsupium. The ovulatory molts of *Tanais cavolinii* alternated regularly with anovulatory molts. Juveniles hatched at a larval stage and remained inside the female's tube until some time after her next anovulatory molt. During this period they molted one or more times into the adult form. Only then did they leave the maternal tube and build their own. Similar observations have been reported for this species by Johnson and Attramadal (1982).

The complete reproductive cycle was longer for the tanaid than for the amphipods. Tube-sharing in *Tanais cavolinii* began about the time the juveniles left the mother's tube. The timing of the beginning of tube-sharing may be an adaptation which prevents males from cannibalizing the female's offspring (which are probably not his own). In contrast, in the amphipods, tube-sharing began before the juveniles were liberated. Cannibalization is probably prevented by the protection afforded the brood while residing within the female's marsupium. The marsupium protects the juveniles from several environmental stresses in another amphipod, *Gammarus palustris* (Borowsky, 1980b).

Reproductive behavior

The reproductive behavior of the three tube-building peracarids studied here is similar to that of *Microdeutopus gryllotalpa*, another tube-builder (Borowsky, 1980a, 1983a). A male and a female are generally in close proximity before, and separate shortly after, the female's molt and ovulation. They are found adjacent to each other (*Jassa falcata*) or in the same tube (the remaining species) just prior to the female's molt. In contrast, the occurrence of heterosexual proximity is unrelated to the male's molt. In *Tanais cavolinii* this pattern of behavior generally occurred at ovulatory, not at anovulatory molts. I have never observed precopulation behavior in these four species, but Nair and Anger (1979a) observed it in *J. falcata*.

Another similarity in the behavior of the four species is that males generally travel to receptive females' tubes, while the females remain in their own tubes. This "cruising male" behavior occurs in *Microdeutopus gryllotalpa* (Borowsky, 1983a), and *Ampithoe valida*. The evidence is only circumstantial for *Jassa falcata*. Males were outside tubes more often than females, and, when in proximity before the female's molt, males were outside, while females were inside the tubes. It seems likely that this species has cruising males, too. Cruising males occur in *Tanais cavolinii* (Johnson and Attramadal, 1982). These authors reported that male *T. cavolinii* travelled to tubes of females before, and left after copulation.

The matter of asynchronous reproduction among female amphipods requires clarification. Populations of some temperate zone species exhibit a fortnightly reproductive rhythm [i.e. *Corophium volutator* and *C. arenarium* (Fish and Mills, 1979); *C. insidosum* (Sheader, 1978); and *Lembos websteri*, and *C. bonnelli* (Moore, 1981)]. Although the majority of females of these species may be at the same reproductive stage at any given point in time, synchrony is far from perfect. Moore (1981, p 24) summarized the situation: "a semi-lunar rhythmicity in egg-laying is then an approximate summer time population phenomenon not a mandatory condition of the individuals' breeding cycle."

Evidence for cruising males in other species

Anecdotal observations of several other tube-building peracarids suggest that these species may also have the cruising male pattern of behavior (summary in Table 4).

Watkin (1941) and Fish and Mills (1979) observed that only adult males of *Corophium volutator* crawled over littoral soil during ebb tide. The latter authors noted that the period of greatest male activity occurred just before the females ovulated. In laboratory experiments, they observed that only females constructed burrows, and that a male and female were often found in the same burrow.

Crawford (1937) observed a male and a female *Corophium arenarium* in the same burrow. Fish and Mills (1979) concluded that this species' pattern of reproduction was similar to that of *C. volutator*.

Nair and Anger (1979b) observed that a male and a female *Corophium insidosum* share a tube just before and separate just after the female molt, and that copulation occurs inside that tube. No precopulatory "riding" behavior was observed. In this species, however, Nair and Anger observed females enter the males' tubes.

Shillaker and Moore (1978) observed that female *Lembos websteri* build tubes "sooner" than males, suggesting female fidelity to tube-sites. Moore (1981) observed: "males and females may cohabit or live apart, in separate tubes, with males entering the tube of a receptive female for only so long as mating necessitates."

Skutch (1926) observed a male and a female *Ampithoe rubricata* in the same tube, and suggested that copulation occurs inside. He noted the absence of carrying or "clasping behavior."

The tube-building tanaid *Leptochelia dubia* may have cruising males. Mendoza (1982) noted the occurrence of tube-sharing, that males are found outside the tubes more often than females, that males build rudimentary shelters as compared with females, and that males, not females, leave their tubes.

Two species of tube-building amphipods do not have cruising males. Both sexes of *Ampelisca abdita* enter the water column simultaneously, possibly in response to a full moon (Mills, 1967). Mills believed that they do this to find mates, and that copulation occurs either during swarming or immediately thereafter. He suggested that *A. vadorum* exhibits the same behavior.

In contrast to other tube-building amphipods, females of these species produce only one brood. Thus it is crucial that males be receptive at the same time as females. This is assured if both sexes become receptive in response to the same external stimulus (or combination of stimuli) such as the full moon. This also results in the absolute synchrony of the females' receptivity. If each female copulates only once, and at the same time as all other females, then after

Table 4. Key reproductive characteristics of tube-building peracarids

	Number of broods per female	Which sex or sexes generally travel between tubes	Is there heterosexual proximity prior to copulation	Reference
Amphipoda				
Family Corophiidae				
Corophium volutator	> 1	males	probably	Fish and Mills, 1979
Corophium arenarium	> 1	males	probably	Fish and Mills, 1979
Corophium insidosum	> 1	females	yes	Nair and Anger, 1979b
Family Aoridae				
Lembos websteri	> 1	males	yes	Moore, 1981
Microdeutopus gryllotalpa	> 1	males	yes	Borowsky, 1980a, 1983a
Family Ischyroceridae				
Jassa falcata	> 1	males	yes	present study
Family Ampithoidae				
Ampithoe rubricata	1	unknown	yes	Skutch, 1926; Bousfield, 1973
Ampithoe valida	> 1	males	yes	present study
Family Ampeliscidae				
Ampelisca abdita	1	both	no	Mills, 1967
Ampelisca vadorum	1	both	no	Mills, 1967
Tanaidacea				
Family Paratanaidae				
Leptochelia dubia	> 1	males	yes	Mendoza, 1982
Tanais cavolinii	> 1	males	yes	Johnson and Attramadal, 1982 and present study

a male copulates there should be no more receptive females available, and searching behavior is pointless. Selection should not favor the cruising male pattern of behavior when females only reproduce once.

It is possible that cruising males are not restricted to the peracarids. Individuals of the snapping shrimp *Alpheus armatus* exhibit fidelity to a fixed site (Knowlton, 1980). Females reproduce several times, some asynchronously, during the reproductive season. Knowlton observed that males move from their sites more often than females. Although some pairs remain together throughout the female's reproductive life, some males move between females and fertilize each female's brood.

Clearly, heterosexual proximity before the female molt is a mechanism that ensures access to the opposite sex during the short period when fertilization can be effected. Separation after copulation is advantageous to the female, because it permits her to utilize all the food that passes by the tube, rather than forcing her to share it with the male (Borowsky, 1980a). Separation is advantageous to the male, because it permits him to find another female that is due to molt shortly after he inseminates the current one. Thus he can produce more offspring per unit time if he moves from female to female than if he remains with the same one through her entire reproductive life.

The cruising male pattern of behavior is not without costs. It is potentially disadvantageous for a female, since there is always the possibility that no male will find her before she ovulates. It is also risky for the male, because moving between tubes subjects him to increased predation. Mendoza (1982) found a relatively high percentage of males of the tanaid *Leptochelia dubia* in fish guts and a low percentage of males in the substratum.

Acknowledgements. I thank P. Fenimore for his help in collecting animals, and R. Borowsky and two anonymous reviewers for careful review and criticism of the manuscript. This work was done, in part, with the aid of ONR Contract no. N00014-82-C-0482.

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Date of final manuscript acceptance: October 1, 1983.
Communicated by J. M. Lawrence, Tampa

Reprint II

J. Exp. Mar. Biol. Ecol., 1983, Vol. 68, pp. 39–51
Elsevier Biomedical Press

BEHAVIORS ASSOCIATED WITH TUBE-SHARING IN *MICRODEUTOPUS GRYLLOTALPA* (Costa) (CRUSTACEA : AMPHIPODA)

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Abstract: An earlier study showed that the onset of precopulatory behavior, or tube-sharing, in the amphipod crustacean *Microdeutopus gryllotalpa* (Costa) generally occurred toward the end of the females' intermolt period. Tube-sharing ended when the female molted and copulation occurred. It was hypothesized that after copulation the male would leave the female's tube, travel to another receptive female's tube, and begin tube-sharing with the new female (the "cruising male hypothesis").

The present study confirms this hypothesis for laboratory cultures. In addition, the study describes a female-typical and male-specific behavior ("blocking" and "intermittent pleopod beats"). These behaviors are only expressed during interactions between one individual who is entering, and another individual who is residing in the tube.

INTRODUCTION

The amphipod crustacean *Microdeutopus gryllotalpa* builds tubes on solid substrata and in sediments along northwestern Atlantic coasts (Bousfield, 1973). Each individual usually resides in its own tube. The tube's diameter approximates that of the animal's cross section and is open at both ends. As in most species of gammarid amphipods in the temperate zone, females generally spawn repeatedly during the reproductive season. Shortly after the female molts, copulation and then ovulation occur. Copulation must occur in the 5 to 20 min between the female's molt and ovulation because there is no sperm storage.

An earlier study established the key features of the pattern of reproductive behavior of this species (Borowsky, 1980a). Females usually reside alone in their tubes during the early intermolt stages, but share their tubes with a single male during the later stages. This tube-sharing ensures the presence of a male during the brief period when fertilization can be effected. Thus, the timing of tube-sharing is coordinated with the females' molt and reproductive cycles (Borowsky, 1980b). Generally, copulation occurs inside the tube. After the female molts, the male leaves and the female remains inside.

The present study reports the results of detailed observations of the mate-searching behavior of male *Microdeutopus gryllotalpa* (Costa) and the behaviors that occur at the onset of tube-sharing. It was hypothesized that after a male left a female's tube, he would attempt to find and enter the tube of another female due to molt and ovulate. This "cruising male hypothesis" was tested in laboratory cultures, and the results are reported here.

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In addition, tube-sharing behavior requires that the male and female tolerate each other's presence in the same tube, a reversal of their behaviors during the female's early intermolt stages. To understand better the factors which control the onset of tube-sharing, the behaviors which occur when one animal enters another's tube were observed. The results of these observations also are reported here.

Field studies on this species' behavior are impractical. The animals are small (mean length 5 mm) and both the tubes and the animals are wonderfully camouflaged. Therefore the present study was performed on animals in laboratory culture. Although mass cultures do not replicate field conditions, they are acceptable models, and, in addition, permit behavioral details to be observed under the microscope.

MATERIALS AND METHODS

GENERAL METHODS

Stocks were cultured at room temperature in 20-cm glass culture dishes in sea water diluted to 24‰ *S*. They were fed sea lettuce (*Ulva* sp.) and bits of ribbed mussel adductor (*Geukensia demissa*). All animals were obtained from these cultures and each was tested only once. All observations consisted of noting whether or not tube-sharing occurred, and the occurrence of each of three behavioral acts by each member of an interacting pair: (1) the use of gnathopods as pincers; (2) "blocking"; and (3) "intermittent pleopod beats" (IPBs).

When an individual extended its first pair of gnathopods, or claw-like limbs (Fig. 1) toward, and distinctly grasped, some part of another individual, the behavior was scored as "use of gnathopods as pincers".

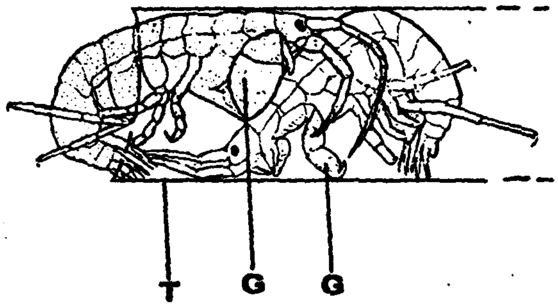

Fig. 1. Typical positions of animals when a male enters the tube of a female in the early intermolt stages (some limbs and setae removed): tube is transparent, as it appears when viewing with substage illumination; animals are on their sides; male (stippled) is pausing at tube's entrance, female is blocking; note sexual dimorphism and different positions of the two animals' first pair of gnathopods; G, first gnathopods; T, outline of tube; modified from plate XLIX, Bousfield, 1973; copyright 1973 by the National Museum of Canada; used by permission of the publisher, Cornell University Press.

During "blocking", the animal flexed its body ventrally while pressing the dorsal part of the thorax against one side of the tube and the head against the opposite side (Fig. 1). This is almost always performed with the dorsal part of the animal's thorax facing its opponent's venter. At most other times the animal's body was extended within the tube so the dorsal part of the head, thorax, and abdomen formed a straight line.

Intermittent pleopod beat behavior involved the use of the pleopods, ventral abdominal respiratory and swimming limbs that generally move in an uninterrupted, coordinated rhythm. During IPBs, the pleopods were stopped for ≈ 1 s, then stopped for another second. This cycle was often repeated three or four times before the regular rate was resumed.

A tube-share was scored when an encounter resulted in both animals remaining in the tube with their heads at opposite openings for at least 1 min. Earlier observations had shown that if these positions were maintained for 1 min, tube-sharing would continue until the female molted. A non-tube-share was scored when either one or both individuals left the tube after an encounter.

Encounters were often brief and occasionally interactions occurred too rapidly to score unequivocally. Only the animals whose behavior was clearly observed were included in the data analysis. The level of significance chosen for statistical analysis was 0.05 or less, and all probabilities were two-tailed, except where noted.

UNSTAGED ENCOUNTERS

Observations without a microscope

Each of five mass culture dishes was placed on a white background and left undisturbed for 15 min before observations were made. Seventy-five encounters that occurred when an adult of either sex attempted to enter another individual's tube were observed. If one animal left the tube, its behavior was observed for an additional 5 min. After the resolution of each encounter, the pair was removed, the sex and relative size of each animal noted, and the animals discarded.

Observations with a microscope

To permit more accurate observations of animals inside tubes, each of seven other mass culture dishes was placed under a dissecting microscope. The same procedure was followed as for observations without a microscope, except that only the results of 41 heterosexual encounters during which a male entered a female's tube were recorded.

STAGED ENCOUNTERS

Animals were placed in individual glass culture dishes (10.5 cm) at 20 °C, 15:9 L:D cycle, to mimic field conditions. The dates of the females' molts were noted. Observations were made on each interacting pair of animals using a dissecting microscope at 12 × magnification. Such interactions occurred when one individual, placed in the dish of another, attempted to enter the latter's tube. Seventy-three isosexual female encounters were staged. To determine the possible effects of the females' intermolt stage on their behavior, about half the females tested were 2 days, and half the females 6 days past their previous molt. (The average length of the females' intermolt period is 6.9 days at 20 °C; Borowsky, 1980b). In addition, 20 isosexual male encounters were staged. The intermolt stages of the males were unknown.

One hundred six heterosexual encounters in which males were introduced to females' tubes were staged. Two-day, 4-day and 6-day post-molt females were employed (30, 46 and 30 females, respectively).

To determine whether IPBs stimulate females to tube-share, 60 tests were performed as follows: each of 30, 2-day post-molt females in individual dishes was presented with a live male who had been immobilized by immersion in a bed of frozen sea-water slush. This treatment eliminated IPB behavior. The male was introduced into the females' tubes head first, as would have occurred if the male was entering the tube on his own. Males were always introduced into the opening the female was currently facing. The females' tube-sharing behavior was then observed. Tube-sharing was scored if the female turned around and remained in the tube for at least 3 min. In addition, the same procedures were performed on 30, 6-day post-molt females.

RESULTS

BEHAVIOR DURING UNSTAGED ENCOUNTERS

Observations without a microscope

The adult sex ratio in the culture dishes was close to 1. Most adults were inside tubes. Of those observed outside, the majority were males (75 observations; 10 females and 65 males outside: binomial test $z = 6.235$, $P < 0.001$). Animals outside tubes attempted to enter the nearest tube. An interaction then occurred between the resident (if present) and the enterer. Then, one of four outcomes ensued: either (1) the entering animal remained inside and the original occupant left; or (2) the entering animal left and the original occupant remained; or (3) both remained; or (4) both left. When any animal left a tube, it walked to and attempted to enter another nearby tube. Then another interaction occurred, which ended in one of the four possible outcomes. Since males were most often outside, it is concluded that males generally travel from tube to tube, while females remain inside.

Males entered other individuals' tubes at random (30 males', 29 females', five juveniles' and one tube-sharing pair's tubes were entered). If the male entered another male's tube, an agonistic encounter occurred after which the larger individual generally remained alone inside (25 encounters involving males of different sizes; 18 larger and seven smaller males were the sole final occupants: binomial test, $P = 0.04$). There was no significant difference between the number of entering males and the number of original occupants who were the final occupants (30 encounters; 16 entering and 14 original occupants were the final occupants: binomial test, $P > 0.05$).

When males entered females' tubes, the females more often remained inside and the males left (18 females and 11 males remained), but the difference was not significant (binomial test, $z = 1.114$, $P > 0.05$). There was no significant relationship between size and final occupancy (24 encounters involving animals of different sizes; 16 males and eight females were larger; seven larger and two smaller males remained, and nine larger and six smaller males left the tubes: Fisher exact probability test, $P = 0.333$). No tube-sharing occurred during these observations.

The presence of some females outside tubes in culture dishes (10 of 75; 13.3%) may be the result of those interactions after which the female, rather than the male, left the tube. Females entered eight other females', one male's, and one juvenile's tube. As in the staged isosexual encounters (see below), the larger female most often remained inside and the smaller left the tube (six encounters involving animals of different sizes, five larger and one smaller female remained inside; but the difference was not significant: binomial test, $P > 0.05$). Tube-sharing occurred when the female entered the male's tube, and the juvenile left when the female entered its tube.

Observations with a microscope

Tube-sharing occurred four of the 41 times a male entered a single female's tube during these observations (Table IA). When there was only one final occupant, it was generally the female (37 observations; females remained 26 and left 11 times; binomial test $z = 2.302$, $P < 0.05$). This result is similar to what was seen during observations without a microscope (see above). Again there was no relationship between size and final occupancy (35 encounters involving animals of different sizes: 27 males and eight females were larger; eight larger and three smaller males remained, 19 larger and five smaller males left: Fisher exact probability test $P = 0.806$).

TABLE I

Relationship between female behaviors and tube-sharing during heterosexual encounters observed under the microscope.

	Blocks	Gnathopod uses	Blocks plus gnathopod uses	Neither	Total
A. Unstaged encounters					
Tube-shares	2	0	0	2	4
Nontube-shares	8	17	3	9	37
Total	10	17	3	11	41
Staged encounters					
Tube-shares	10	5	4	32	51
Nontube-shares	22	9	10	14	55
Total	32	14	14	46	106

Males exhibited IPBs during each of the four tube-shares observed and only once during the other 37 encounters in which tube-sharing did not occur.

Thirteen of the 41 females exhibited blocking behavior (Table IA) and not one of these left their tubes. In contrast, of the 28 cases when females did not block, 11 ended with the female's eviction (females block 13 and leave zero times; females do not block 28 and leave 11 times; Fisher exact probability test, $P = 0.006$). This suggests that the function of blocking is to inhibit males from ejecting resident females.

BEHAVIOR DURING STAGED ENCOUNTERS

Isosexual encounters

Females. When one female entered another female's tube an agonistic interaction occurred after which one animal remained inside and the other left. The larger female was generally the final resident (52 times out of 68 encounters involving animals of different sizes: binomial test, $P < 0.001$). There was no significant difference between the number of original occupants and the number of entering females that were the final occupants (73 encounters in which one animal remained inside; 42 entering and 31 original occupant females were final occupants: binomial test, $z = 0.590$, $P > 0.05$). Therefore, as in the unstaged encounters, size, not original possession of the tube, was the principal determinant of final occupancy.

Some females left their tubes immediately after another female entered. Typically, however, most original occupants remained and extended their gnathopods toward the entering female. Both animals then used their gnathopods against each other. Females blocked occasionally during isosexual encounters, but the frequency of this behavior

TABLE II

Behavior of females during staged encounters.

Blocks	Gnathopod uses	Blocks plus gnathopod uses	Neither	Total	% Blocks
A. Isosexual encounters					
1. Original occupants					
3	52	4	13	72	9.7
2. Enterers					
2	57	0	7	66	3.0
B. Heterosexual encounters					
1. 2 days post-molt					
17	3	4	6	30	70.0
2. 4 days post-molt					
10	9	9	18	46	41.3
3. 6 days post-molt					
5	2	1	22	30	20.0
4. Totals					
32	14	14	46	106	

was low (nine of 138 females, Table IIA). Females never expressed IPBs in isosexual encounters.

These data also show that original occupants used their gnathopods about as often as entering females (Table IIA; 56 of 72 residents and 57 of 66 entering females employed their gnathopods: $X^2_2 = 1.711$, $P > 0.05$). But regrouping the data on gnathopod use by the females' intermolt stages shows that 2-day post-molt females used them significantly more often than 6-day post-molt females (Table IIIA: $X^2_2 = 7.943$, $P < 0.01$). Interestingly, there was no difference in the use of gnathopods between entering females of the two intermolt stages, but the difference between original occupants of the two stages was highly significant (Table IIIB, C: Fisher exact probability test, $P = 0.518$ and $P = 0.001$, respectively). Thus, females in tubes behave less agonistically to entering females when they are in the later intermolt stages than they do when they are in the early intermolt stages.

TABLE III

Use by females of gnathopods during staged isosexual encounters.

Days post-molt	Use of gnathopods	
	Yes (%)	No (%)
A. All females		
2	62 (92.5)	5 (7.5)
6	53 (74.6)	18 (25.4)
B. Entering females		
2	29 (90.6)	3 (9.4)
6	29 (87.9)	4 (12.1)
C. Resident females		
2	33 (94.3)	2 (5.7)
6	24 (63.2)	14 (36.8)

Males. The behavior of males was similar to the females during isosexual encounters. Of 39 males clearly observed, 23 employed their gnathopods, one blocked, and none exhibited IPBs. Final occupants tended to be larger (after 18 encounters 13 final occupants were larger) and/or enterers (after 20 encounters 14 final occupants were the enterers), but the differences were not significant (binomial tests, $P > 0.05$).

Heterosexual encounters

Females. Females behaved differently to entering males than they did to entering females. First, females tube-shared with males, but never with females. Secondly, they employed their gnathopods toward females significantly more often than toward males (Table II: original occupant females only; 56 of 72 vs. 28 of 106 females, respectively:

$X_2^2 = 45.389, P < 0.001$). And thirdly, females blocked significantly more often to males than to females (Table II: original occupant females only; 46 of 106 vs. 7 of 72 times, respectively: $X_2^2 = 23.252$, $P < 0.001$). The frequency of blocking decreased as the females' intermolt stage advanced (Table IIB: $X_2^2 = 15.411$, $P < 0.001$). But, as in isosexual encounters, females never expressed IPBs.

Males. Males behaved differently toward resident females than they did to males. IPBs and tube-sharing occurred in heterosexual, but not in isosexual encounters. In addition, the frequency of IPBs increased as the females' intermolt stage advanced (Table IV: $X_2^2 = 19.974$, $P < 0.001$). When only one animal remained, it was most often the male (40 of 55 encounters: binomial test, $z = 3.260$, $P < 0.001$).

TABLE IV

Relationship between intermittent pleopod beats (IPBs) and tube-sharing in staged heterosexual encounters.

	IPBs	No IPBs	% IPBs	% Tube-shares
Females 2 days, post-molt			30	30
Tube-shares	9	0		
Nontube-shares	1	20		
Females 4 days, post-molt			77	43
Tube-shares	19	1		
Nontube-shares	1	25		
Females 6 days, post-molt			87	73
Tube-shares	22	0		
Nontube-shares	4	4		
All days combined				
Tube-shares	50	1		
Nontube-shares	6	49		

In contrast, in unstaged encounters, the sole final occupants were most often females (44 of 66 encounters, observations with and without a microscope combined). The difference between the sexes of the winners in staged vs. unstaged encounters was significant ($X_2^2 = 18.634$, $P < 0.001$).

Function of blocking during heterosexual encounters. Tube-sharing increased as the females' intermolt stage advanced in this study (Table IV: $X_2^2 = 11.982$, $P < 0.001$), as in an earlier one (Borowsky, 1980a). Blocking was shown to be a female-typical behavior which was expressed more often when males, rather than females, entered a resident female's tube. Also, blocking decreased in frequency as the females' intermolt advanced. Further, in staged encounters, females who tube-shared generally did not block (Table IB; blocking and gnathopods plus blocking alone classes combined vs. gnathopods alone and neither behavior classes combined: $X_2^2 = 10.174$, $P < 0.01$). Finally, the female appeared to physically occlude the tube when she blocked (Fig. 1).

These observations suggested that the female limited tube-sharing by blocking. That is, the absence of blocking behavior allowed the male (seeking to tube-share with any female, regardless of her intermolt stage) to enter the tube. However, this hypothesis can not be fully correct, because (1) tube-sharing occurred after 10 of the 32 encounters during which the female expressed only blocking behavior, and (2) did not occur after 25 of the 60 encounters during which females did not block (according to the hypothesis 31 and 42% "incorrect" responses to blocking, respectively, Table IB).

The function of IPBs during heterosexual encounters. IPBs were only observed in males, and then only in the presence of females. Further, the frequency of IPBs increased as the females'intermolt periods advanced. Finally, tube-sharing and IPBs were closely correlated (Table IV, all days combined). This suggested that IPBs might stimulate the females' tube-sharing behavior.

This hypothesis cannot be fully correct, however, because females tube-shared with ice-immobilized males. Six-day post-molt females tube-shared with such males significantly more often than did 2-day post-molt females (Table V: $X_2^2 = 8.540$, $P < 0.01$). Most important, however, observations on 6-day post-molt females showed that there was no difference in the frequencies of tube-sharing with immobilized vs. untreated males (Table V: $X_2^2 = 0.373$, $P > 0.05$).

Thus, while IPB behavior is closely associated with the onset of tube-sharing it is not an important stimulus for the females' tube-sharing behavior.

TABLE V

Female tube-sharing behavior with ice-immobilized males.

Days post-molt	Number that tube-share (%)	Number that do not tube-share (%)
Ice-imobilized males		
2	13 (43.4)	17 (56.7)
6	24 (80.0)	6 (20.0)
Untreated males		
6	22 (73.3)	8 (26.7)

DISCUSSION

CRUISING MALES IN *MICRODEUTOPUS GRYLLOTALPA*

Data from the present and an earlier study show that the "cruising male" pattern of reproduction occurs in mass cultures of *M. gryllotalpa*. In an earlier study (Borowsky, 1980a), it was shown that most individuals were found inside tubes. The few individuals outside tubes were generally males. The present study corroborates this and, in addition, shows that these outside, or cruising males, walk from tube to tube attempting to enter each regardless of the sex or age of the occupant. Once at least partially inside, a male's behavior depends upon the sex and the intermolt stage of the occupant (if female).

If a single male is the occupant, an agonistic encounter occurs after which the smaller male leaves the tube. If a female at the beginning of her intermolt is encountered, the male generally leaves the tube even if he is the larger of the pair. If a solitary female at the later intermolt stages is encountered, tube-sharing occurs. If a tube-sharing pair is encountered, the female moves aside, and the two males engage in an agonistic exchange, after which the larger one remains and the smaller male leaves the tube (Borowsky, 1980a). In single pair cultures, once tube-sharing was begun, the male usually did not leave the tube until the female molted. Thus, unless he is evicted by a male larger than himself, the successful male probably remains with the female until copulation occurs after she molts. Most males left the tube within 24 h after the molt, thus becoming active cruisers again.

Entering males attempted to evict both tube-sharing and lone resident males. It is obviously worthwhile to evict a resident male that is tube-sharing, for if this is accomplished, the entering male will gain access to a competent female. But why did males attempt to evict solitary males? The most likely answer is that under laboratory conditions an entering male does not know the nature of the tube's occupant(s) before he enters. Cruising males entered tubes at random. In addition, the resident male of a tube-sharing pair usually rushed to the opening through which the entering male was passing, thus engaging him immediately in an agonistic encounter, and possibly preventing him from determining whether or not a female was also present.

Females left their tubes significantly more often after staged rather than unstaged encounters with males. This is probably explained by the difference in treatment of the males. Males were agitated during pipetting to the female's dish in staged encounters, but they were relatively undisturbed in culture dishes. Agitated males may enter a tube to seek protective shelter rather than access to a female. In the former case non-receptive females probably either leave or are ejected, but in the latter case non-receptive females probably would remain and males move on. In the field males may enter a tube for either motive.

Briefly, the advantage of cruising behavior to the male is that he can fertilize more broods per unit time by passing from one receptive female to another than if he remained with the same female through her reproductive life. The disadvantage to the male is that moving between tubes submits him to increased predation pressure. Residence within the camouflaged tubes undoubtedly affords protection against such visual amphipod predators as the flounder *Pseudopleuronectes americanus* (Kennedy & Steele, 1971).

There are also advantages to the female. First, she normally does not have to leave the tube to have her brood fertilized, thus minimizing the chances of predation. And second, since she feeds upon whatever passes by her tube, she has access to all of the food at the beginning of the intermolt, when she is alone, and only has to share it toward the end, when the male is also in residence.

Field and laboratory studies have shown that several other species of tube-dwelling gammarids also exhibit behaviors consistent with the cruising male hypothesis (reviewed by Borowsky, in prep.). The pattern may ultimately be demonstrated in many territorial crustaceans, and possibly in territorial species of other taxa as well.

FUNCTIONS OF THE THREE BEHAVIORS OBSERVED

Gnathopod uses

Both sexes used their gnathopods during isosexual encounters. Since all isosexual encounters resulted in at least one animal leaving the tube, these encounters may be considered agonistic, and the gnathopods, which are claw-like, may be considered the chief means of determining the winner of such encounters. Since both males extended their gnathopods toward each other during these encounters, the gnathopods of one male generally grasped the gnathopods of the other. However, any exposed part of an opponent was occasionally grasped.

The larger the animal, the larger the gnathopods, which grow linearly with increasing body length in females, but geometrically with length in males (unpubl. data). Since the larger animal was generally the one that remained inside the tube, winning may be the direct result of possessing the larger set of gnathopods.

Individuals of both sexes used their gnathopods during heterosexual encounters as well. But when tube-sharing did not occur the outcome of these encounters was related to the animals' sex and the conditions of the experiment, not to the size of the animals; males most often remained alone in the tubes after staged encounters, and females most often remained alone after unstaged encounters.

Blocking

Blocking behavior was expressed almost exclusively by females, and generally directed at males. In addition, its frequency decreased as the female's intermolt advanced. The data show that blocking did not physically prevent male entries (tube-sharing occasionally occurred in spite of blocking) but did prevent female evictions (in unstaged encounters). Thus, it appears that, in general, females in the early intermolt stages, who are not receptive to tube-sharing, indicate their non-receptivity by blocking, and this prevents males from evicting them from their tubes. It should be noted that blocking did not prevent evictions when males entered females' tubes to gain access to the tube itself, rather than to the female.

Preventing female evictions may save energy for both sexes. If a female is at the early intermolt stages, a male would waste energy by evicting the female since all that would be gained would be an empty tube which would soon be abandoned in his search for another female. If a female is at the later intermolt stages, a male would be defeating the purpose of his entering the tube if he evicted her. Evictions waste the females' energies too, for after evictions they must either search for or build new tubes.

If blocking prevents evictions, why don't males block, and why don't females block more often to entering females? The most probable explanation is that blocking prevents evictions by unequivocally identifying its performer as a non-receptive female. Thus, males do not block to males because while blocking might prevent their eviction, it might also lead to the entering male's remaining inside to tube-share. Tube-sharing occasionally followed when a female blocked (Table I). Neither males nor females block to entering females, because this would not inhibit evictions; isosexual tube-sharing is eschewed.

Why has blocking, rather than some other behavior evolved to indicate the female's non-receptivity? Perhaps this behavior protects the female's relatively delicate ventral structures (the gills, brood pouch, and especially the brood) from damage from the male's gnathopods, should he use them.

Intermittent pleopod beats

This behavior was expressed exclusively by males during heterosexual encounters. In addition, the frequency of IPBs increased as the female's intermolt stage advanced. IPBs and tube-shares were closely correlated. IPBs were expressed during all but one of the 55 encounters that ended in tube-sharing, and during only seven of the 92 encounters that ended in non-tube-sharing (98.2 and 7.6%, respectively; staged and unstaged encounters combined). However, observations of females presented with males who did not express IPBs clearly demonstrated that IPBs do not stimulate the female's tube-sharing behavior.

The close correlation between the onset of tube-sharing and IPBs suggests that IPBs indicate the male's perception of the female's receptivity. Thus, although its function is unknown, IPB behavior should prove a powerful diagnostic tool in attempts to determine how the female stimulates the male to tube-share.

CONCLUSION

One of the objectives of this study was to learn whether the behaviors observed when one individual attempted to enter another's tube stimulated either or both sexes to tube-share. The data show that none of the three behaviors observed stimulate tube-sharing. However, the data do offer clues about how the onset of tube-sharing is regulated.

It is possible that the stimulus for the female's tube-sharing behavior is an internal physiological trigger. Females were less agonistic to individuals entering their tubes at the end as opposed to the beginning of their intermolt period. This was true for entering males, females and anesthetized males. Since, in the field, the entering animal would most often be a male, the physiological stimulus would most often result in successful tube-sharing.

But the stimulus for the male's tube-sharing is something other than the female's passive behavior. Males tube-shared with some females who blocked and/or used their gnathopods.

Based on the data, the most parsimonious hypothesis to explain how the tube-sharing behavior of both sexes is controlled is as follows: as the female's intermolt advances, she becomes increasingly more receptive to tube-sharing as the result of physiological changes. But, in addition, these physiological changes cause the expression of another factor which stimulates the males to tube-share. Efforts to identify this unknown female factor are currently underway.

ACKNOWLEDGEMENT

I thank an anonymous reviewer for very constructive criticisms.

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Reprint III

Mar. Behav. Physiol. 1983, Vol. 9, pp. 319–325
0091-181X/83/0904-0319 $18.50/0

Printed in Great Britain

Placement of Eggs in their Brood Pouches by Females of the Amphipod Crustacea *Gammarus palustris* and *Gammarus mucronatus*

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(Received January 15, 1983; in final form March 28, 1983)

Unhatched eggs removed from the brood pouches of two species of littoral amphipods were placed in dishes either with their mothers, other mothers of the same species, or mothers of the other species. Females either ate the eggs, left them alone, or placed them in their brood pouches. In contrast, males of both species ate every egg presented to them. *Gammarus mucronatus* females treated all classes of eggs about the same way. But *G. palustris* replaced more and ate fewer *G. palustris* than *G. mucronatus* eggs.

INTRODUCTION

The amphipods are among the most successful species that occupy the littoral zone, both in numbers of individuals and in numbers of species (Green, 1968), yet their life histories differ markedly from the majority of other littoral species. Other species generally lay eggs which hatch at an immature stage, enter the plankton for a period of development, and then metamorphose into a benthic adult (Thorson, 1950). In contrast, amphipod females deposit their eggs in a ventral brood pouch, where the eggs develop directly and hatch into juveniles which resemble the adults. In addition, the hatched juveniles spend some time within the marsupium before leaving or being cast off from the mother simultaneously with her molt.

Amphipod females thus exhibit brood care, while most other littoral species do not. But the type of care just described does not require female behaviors directed toward the developing brood. The present study was undertaken to learn whether females take a more active part in caring for their broods. The brood pouch is open to the environment, and an egg can be dislodged, especially when part of a large brood. The following questions were asked: do females replace eggs in their brood pouches? And, if so, do females replace their own more often than other individuals' eggs?

MATERIALS AND METHODS

All experimental animals were taken from the intertidal zone at low tide at Jamaica Bay, Gateway National Park, Brooklyn, New York, in May and June of 1981 and 1982. Seventy *Gammarus palustris* and 70 *Gammarus mucronatus* females were used. Within 24 hours of capture all the unhatched eggs from each female's marsupium were removed by anaesthetizing the female with frozen sea water, then forcing the eggs out between the oostegites with a stream of water from a glass pipette. The female and the eggs were placed immediately either in the same or different 10.5 cm diameter glass culture dishes containing water from the collection site. The broods of the 70 *G. palustris* females were distributed in the following way: each of ten broods was placed in the dish of one of ten *G. palustris* males; each of 20 broods was placed in their mothers' dish; each of 20 broods was placed in the dish of one of 20 *G. palustris* females not their mothers; and each of the remaining 20 broods was placed in the dish of one of 20 *G. mucronatus* females. The 70 *G. mucronatus* broods were distributed similarly: ten to male *G. mucronatus'; 20 to their mothers' 20 to other G. mucronatus* females'; and 20 to *G. palustris* females' dishes. Broods were always placed in the dishes of females whose body sizes matched that of the broods' mothers.

Adults and their dishes were examined 24 hours after removing the broods. All the eggs were again removed from the females' brood pouches and counted. In addition, any eggs remaining free in the dishes were counted.

RESULTS

The average number of eggs per brood for *Gammarus mucronatus* and *Gammarus palustris* was: $\bar{x} = 22.8 \pm 11.0$, and $\bar{x} = 6.4 \pm 25.7$, respectively; *G. palustris* had significantly fewer eggs per brood although females of the two species were matched for size ($t = 7.035$, $df = 135$, $p < 0.001$). Other than broods, nothing was found in *G. mucronatus* brood pouches. The same was true for *G. palustris*, except for the presence of a few individuals of an as yet unidentified nematode in many brood pouches.

Males of both species ate every egg placed in their dishes (230, and 205 eggs, *G. palustris* and *G. mucronatus*, respectively). Females treated eggs in one of three ways: either they ate them, placed them in their brood pouches or left them lying in the bottom of the dish.

The method of the females' treatment of eggs was observed frequently. A female moving about the dish would encounter an egg, pick it up with the gnathopods and bring it to the mouth. The egg was rotated for a few seconds between the mouthparts and the gnathopods, and then either dropped, eaten, or passed ventrally and posteriorly into the marsupium through its anterior ventral gap behind the head.

G. mucronatus treated the three classes of eggs in the same way. The proportions of eggs remaining in the dishes, placed in the brood pouches, and eaten, were not significantly different among the three types of eggs (Kruskal–Wallis tests: $H = 4.6812$, $H = 0.1382$, $H = 0.3158$, respectively; all $dfs = 2$, all $ps > 0.05$). (Comparisons were made using proportions rather than actual numbers of eggs per brood because of the range of brood sizes (see Table I).)

In contrast, *G. palustris* treated the different types of eggs differently. First, there was a significant difference among the proportions of the types of eggs replaced (Kruskal–Wallis test: $H = 10.1636$, $df = 2$, $p < 0.01$). The more distantly related the species of the eggs were to the female, the less the proportion of eggs that

Table I

Treatment of experimentally removed eggs by female gammarids

	Number females tested	Mean number eggs in brood	Mean number eggs remaining in dishes	Mean percent eggs remaining in dishes
Gammarus palustris (*G. p.*)				
own eggs	20	16.3 ± 9	7.0 ± 6	18.15 ± 22.9
other *G. p.* eggs	20	18.6 ± 6	7.2 ± 5	24.05 ± 22.7
G. m. eggs	20	60.3 ± 29	15.7 ± 15	19.30 ± 16.4
Gammarus mucronatus (*G. m.*)				
own eggs	20	54.0 ± 23	13.0 ± 8.4	20.40 ± 14.7
other *G. m.* eggs	20	32.8 ± 14	8.7 ± 6	9.95 ± 6.7
G. p. eggs	20	29.7 ± 12	9.4 ± 6	12.95 ± 7.0

	Mean number eggs replaced in brood pouches	Mean percent eggs replaced in brood pouches	Mean number eggs eaten	Mean percent eggs eaten
Gammarus palustris (*G. p.*)				
own eggs	11.6 ± 9	65.45 ± 23.9	4.8 ± 4	21.85 ± 17.4
other *G. p.* eggs	10.2 ± 6	53.30 ± 30.2	7.1 ± 6	28.35 ± 25.3
G. m. eggs	26.7 ± 15	34.55 ± 29.5	25.1 ± 15	48.65 ± 32.0
Gammarus mucronatus (*G. m.*)				
own eggs	28.4 ± 24	44.8 ± 30.0	20.4 ± 15	43.25 ± 28.3
other *G. m.* eggs	20.8 ± 15	48.55 ± 36.4	11.7 ± 9	46.35 ± 35.8
G. p. eggs	17.8 ± 14	52.70 ± 35.8	11.8 ± 10	42.40 ± 36.8

were replaced (see Table I). The difference between the females' own eggs and other *G. palustris* eggs was not significant (Mann–Whitney U test; $U_{20,20} = 157$, $p > 0.05$), but the difference between all *G. palustris* eggs and *G. mucronatus* eggs was significant (Mann–Whitney U test: $U_{20,40} = 211.5$, $z = 2.9559$, $p < 0.01$).

Second, there was a significant difference among the proportions of eggs eaten (Kruskal–Wallis test: $H = 9.740$, $df = 2$, $p < 0.01$). The more distantly related the eggs were to the female, the *greater* the proportion consumed (see Table I). Again, the difference between the females' own eggs and other *G. palustris* eggs was not significant (Mann–Whitney U test: $U_{20,20} = 200.5$, $p > 0.05$), but the difference between all *G. palustris* eggs and *G. mucronatus* eggs was significant (Mann–Whitney *U* test: $U_{20,40} = 201$, $z = 3.434$, $p < 0.001$). In contrast, there was no difference in the proportions of eggs remaining in the dishes among the three types of eggs (Kruskal–Wallis test: $H = 1.0345$, $df = 2$, $p > 0.05$).

Thus, *G. mucronatus* females did not distinguish among the three types of eggs, but *G. palustris* females replaced more and ate fewer *G. palustris* than *G. Mucronatus* eggs.

DISCUSSION

The present study shows that *Gammarus palustris* and *Gammarus mucronatus* replace their own eggs in their brood pouches. Another gammarid amphipod, *Melita zeylandica* (Stebbing), also exhibits this behavior (Krishnan and John, 1974). Since newly captured females had only eggs (and for *G. palustris*, a nematode) in their brood pouches, it seems likely that these females do not place objects other then eggs in their brood pouches in the field.

Newly hatched juveniles of several species can reenter the brood pouches (*Marinogammarus obtusatus*, Sheader and Chia, 1979; *Neohaustorius schmitzi*, Croker, 1968; *G. palustris*, Borowsky, 1980a), but there is no evidence that the mother assists the juveniles' reentries. Instead, juveniles encounter the female's body during the course of their movements in the dish and enter the brood pouch because it is an available hiding place. In *Marinogammarus obtusatus*, it was observed that juveniles did not necessarily prefer their mother's brood pouches, but preferred the brood pouches of any female in the later intermolt stages (Sheader and Chia, 1970). Thus, it appears that the motive force in egg replacement is the female, but the motive force in juvenile reentries is the juvenile.

Displaced eggs which are not replaced in the brood pouch are probably lost, either because they fail to develop, or as the result of predation pressure. The majority of eggs removed from the brood pouches of *Marinogammarus obtusatus* became diseased and failed to develop (Sheader and Chia, 1980). Predation may be particularly significant during the eggs' later developmental stages, at least for the two species studied here, because at that time precopulation, a behavior which involves a male carrying a female about, generally occurs (Borowsky, 1978; personal observation). As the present study suggests, the male would readily eat the displaced egg if it was encountered.

The frequency of egg replacement in the field is unknown. Neither the number of healthy eggs displaced from the brood pouch nor the number returned is known. It is unlikely that, as in the experiments described here, all the eggs of a given brood are lost from the brood pouch. But displacement of a few eggs may be common. I have often observed eggs displaced from the brood pouches of females with large broods during field collections. Females may displace eggs when agitated for other reasons as well. Replacement might occur when the female was not actively swimming; when feeding on benthic algae or during periods of exposure.

The two species investigated here differed in their treatment of the three classes of broods. In *G. palustris,* the more distantly related the female was to an egg, the less likely she was to replace it, and the more likely she was to eat it. In contrast, *G. mucronatus* did not treat the different types of eggs differently.

G. palustris' treatment of the different types of eggs is consistent with the hypothesis, suggested earlier, that this species exhibits a greater degree of maternal care than does *G. mucronatus* (Borowksy, 1980b). Additional support for this idea is that *G. palustris* eggs are larger than *G. mucronatus* eggs (Borowsky, 1978), the mean number of eggs per brood is smaller (present study) and hatched juveniles remain in the brood pouch longer in *G. palustris* than in *G. mucronatus* (Borowksy, 1980b). The explanation for the greater extent of maternal care may be that *G. palustris'* habitat is at the high tide mark, and *G. mucronatus'* habitat is at the mean tide mark extending into the subtidal zone. The former habitat is more stressful to marine organisms, and it has been observed in other groups of animals that stressful environmental conditions tend to select for maternal care (Wilson, 1975).

Acknowledgments

This study was supported, in part, by ONR Contract No. N00014-82-C-0482.

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Marine Biology 75, ■ ■ (1984)

Marine
Biology
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Preprint I

Effects of receptive females' secretions on some male reproductive behaviors in the amphipod crustacean *Microdeutopus gryllotalpa*

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Abstract

The use of a choice test apparatus has shown that receptive females of the tube-building amphipod crustacean *Microdeutopus gryllotalpa* (Costa) secrete a water-borne substance which attracts males. This substance (1) increases the level of activity of males, and (2) orients the males' movements towards its source. Males do not secrete this substance. In this species, females tend to remain inside their residential tubes, and males generally travel from receptive female to receptive female. Thus, in the field, the attractant may stimulate the male to accelerate his movements toward, and guide him directly to a receptive female. On the other hand, at the concentration tested, the substance alone does not elicit "intermittent pleopod beats", a male-specific behavior that only occurs in a reproductive context. The expression of "intermittent pleopod beats" is shown to require the presence of a female plus its tube.

Introduction

Female secretions have been implicated as sex pheromones in many aquatic Crustacea. They have been shown to function as attractants in some species (i.e. in the amphipod *Gammarus duebeni:* Dahl *et al.*, 1970) and as stimuli for courtship behaviors in others (i.e. in the crab *Carcinus maenas:* Eales, 1974). However, the ranges of the function(s) of specific pheromones have not been clearly defined (Dunham, 1978). In part, this is because some of the species studied do not adapt well to laboratory conditions, which hampers efforts to describe the repertoire of their typical reproductive behaviors. In contrast, the amphipod crustacean *Microdeutopus gryllotalpa* adapts well to the laboratory, and its reproductive behavior has been described in detail (Borowsky, 1980, 1983a). Therefore, it was thought that studies of this species might permit the specific definition of any effects that receptive female secretions have on the males' reproductive behavior.

Microdeutopus gryllotalpa is a marine species that builds individual residential tubes. Females breed several times in succession, but do not store sperm. Thus, a male and female must have access to each other when the female molts, for ovulation occurs shortly thereafter. To guarantee this access, a male generally enters and remains in a female's tube a few days before the female molts. The onset of tube-sharing is generally accompanied by a male-specific behavior, "intermittent pleopod beats", or "IPBs" (Borowsky, 1983a). Then, within three to four hours after the molt, copulation occurs. Shortly after that, the male leaves. The female remains alone until she is due to molt again, whereupon tube-sharing is reinitiated with another male. Upon leaving the female, the male travels immediately to other individuals' tubes, testing the nature of each occupant, until he finds another female with which to tube-share (called "cruising behavior", Borowsky, 1980, 1983b).

Thus, males exhibit three reproductive behaviors that occur in sequence: first, travel to the female's tube; second, IPBs and tube-sharing; and third, copulation. It was reasoned that female secretions might stimulate any or all of these behaviors, either entirely or in part. This paper reports the results of studies designed to determine whether receptive female secretions, by themselves, affect males' travels and stimulate IPBs.

Materials and methods

Effects of different individuals' secretions on the level of males' activities and as male attractants

The choice test apparatus

Description. The test apparatus was based on a glass Y-tube (see Fig. 1). The base of the Y was connected to a

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one-liter aspirator bottle (the "departure bottle") by Tygon tubing (7 mm i.d.) while each of the arms was similarly connected to two 2-l aspirator bottles in series. The distal bottles were slightly taller than the proximal bottles (28.5 and 24.5 cm high; the "tall aspirator bottles" and the "arrival bottles", respectively). *Microdeutopus gryllopta* (Costa) whose secretions were to be tested for their attractiveness to males (the "test individuals") were placed in the tall aspirator bottles. During a test, fresh sea water dripped into the tops of these bottles at the rate of 100 ml h^{-1} each, and continued out at the same rate through their spouts. Thus, water flowing out of these bottles had bathed the test individuals. This water then passed through two 47-cm lengths of tubing, which led into the tops of the arrival bottles. The water flowed out of the arrival bottles' spouts through 8-cm lengths of tubing, and continued into the arms of the Y-tube. A single 13-cm length of tubing carried the water from the base of the Y to the spout of the departure bottle. The water exited the system through the top of the departure bottle. To prevent individuals from passing between the tall and arrival bottles, the ends of the connecting tubes were covered with nylon mesh netting, which did not impede the flow of water.

Twenty-four hours before each test the apparatus was assembled, and the appropriate test individuals were placed in the tall aspirator bottles. *Ulva lactuca* was provided to simulate natural conditions by allowing tube-building and feeding. *U. lactuca* was also placed in the arrival bottles, but not in the departure bottle. Water flowed through the system for the next 24 h. This permitted the concentration of water-borne substances to equilibrate. At the beginning of each test, the water was turned off, and the current halted. Then 15 males (the "travelling males") were placed in the departure bottle, and its mouth was capped with nylon mesh netting to prevent travelling males from leaving the apparatus.

All individuals were isolated from mass cultures maintained as described by Borowsky (1980). Males were kept isolated for two or more days and were tested without regard to their intermolt stages. Females were tested when they were "receptive", "non-receptive", [six days and two days past their previous molt, respectively (Borowsky, 1983a)], or when their intermolt stages were unknown. The type of female used in any test depended upon the experimental design. Each individual was used only once.

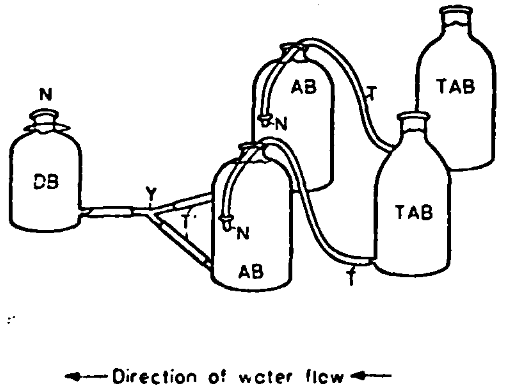

Fig. 1. The choice-test apparatus employed to test for the existence of attractants in different types of individuals' secretions (see "Materials and methods" section for detailed explanation of its use) Abbreviations: AB-arrival bottles, DB-departure bottle. N-nylon mesh netting. T and T-tubing. Y-glass Y-tube

Each test was begun by starting the flow of water through the system. During a test, travelling males could pass freely among the two arrival bottles and the departure bottle. After 5.5 h, the test ended and the number of travelling males left in the departure bottle as well as the number in each arrival bottle were counted. The tests were conducted in dim light. The entire apparatus was disassembled, washed in hot tap water, and allowed to air-dry between tests.

Rationale of data analysis. The apparatus offered males in the departure bottle two choice points. The first choice, available to all travelling males, was whether or not to leave the departure bottle and enter the tube leading to the Y-tube. The second choice was available only to those males that left the departure bottle. When they arrived at the fork of the Y they could go through the left or right arm of the Y, or turn around and return to the departure bottle. A comparison of differences at the first choice point would reveal whether test individuals in the tall aspirator bottles secreted a substance which altered travelling males' general level of activity. A comparison of differences at the second choice point would reveal whether travelling males distinguish between different stimuli (i.e. males, non-receptive, or receptive females).

Hypotheses were tested using χ^2 and binomial statistics. The overall significances of the Experiments (procedures described below) were obtained by combining probabilities from separate replicates using the method of Fisher (1946). Individual probabilities for each replicate were obtained from the binomial distribution (one-tailed test) and -2 times the sum of their natural logarithms was taken as χ^2 with eight degrees of freedom (referred to below as the "aggregate χ^2").

Experiments in the apparatus

Four experiments were performed, each consisting of four identical tests. The objectives of the four experiments were as follows: Experiment I, to learn whether travelling males would respond to male secretions; Experiment II, to learn whether they would respond to female secretions; Experiment III, to learn whether they could distinguish between secretions of receptive and non-receptive females; and Experiment IV, to learn whether the attractive secretions remain potent ~~to~~ for 1.9 h.

In each test of Experiment I, 60 males were placed in one tall aspirator bottle, and no individuals were placed in the other one. Thus, the secretions from 60 males were in the water that entered one of the arms of the Y-tube.

However, the concentration of these secretions was reduced by one half in the water entering the departure bottle from the tube (equal to the amount secreted by 30 males), because this water was mixed, in equal parts, with water from the other tall aspirator bottle, which contained no test individuals.

Experiment II was conducted in the same way as Experiment I, except that 60 females were placed in one of the tall bottles. The females' intermolt stages were unknown.

Experiment III was conducted as were the other two, except that 30 receptive females were placed in one tall aspirator bottle, and 30 non-receptive females were placed in the other. Thus the water in both arms and the base of the Y tube contained secretions from 30 individuals. In this way males' responses to the nature of the females' secretions, rather than to their concentrations, were tested.

To learn whether the receptive female attractant stimulated specific reproductive behaviors in males, the possibility that the attractant lost its potency immediately after being secreted had to be eliminated. Thus an experiment was run in which the secretions of the females' passage through the apparatus was delayed. Experiment IV was identical to Experiment II, except that in the former, the 47-cm long tubes connecting the tall aspirator and the arrival bottles (labelled "T" in (Fig. 1) were replaced with tubes that were 15.24 m long. It was determined empirically that at a flow rate of 100 ml h^{-1}, it would take approximately 1.9 h for water to pass through the longer tube, but only 3.5 min to pass through the shorter one. If males behaved the same way in Experiments II and IV, one could conclude that the attractant remained potent for at least 1.9 h.

Test individuals were placed in the right and left bottles alternately in all the replicates.

Effects of receptive female secretions alone on "intermittent pleopod beats"

Having determined that the receptive female attractant was potent for at least 1.9 h, it became possible to test for its ability to elicit male IPBs. This behavior is easily recognized, and is defined as the strong, rapid beating of the pleopods for one second, followed by a rest of one or two seconds. The cycle is often repeated a few times. IPB behavior is only expressed by males to females and is almost always followed by tube-sharing (Borowsky, 1983a).

Each of the three possible sources of stimuli, receptive female secretions, receptive female tubes, and the receptive female itself, was first tested alone, then tested in combinations of two. Each experiment consisted of 20 tests, and each individual was tested only once. All individuals were kept isolated in separate 10.5-cm-diameter glass culture dishes until they had built tubes. To make sure that a tested pair was competent, a male was introduced into a receptive female's dish. If the male did not exhibit IPBs or tube-sharing did not occur, the pair was discarded. However, if both behaviors occurred, the pair was removed from the female's dish and employed in the tests immediately. The individuals were observed with substage illumination at 12× magnification. The procedures for each type of test and its results are presented in Table 2.

Results

Effects of different individuals' secretions on the males' movements: Experiments I to III

Behavior of males at the first choice point: differences in levels of activities

The number of male *Microdeutopus gryllotalpa* in the arrival vs the number of males in the departure bottles was significantly different among the three experiments (Table 1; $\chi^2_2=32.578$, $p<0.001$). More males were in the departure bottles (38) than in the arrival bottles (21) in Experiment I when the test individuals were males, but significantly more males were in the arrival than in the departure bottles in Experiments II and III combined (86 vs 32), when the test individuals were females ($\chi^2_1=22.875$, $p<0.001$). This suggests that female secretions increase the males' level of activity more than males' secretions do.

Behavior of males at the second choice point: effects of different individuals' secretions as male attractants

Experiment I. There was no difference in the number of males in the male water arrival bottles and the number in the arrival bottles which had no test individual secretions (aggregate $\chi^2=11.676$, df=8, $p>0.05$). This suggests that

Table 1. *Microdeutopus gryllotalpa.* Means and standard deviations of the number of travelling males in arrival and departure bottles after the four tests of each experiment in the apparatus. Nature and number of test individuals whose secretions passed into the arrival bottle indicated

	Means (SD)	Nature (no.)	Bottle
Experiment I:	No individuals (3±1.7)	Males (60) 3±2.4	Departure bottle 10±2.6
Experiment II:	No individuals (3±1.7)	Females (60) 6±2.4	Departure bottle 6±3.5
Experiment III:	Non-receptive females (30) (3±1.7)	Receptive females (30) 11±2.1	Departure bottle 2±1.6
Experiment IV:	No individuals (3±1.5)	Females (60) 8±2.6	Departure bottle 5±3.6

Table 2. *Microdeutopus gryllotalpa.* Number of males (of twenty) that exhibit IPBs

Stimulus	Methods	Results
A. When males are presented with one possible stimulus		
	Male introduced into:	
Receptive females' water	clean dish containing water in which receptive female has resided	0
Receptive females' tubes	dish containing receptive female's tube after removing female, rinsing and replacing old with fresh sea water	0
Receptive females' bodies	clean dish with fresh sea water and receptive female introduced 2 minutes earlier	0
B. When males are presented with two possible stimuli combined		
	Male introduced into:	
Receptive females' water plus females	clean dish containing receptive female plus water from her dish	1
Receptive females' water plus their tubes	Receptive female's dish after removing female	0
Receptive females plus their tubes	Receptive female's dish after removing water, rinsing and then replacing with fresh sea water	18
C. When males are presented with all three possible stimuli combined		
	Male introduced into:	
Receptive females plus their tubes and their water	Receptive female's dish containing tube and female's water	17

male water did not orient males either toward or away from its source. Thus, observations of the males' behavior at the two choice points of Experiment I suggest that males' travels were not influenced by other males' secretions.

Experiment II. There were more males in the female water arrival bottles than in the arrival bottles which had no test individual secretions (aggregate $\chi^2 = 16.345$, df=8, $p < 0.05$). This suggests that female water stimulated males to orient their movements toward its source.

Experiment III. There was a highly significant difference in the number of males in the receptive female water arrival bottles and the number of males in the non-receptive female water arrival bottles (aggregate $\chi^2 = 34.182$, df=8, $p < 0.001$). This suggests that receptive female water was more attractive to males than was non-receptive female water.

Effects of female secretions as male attractants after aging for 1.9 h

In Experiment IV, as in Experiment II, more males went to the side with receptive females than to the side with non-receptive females (aggregate $\chi^2 = 17.859$, df=8, $p = 0.01$). This suggests that the attractant remains potent for at least 1.9 h after being secreted.

Effects of receptive female secretions alone on "intermittent pleopod beats"

Table 2 shows that IPBs were almost never expressed by males, except when both the female and her tube were available. When they were available, however, almost all the males expressed IPBs, regardless of the type of water in the test dish. Thus the ambient water around receptive females does not elicit IPBs by itself.

Discussion

The apparatus employed in the present study was designed to generate objective, quantitative information to test whether *Microdeutopus gryllotalpa* female secretions affect males' travels. It was based on a standard choice test model, modified to mimic the typical field conditions of this species. The results demonstrate that receptive females secrete a male attractant, and that this substance both increases the males' level of activity, and attracts them to its source. On the other hand, the data show that male secretions have no detectable effect upon other males' travels. Thus, the attractant is not species-specific, but is receptive-female-specific.

Receptive female secretions may influence males' movements in the field in such a way that they are more likely to arrive at receptive females' tubes than at the tubes of other individuals. This is of advantage to both sexes: for the female, it may enhance the likelihood that she will be attended by a male during the brief period of time when fertilization can be effected, and for the male, it may minimize energies expended on the search for receptive females.

Female effluents that increase the general level of activity and/or orient males' movements have been reported for many other Crustacea [see Dunham (1978) for a summary of earlier reports; more recent reports include the existence of such a substance in the crabs *Carcinus maenas* (Seifert, 1982), *Portunus sanguinolentus* (Christofferson, 1978), and *Callinectes sapidus* (Gleeson, 1980); and the shrimp *Hept~~[illegible]~~carpus paludicola* (Bauer, 1979)]. a/ In contrast, Hartnoll and Smith (1979) found no evidence for such a substance in the crab *Cancer pagurus.* In one case, two studies have yielded contradictory results. Dahl *et al.* (1970) found that in the amphipod *Gammarus duebeni,* female secretions increased males' general level of activity and attracted them to a double mesh net separating the two sexes. But Hartnoll and Smith's (1980) studies, which were similar in design, revealed no such effects.

The results of the present study suggest that a combination of the receptive female and her tube release male IPBs, but that neither will elicit this response by itself. In addition, the data suggest that the concentration of the attractant that the female secretes when unattended by a male does not release IPBs by itself. Thus, in this species, ambient concentrations of receptive female secretions do not, by themselves, elicit the males' entire reproductive behavioral repertoire; their effect is limited to guiding the males' travels.

This does not rule out the possibility that female secretions play a role in eliciting IPBs, however. There are at least three ways it could act. First, it is possible that a higher concentration of the attractant elicits IPBs. It is conceivable that under natural conditions a female increases the amount of the substance secreted when she preceives a male entering her tube, and that this higher concentration elicits IPBs. Christofferson (1978) showed that the female sex pheromone of the crab *Portunus sanguinolentus* was secreted via the urine, and that the rate of urine secretion could be greatly altered by the female. Further, Rust (1976) showed that in the American cockroach *Periplaneta americana* "males responded to (female) sex pheromone in three distinct levels: arousal, running and courtship displays" and "each level was initiated by increasing concentrations of sex pheromone". The second way female secretions might participate in eliciting IPBs is if another substance (different from the attractant) is secreted by the female when she perceives the male, and that this stimulates IPBs. Finally, it is possible that IPBs are stimulated by the tube plus the female's body plus the attractant combined.

Whether or not female secretions contribute to stimulating IPBs, it has been shown that the male must have access to the female's body before they are expressed. Intuitively this makes sense, for it seems a waste of the male's energy to express specific reproductive behaviors unless the female can witness and be influenced by them. A similar situation is found in other arthropods. For example, Atema *et al.* (1979) report that male reproductive behaviors in *Homarus americanus* appear to be influenced by a female pheromone and other female stimuli combined. In addition, the full range of courtship behaviors in *Periplaneta americana* is expressed only in the presence of a female (Rust, 1976).

The present study has provided some insight into the mechanism which controls the accurate timing of the onset of tube-sharing in *Microdeutopus gryllotalpa*. Receptive females broadcast a substance which attracts males to their tubes. Once having arrived at the tube, however, other female factors must be present to induce the male to remain and initiate tube-sharing. The nature of this factor or factors is currently under investigation.

Acknowledgements. I thank Dr. G. Ruggieri for making available the facilities necessary to perform this work. I also thank R. Borowsky for a critical review of the manuscript. This work was done with the aid of ONR Contract NOOO14-82-C-0482.

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Date of final manuscript acceptance: September 18, 1984.
Communicated by S. K. Pierce, College Park

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Preprint II

THE USE OF THE MALES' GNATHOPODS DURING PRECOPULATION IN SOME GAMMARIDEAN AMPHIPODS

BY

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INTRODUCTION

The typical pattern of reproductive behavior in epibenthic gammarid amphipods involves a male's carrying a female about for a few days prior to the female's molt. Carrying, or precopulation, guarantees a male and female access to each other's gametes during the brief interval after the female's molt when a viable brood can be produced. The interval is brief because ovulation must occur while the female's exoskeleton is still flexible enough to permit eggs to pass through the oviducts, and, since there is no sperm storage, copulation must immediately precede ovulation. After copulation the male and female separate.

In most gammarid amphipods the firt two pairs of peraeopods are modified into claw-like structures, or gnathopods. The posterior pair is generally larger than the anterior pair in individuals of both sexes. In addition, males tend to have larger and more elaborate posterior gnathopods than females of the same species.

The general belief has been that males use their larger gnathopods to attach to the female during precopulation (i.e., Barnard, 1969; Bousfield, 1973). Thus it was surprising to learn that some of the species whose males have the largest and most elaborate gnathopods never precopulate at all (i.e., *Microdeutopus gryllotalpa* (Costa, 1853), cf. Borowsky, 1980, and also *Jassa falcata* Montagu, 1818) *Ampithoe valida* Smith, 1873, cf. Borowsky, ~~in prep.~~). This made me wonder whether precopulating males did indeed use their larger gnathopods to carry females. Therefore I examined many precopulating pairs of individuals belonging to six species. The results are presented here.

1983 B

MATERIALS AND METHODS

The species studied were chosen arbitrarily; they were abundant and accessible. *Gammarus palustris* Bousfield, 1969, *G. mucronatus* Say, 1818, and *Melita nitida* Smith, 1873 (50 pairs each) were taken from underneath rocks in the intertidal zone during low tide at the Cross Bay Boulevard Bridge, Jamaica Bay, New York. *G. lawrencianus* Bousfield, 1956 (15 pairs) was taken from red algae thalli floating in the water at low tide at Coney Island, New York. *Hyalella azteca* Saussure, 1857, and *G. fasciatus* Say, 1818 (50 pairs and 10 pairs, respectively), were taken from benthic debris and algae in 1.5 meters of fresh water, off Nobles Island, Lake George, New York. Observations were either made immediately, or within 24 hours of capture.

RESULTS

Males of all six species used the smaller, anterior gnathopods to carry females and left the posterior ones free. But the orientation of the gnathopods and points of attachment on the females were different among the three genera. *Hyalella azteca* male held onto the females' second coxal segment (fig. 1, above). Females, but not males, have a postero-ventral process on this segment (fig. 1, below) which is grasped between the propodus and the dactyl of the male's gnathopod.

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In *Melita nitida*, the point of attachment is the sixth coxal plate (fig. 2, above). As in *H. azteca*, this site was modified in the female, but not in the male (fig. 2, below). The male's gnathopod and the female's point of attachment fit together like two puzzle pieces. In four pairs, males held onto females at other sites with their large claws in addition to the principal attachment site. In each case, the males were smaller than the females they were carrying.

Males of all four *Gammarus* species used their smaller gnathopods the same way: one was attached to the female at the dorsal part of the first coxal segment, and the other, rotated posteriorly 180°, was attached to the fifth coxa (fig. 3). The attachment was effected by inserting the dactyl under the segment where it overlapped an adjacent segment. In contrast to the other genera observed, there was no sexual dimorphism in the points of attachment in *Gammarus*. In addition, there was no preference for which of the gnathopods would be inserted at the first, and which at the fifth coxae (*G. mucronatus* had 24 of 50 left gnathopods inserted at the first coxa; *G. palustris* had 19 of 50 left gnathopods inserted there; *G. fasciatus* had 8 of 10, and *G. lawrencianus* had 7 of 15).

DISCUSSION

The sexual dimorphism of the points of attachment of *Hyalella azteca* and *Melita nitida* have not been reported before, to my knowledge. However, Sars (1895) noted that *Melita palmata* G. O. Sars, 1895, had a process which appears identical to that observed for *Melita nitida*.

Males of the six species observed here used the smaller of the two pairs of gnathopods to carry females, leaving the larger pair free. This may be typical of precopulating species. For example, Sheader & Chia (1970) observed this in *Marinogammarus obtusatus* Dahl, 1936, and I have observed it in a few pairs of *Hyale plumulosa* (Stimpson, 1853).

What, then, is the function of the larger gnathopods? Perhaps they are used against other males during agonistic encounters. Males of three species which have sexually dimorphic posterior gnathopods but which do not precopulate use the larger pair of claws during agonistic encounters. Such encounters are most likely to occur when one male is defending a sexually receptive female against another male (Borowsky, 1983 and unpublished data). Casual observations of encounters between males of *G. palustris* suggest a similar function for these males' larger gnathopods. When a male was placed in a dish with a precopulating pair whose male was smaller, the larger male dislodged the smaller one and took its place, carrying the female itself. The larger male first attached itself to the female with its smaller gnathopods placed at the usual points of attachment. Then the larger male used its larger gnathopods to pry off the small gnathopods of the precopulating, smaller male while pushing the smaller male away with its uropods.

The details of the smaller gnathopods' orientation and points of attachment to the females differed among the three genera, but were identical within the genus *Gammarus*. Interestingly, the four *Gammarus* species were found in three different habitats (see Materials and Methods section). This suggest that precopulation appeared in the ancestral *Gammarus* before speciation ensued.

On the other hand, the differences among the genera suggest that precopulation may have evolved independently more than once. The nature of the founding species of the Gammaridea is at present unknown. Unfortunately the fossil record begins after the suborder was fully formed (Just, 1974). Barnard (1974) suggested two possible alternative ancestors of the Gammaridea, one of which was a *Corophium*-like species, and the other a *Gammarus*-like species. Recent corophioids are benthic, tube-building species which apparently do not precopulate (i.e. *Corophium volutator* (Pallas, 1766) and *C. arenarium* Crawford, 1937, cf. Fish & Mills, 1979, and *C. insidosum* Crawford, 1937, cf. Nair & Anger, 1979), while recent gammarids are generally epibenthic and exhibit precopulation. If, as suggested by the observations reported here, precopulation evolved more than once, it seems likely that epibenthic life-histories evolved more than once. In other words, it is possible that the ancestral gammaridean was a benthic, tube-dwelling species, from which many lines have radiated in response to selection for epibenthic life-histories. Perhaps the sequence from benthic to epibenthic forms, rather than the reverse, warrants some additional consideration.

ACKNOWLEDGEMENTS

I thank B., R., and S. Borowsky for collecting animals at Lake George. The figures were modified from several plates from Bousfield, 1973. (Copyright 1973 by the National Museum of Canada. Used by permission of the publisher, Cornell University Press.) This work was supported, in part, by ONR contract N00014-82-C-0482.

RÉSUMÉ

L'usage des gnathopodes par les mâles, pendant la précopulation, a été observée chez six espèces d'amphipodes appartenant à trois genres. Les mâles des six espèces utilisaient la paire antérieure, la plus petite, pour s'accrocher à la femelle, laissant libre la paire postérieure, la plus grande. De plus, le placement exact et l'orientation de ces appendices étaient stéréotypés dans chaque espèce, mais différaient suivant les genres.

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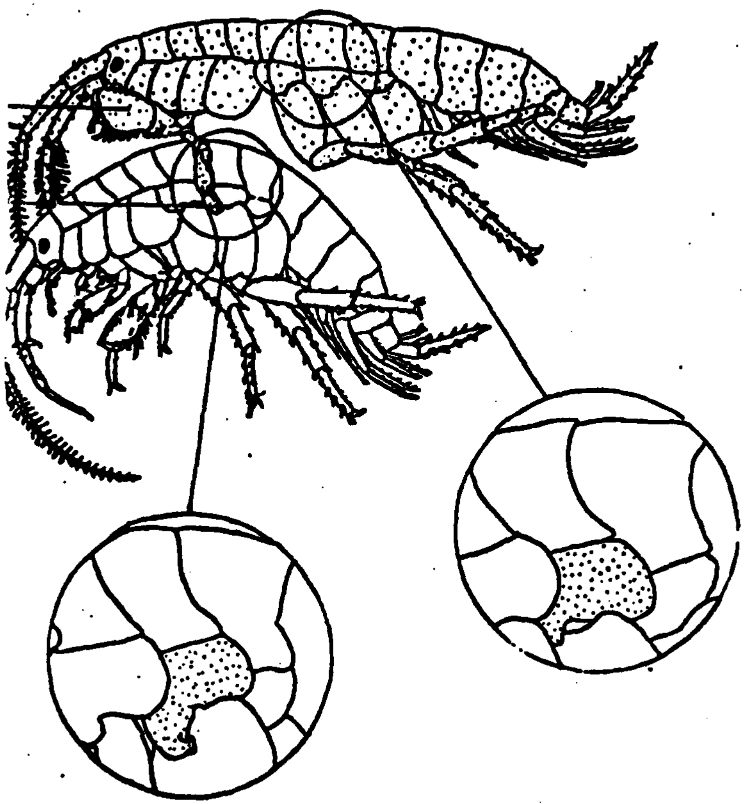
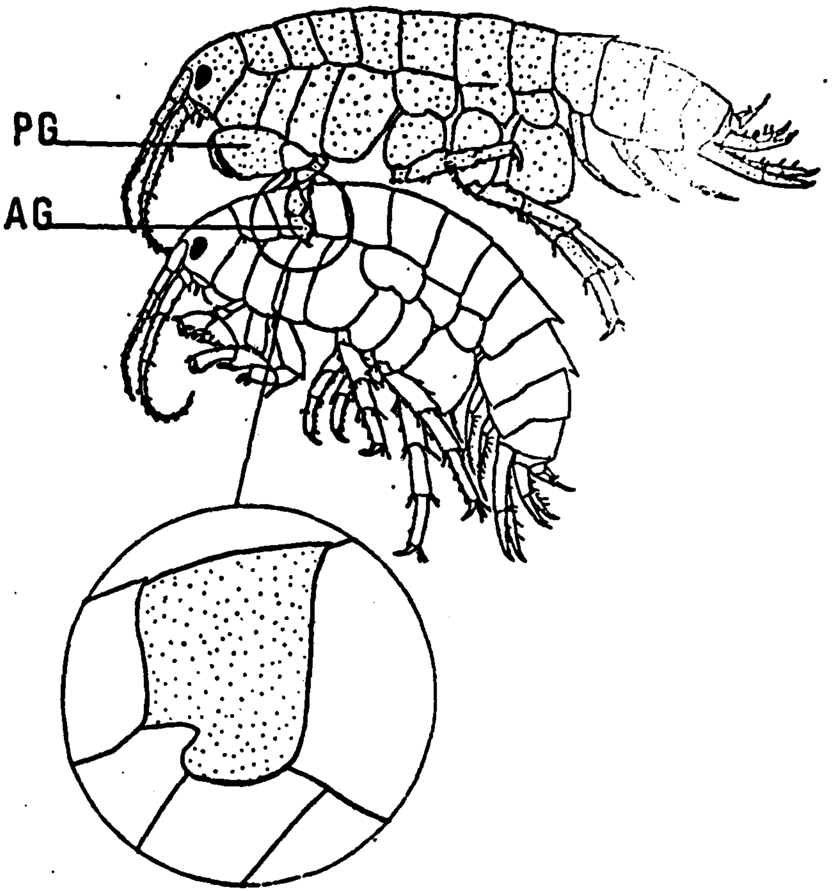
Received for publication 25 February 1983.

Fig. 1. Lateral view of a precopulating pair of *Hyalella azteca* Saussure, 1857. Above: the male (stippled) is using its smaller, anterior gnathopods (AG) to hold onto the female's second coxal segment. The male's larger posterior gnathopods (PG) are held freely. Below: the female's second coxal segment magnified to show the morphology of the postero-ventral point of attachment which is missing in the male.

Fig. 2. Lateral view of a pair of precopulating *Melita nitida* Smith, 1873. Above: the male (stippled) is using its smaller, anterior gnathopods (AG) to hold onto the female's sixth coxal plate. The male's larger posterior gnathopods (PG) are held freely. Below: the female's (left) and male's (*right*) *sixth coxal plate enlarged to show the morphology of the point of attachment* which is present in the female and absent in the male.

Fig. 3. Lateral view of a pair of precopulating *Gammarus* sp. The male (stippled) is using its smaller, anterior gnathopods (AG) to grasp the female. The larger, posterior gnathopods (PG) are held freely.

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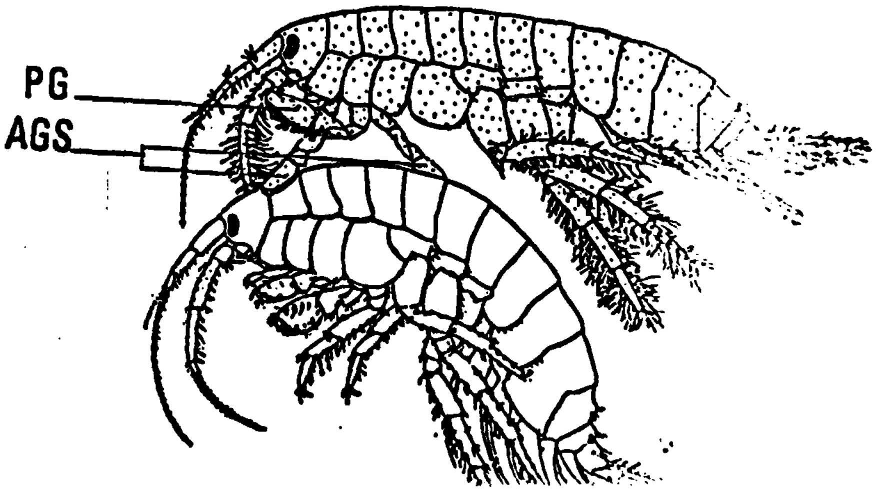
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In Preparation I

DIFFERENCES IN REPRODUCTIVE BEHAVIOR BETWEEN TWO MALE MORPHS OF THE AMPHIPOD CRUSTACEAN *JASSA FALCATA* MONTAGU

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Although the two adult male morphs of the amphipod crustacean *Jassa falcata* are equally fertile, they probably differ markedly in their reproductive success. Those without "thumbs" on their second pair of chelae tend to remain in their own residential tubes. On the occasions when they are outside their tubes, they tend to evict receptive females from the latters' tubes, and lose agonistic contests with "thumbed" males. In contrast, thumbed males tend to abandon their tubes to travel to receptive females, and attend them closely until they copulate. They do not evict females. In addition, receptive females are less tolerant of the presence of a thumbless than a thumbed male. It is important for males to maximize their size, because larger individuals tend to win contests with other males for access to receptive females. Since thumbless males will continue to grow but thumbed males will not, it is hypothesized that the differences in their behavior are adaptations which maximize the formers' growth and the latters' reproductive success given a particularly harsh environment (a swift current which can dislodge animals outside their tubes).

Considered in toto, this species may be evolving toward a metamorphic pattern of development. At present, there is no apparent sexual physiological difference between the two male morphs, but their behavioral differences are such that in the field the vast majority of matings must be effected by thumbed

males. If selection continues to favor the separation of the males' development into an early growth-intensive and a later reproductive-intensive period, additional differences may accumulate until pre-terminal molt males are unable to reproduce and a true metamorphosis is attained.

INTRODUCTION

Most crustaceans pass through one or more distinct larval stages (Williamson, 1982). In contrast, the morphology of newly hatched amphipods closely resembles the adults', except for their sexual characteristics, which develop gradually through several successive molts (Kaestner, 1970). In addition, all amphipods whose life-histories have been examined have been shown to have indeterminate growth. Thus, it was interesting to learn that one amphipod, Jassa falcata , differs from the general amphipod pattern of development in the direction of the other crustaceans. The males of this species have terminal molts, and at least one sexually dimorphic feature appears de novo at the terminal molt (Borowsky, 1983). The feature is a non-prehensile projection, or "thumb", on the males' larger pair of chelae, or gnathopods (figured in Sexton and Reid, 1951). Thus, a single population contains two types of males; those without thumbs, which are still growing, and those with thumbs, which have ceased growth.

The present study was undertaken to learn why 1) Jassa falcata males' development differs from most other amphipods' and 2) why their terminal molts are marked by the appearance of the thumb. Since the amphipods are currently undergoing an explosive adaptive radiation (Bousfield, 1973), it was thought that further observations on J. falcata might provide insights into what environmental pressures select for metamorphic development.

Jassa falcata builds individual residential tubes comprised of thread-like secretions and local debris, and typically forms dense colonies on marine substrata exposed to swift currents. Laboratory studies showed that adult females tend to remain inside their residential tubes while males tend to abandon theirs to seek out receptive females. Males attend such females by clinging to the outside of their tubes until copulation occurs. After copulation the males leave to seek other receptive females (Borowsky, 1983). In the field, their habitat may challenge the animals' ability to reproduce. The tubes are affixed firmly to the substratum and animals inside them are relatively sheltered from the current. But during their travels and their attendance upon receptive females, males are directly exposed to the shearing forces of the current and to predation (e.g. Homes and Gibson, 1983). Several specific hypotheses to account for the dimorphism of the males' gnathopods were tested: 1) that only thumbed males are sexually competent, and the thumb functions as a label to identify them; 2) that the thumb facilitates the males' adherence to the substratum, which permits them to travel between tubes and attend receptive females and 3) that the gnathopods are used as weapons during encounters with other males and the thumb increases the probability of winning contests.

MATERIALS AND METHODS

All animals were collected either from the cold water tanks at the New York Aquarium, Coney Island, New York, or from the submerged surfaces of the docks at the Barren Island Marina, Jamaica Bay, New York, and maintained in glass culture dishes in 29 ppt sea water at 20°C. Animals were supplied, *ad libitum* with chopped *Ulva lactuca* for food and materials with which to build their tubes, and brine shrimp nauplii every other day. It should be noted that individuals of this species are very delicate, and significant mortality could only be avoided *if handling was minimized* during the observations.

Body lengths were measured under the microscope with an optical micrometer. The measurements were taken from the anterior tip of the rostrum to the most posterior tip of the telson. Animals were measured only after observations.

It was reasoned that the best indicator of a male's physiological sexual maturity is its ability to fertilize a brood. To determine whether thumbless males were physiologically sexually mature, 40 heterosexual pairs (20 with thumbed and 20 with thumbless males) were placed in individual 10.5 cm glass culture dishes and observed once a day. Females were examined for the presence of a brood two days after their molts and then discarded. This procedure was adopted because animals were adversely affected by the manipulations necessary to examine them under the microscope. As controls, 80 additional females were maintained in isolation. Twenty of these were examined the day of their molt, and 20 more each day thereafter, until day four. In addition, 20 other females were maintained in isolation until they completed their second molts, and their dishes examined for the presence of juveniles.

To determine whether thumbless males were behaviorally sexually mature, their behavior to females was observed 1) through time and 2) upon initial contact, and their behavior to other males was observed upon initial contact. Observations through time were conducted in the following way: 40 heterosexual pairs were placed in individual culture dishes. In twenty of the pairs the male had thumbs, and in 20 it did not. Each pair was observed daily until one or both members died, noting the location of both individuals relative to each other and the day of the female's molt. In a complementary experiment, the animals' behavior in the excurrent pipes of the cold water tanks of the New York Aquarium was observed. This experiment was performed because the animals' living conditions in the pipes mimic field conditions. They form dense colonies and each animal is subject to swift currents in the tanks (about 200 ml/min). The first 50 animals that were observed outside their tubes and seen moving about were removed, sexed, their body lengths measured, and, in the case of the males, the presence or absence of a thumb noted.

Observations on the males' behaviors to newly encountered receptive females were conducted in the following way: 40 individual pairs of females and thumbed males were placed in culture dishes and observed daily until the male was attending the female. Females were considered receptive and therefore were tested, on that day. The resident male was removed, and either another thumbed male or a thumbless male was introduced into the dish (20 replicates each). The behavior of the introduced male and resident female was recorded.

Observations of the males' behaviors to other males upon initial contact were conducted as follows: another 40 female-thumbed male pairs were set up, and tests were conducted on days when the males were attending the females. In these tests the resident male was allowed to remain in the dish, and another male was introduced (20 thumbed and 20 thumbless males were introduced). Ten of the introduced males were larger, and 10 smaller in each of the two sets of 20 observations. The behavior of all three individuals was noted. The experiment was designed to maximize the frequency of expression of reproductive behaviors; males generally do not interact in the absence of a receptive female.

To determine whether males with thumbs adhered to the substratum better than did males without thumbs, 50 males with thumbs and 50 males without thumbs were pipetted, one at a time, directly onto the colonies in the excurrent pipes. The number of males that were washed away vs. the number that adhered to the surface was noted.

RESULTS

Differences in Physiological Sexual Maturity

Although many females ovulated when maintained in isolation (at least 19 of 100), the eggs were probably not fertile. By four days past their molt no female had eggs in its brood pouch (Table I.A). In addition, no juveniles were found in any of the dishes of the isolated females that were maintained until their subsequent molt. Thus, some females ovulated even when copulation did not occur, but the eggs didn't develop, and they were discarded from the brood pouch after a few days. This shows that there is no sperm storage in this species.

The observations suggest that thumbless males are physiologically sexually mature. First, thumbless males were observed copulating with females on four occasions. And second, thumbless males fertilized about the same number of broods as thumbed males did. The number of females with eggs in their brood pouches two days after they molted was about the same when they were maintained with either type of male (Table I.B and C). However, significantly fewer females had eggs two days after their molt when they were isolated (two of 20 isolated females and 31 of 40 paired females had eggs: $\chi^2_1=24.5$, $p<0.001$). Since there is no sperm storage in this species the thumbless males maintained with females must have fertilized those females' broods.

Differences in Behavioral Sexual Maturity

Observations Through Time

In culture dishes, on the day before and/or the day of the female's molt males with thumbs were generally found on top of receptive females' tubes, while males without thumbs were generally found inside their own tubes (20 of 26, and 4 of 32 times, respectively: $\chi^2_1=24.6$, $p<0.001$). Thus, males with thumbs attended receptive females more closely than did males without thumbs.

Observations of animals in the excurrent pipes of the cold water tanks supported the results of observations of animals in culture dishes. Of the 50 animals observed walking outside the tubes 32 were males with thumbs, 10 were males without thumbs, seven were females, and one was a juvenile. Thus, there were significantly more males than other individuals walking outside tubes, and significantly more thumbed males than thumbless males observed there (binomial tests; $z=4.67$, $p<0.001$, and $z=2.93$, $p<0.01$, respectively). The results of these two experiments suggest that thumbed males travel more often to females' tubes than thumbless males do.

Differences in Adhesion to the Substratum

There was no difference in the number of thumbed and thumbless males that clung to the substratum after they were introduced into the excurrent pipes (40 of 50 and 43 of 50 times, respectively, $\chi^2_1=0.638$, $p<0.05$). This suggested that thumbs are irrelevant to the males' ability to grip the substratum, which, in turn, suggests that the thumbless males' failure to travel to receptive females' tubes is a matter of behavioral choice, rather than a mechanical constraint.

Observations of Behaviors Upon Initial Contacts

Single Males and Receptive Females

Males. Thumbless males evicted receptive females from their tubes significantly more often than thumbed males did (13 of 20 vs 4 of 20 times, respectively; $\chi^2_1=8.29$, $p<0.01$).

Females. Receptive females generally avoided agonistic encounters with males. They never employed their gnathopods against thumbed males. However, they employed them occasionally against thumbless males both when only thumbless males were present (3 of 20 times) and when a thumbless and a thumbed male were present (3 of 20 times; see below). Females employed their gnathopods against thumbless males significantly more often than against thumbed males (6 of 40 vs 0 of 80 times, all thumbless and thumbed males encountered combined, respectively; Fisher Exact Probability Test, $p=0.01$). After three of these six encounters the male left the tube; after two of them the female left, and both remained after the final encounter. Thus, some females chose not to remain adjacent to thumbless males even though both individuals were probably physiologically sexually competent.

Two Males on a Receptive Female's Tube

When one male was introduced into the dish of a heterosexual pair, agonistic contests generally occurred between the males, after which only one remained on the receptive female's tube (17 of 20 and 19 of 20 times in thumbless vs thumbed contests and in thumbed vs thumbed contests, respectively). The contests involved physical contact. A typical encounter between a thumbed and thumbless male involved the thumbed male grasping and pinching the thumbless male with its two pairs of gnathopods, without the thumbless male's responding in kind. In contrast, a typical encounter between thumbed males involved their grasping each others' large gnathopods and pushing against each other with their antennae while pinching each others' heads with their first, small pair of gnathopods.

Most of the contests between thumbed and thumbless males were won by the thumbed males. Sixteen thumbed and one thumbless male remained alone on the female's tube after these encounters, and both males remained there three times. Thumbed males won contests regardless of their sizes (7 of the thumbed males were larger, and 9 smaller than their thumbless opponents in the contests won by thumbed males.)

In contrast, when both of the males posessed thumbs, it was generally the larger animal that retained possession of the female's tube (16 encounters after which only one animal remained; 15 larger, one smaller: Fisher exact probability test, $p < 0.001$). Prior residency had no effect on the outcomes (7 residents and 9 introduced males won: $\chi^2_1 = 0.500$, $p > 0.05$).

Thumbed males used their large gnathopods more often than did thumbless males during these encounters (36 of 60 thumbed males and 3 of 20 thumbless males used these limbs; $\chi^2_1 = 12.2$, $p < 0.00$). Thumbed males used their gnathopods about as often in contests with thumbless as in contests with thumbed males (14 of 20 vs 22 of 40 times, respectively; $\chi^2_1 = 1.25$, $p > 0.05$). They used them whether they were larger or smaller than their opponents (9 of 20 smaller males vs 13 of

20 larger males used their large gnathopods in the thumbed male vs thumbed male contests; $\chi^2_1=1.62$, $p>0.05$). Thus, thumbed males fought for and retained posession of receptive females' tubes more often than did thumbless males, regardless of relative sizes. But when two thumbed males engaged in agonistic interactions, the larger individual generally retained or gained posession of the female.

DISCUSSION

The data show that although both thumbed and thumbless males are physiologically sexually mature they exhibit different reproductive behaviors. Thumbed and thumbless males fertilized about the same number of broods. Therefore they were both physiologically mature. In addition, the two morphs were equally able to cling to the substratum in the face of swift currents, so theoretically they could travel to receptive females with equal facility. Thus, there is no apparent physiological or morphological bar to thumbless males' ability to reproduce.

In contrast, the two types of males displayed quite distinct reproductive behaviors. Observations of the colonies resident in the excurrent pipes showed that males were outside tubes more often than females. More importantly, most of the males outside tubes were thumbed. Another difference in the two types of males' behavior was their location relative to the receptive females' tubes. Thumbed males were generally on top of the females' tubes, but thumbless males were generally in their own tubes. Another difference was in the males' behavior toward receptive females when they were placed in the females' dishes. Thumbless males tended to evict the females, while thumbed males tended to remain in attendance on top of the female's tube.

Males in the terminal molt stage may have the thumb to identify them to females as animals engaging in reproductive activities. Females treated thumbless males differently than thumbed males. They tolerated all the thumbed males' presences, but would not tolerate the presence of 15% of the thumbless males they encountered. While this is not a difference of great magnitude, it shows that females can distinguish between the two types of males and may discriminate against the thumbless ones. Perhaps females can not distinguish between thumbless males and females, and exhibit agonistic behaviors to both of them to inhibit their own evictions.

It is worthy of note that some females ovulated even though they had not been inseminated. In general, gammarid amphipods do not ovulate if they do not copulate (Kaestner, 1970), but at least one other amphipod *Microdeutopus gryllotalpa* also ovulates in the absence of copulation (Myers, 1971). This species also builds tubes and has a pattern of reproductive behavior similar to *J. falcata* . ¶ The data suggest that receptive females rarely copulate with thumbless males in the field 1) because the latter tend to remain inside their own tubes 2) they tend not to cling to the outside of receptive females' tubes and 3) should they travel, they tend to evict lone receptive females or 4) retreat from receptive females' tubes if they are already attended by thumbed males, and finally, 5) the receptive female may not tolerate the thumbless male's proximity.

Thus, the males' passage from the thumbless into the thumbed stage is accompanied by a marked change in their reproductive activities. Significantly, this passage also marks an important physiological change; thumbed males have stopped growing. A logical explanation for these phenomena is that before their terminal molts males exhibit behaviors which will maximize their growth and survival (they remain sequestered in their tubes), but after their molts they exhibit behaviors which will maximize their reproductive potential (they travel to receptive females' tubes and engage in contests with other males.) This may be an adaptation which permits males to grow to the largest size possible before they attempt to reproduce. As shown here, large size is important, because larger males tend to win contests with other males for access to receptive females.

J. falcata may be a species in transition from direct to metamorphic development. Unlike other amphipods, males exhibit an abrupt physiological change (the terminal molt) accompanied by the sudden appearance of a distinct morphological feature, the thumb. In addition, their reproductive behaviors change at their terminal molts. Mayr (1970) has suggested that morphological changes may be preceded by behavioral changes directed by the environment. The effects of *J. falcata's* harsh habitat may have selected for modifications of the males' development to permit the species to engage in the ancestral cruising male pattern of reproduction (as described in Borowsky, 1983). It is possible that continued selection for differences between the two male morphs will lead to differences in their reproductive physiology as well, until thumbless males are incapable of reproducing, and true metamorphic development is attained.

I thank Y. Mohamed for invaluable assistance, and R. Borowsky for a critical review of the manuscript. This work was supported by Office of Naval Research Contract N00014-82-C-0482.

Table I: Number of females (of twenty) that have eggs in their brood-pouches 0 to Four days after they molt

	Days after the molt: 0	1	2	3	4
A. Isolated females	5	11	2	1	0
B. Females paired with males with no thumbs			15		
C. Females paired with males <u>with</u> thumbs			16		

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In Preparation II

THE EFFECTS OF RECEPTIVE FEMALE SECRETIONS ON MALE COURTSHIP IN MICRODEUTOPUS GRYLLOTALPA (AMPHIPODA:CRUSTACEA)

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Abstract. Receptive female water-borne secretions of the amphipod crustacean Microdeutopus gryllotalpa attract males. The present study was conducted to learn whether these substances stimulate male courtship behaviors as well. The results show that the attractant has no discernible affect upon male courtship. Instead, five factors, whose affects are additive, elicit courtship: the receptive female inside its tube, the female's movements, its exoskeleton, its tube, and some as yet unidentified factor. The observation that the female incorporates information about its reproductive stage into its residential tube is a novel finding for this group.

Water-borne pheromones are a common solution to the constraints imposed by aquatic environments on interspecies communication (Wilson, 1975). In particular, sex pheromones are widespread among the crustaceans (see Dunham, 1978 for earlier studies; more recent studies described in Borowsky, 1984). But although most investigations suggest that sex pheromones do not stimulate all aspects of these species' reproductive behaviors (see Salmon, 1983, Table 1, for a good synopsis of the literature), the specific functions of none of these substances has been resolved. The experiments described here were conducted as part of an ongoing effort to 1) define the function of the water-borne attractant of the amphipod crustacean Microdeutopus gryllotalpa and 2) determine what factors elicit those reproductive behaviors which are not stimulated by the attractant.

Individuals of this species build residential tubes in the shallow subtidal and low tide mark of the intertidal zone. Males travel from tube to tube in search of a receptive female. When one is found, a male generally expresses a courtship behavior ("intermittent pleopod beats", or "IPBs") and moves into the receptive female's tube ("tube-sharing"). The male and receptive female continue to tube-share until the female molts, at which time copulation occurs. Within a few hours after copulation the female ovulates and the male leaves to seek another receptive female. The female remains alone in her home tube until she is receptive again, when tube-sharing is reinitiated. The males' behaviors to any given female are determined by the female's reproductive state (Borowsky, 1980 a and b)

This pattern of behavior may be conveniently divided into three sequential stages. First, males seek receptive females. Second, courtship, followed by tube-sharing occurs. And third, copulation occurs.

The first stage is undoubtedly facilitated by the male attractant which is secreted by receptive females (Borowsky, 1984). The experiments described here concern the second stage. In addition to the water-borne secretions there are two additional broad categories of potential stimuli: 1) the tube; and 2) the female's body (including morphology and behaviors). The effects of each of the three potential stimuli on competent males was investigated.

MATERIALS AND METHODS

General Procedures

"Intermittent pleopod beats", or "IPB"s is a distinct, easily recognized behavior which males generally express to females with whom they intend to tube-share. It involves the strong, rapid beating of the pleopods (ventral limbs employed to generate a respiratory current) for one second, followed by a rest of one or two seconds. The sequence is usually repeated a few times. The second stage of the male's reproductive behavior entails both tube-sharing and IPBs. IPBs are only observed in a reproductive context (Borowsky, 1983) and so were used as an index of reproductive activity in the following experiments.

Animals were taken from mass cultures maintained in dishes of 20 cm diameter, in sea water reduced to 24 ppt (Borowsky, 1980). Each individual was isolated in its own 11 cm diameter dish and maintained at 20°C until tested. Each dish was supplied with about 1/2 gm of chopped Ulva lactuca thalli, which served as food and tube-building materials. No additional Ulva was supplied, and the water was not changed before the animals were tested. Unless stated otherwise, animals were "pretested" before each test, to establish 1) that the males were sexually competent, and 2) to confirm the reproductive stage of the female. All the animals and their tubes had been isolated for the same number of days. First, a male was introduced into the dish of a six day post-molt female. If IPBs were expressed, both individuals could then be scored in tests employing receptive females, or the male could be introduced into the dish of a presumptive non-receptive female and the expression of IPBs noted. Thus, males were considered competent if they expressed IPBs. Females were considered "receptive" if: 1) they were six or seven days past their previous molt; and 2) the test male expressed both IPBs and tube-sharing to that female during the pretest. Females were considered "non-receptive" if: 1) they were two days past their previous molt; and 2) males did not express IPBs and tube-sharing did not occur during the pretest.

Immediately after the pretest established the female's reproductive stage, the "test" was conducted. Both individuals were introduced into a dish different from the one employed for the pretest. The males' expression of

IPBs was then scored. Each experiment consisted of 20 tests.

Observations of Unaltered Animals

Expression of IPBs to Receptive Females Inside vs On Top of Their Tubes.

Males were introduced into the dishes of receptive females that were either inside their tubes or outside, on top of them. The latter females had been evicted from their tubes, and the ends of the tube closed off by pressing them down with glass slides. Males were permitted to encounter these females only when the latter were standing on the outside of their tubes, so the tube and the female were encountered at the same time.

Expression of IPBs to Different Types of Individuals in Their Home Dishes.

Males were introduced into the dishes of receptive females, non-receptive females, or males, and the frequency of IPBs recorded. The animals were not pre-tested.

Expression of IPBs to Receptive Females in Different Types of Individuals' Dishes.

In these experiments the frequency of IPBs expressed to receptive females placed either in other receptive females', non-receptive females' or males' tubes was observed. The original residents were removed from the dish a few seconds before the tests.

Observations of Freeze-Fixed Animals

Males will not express IPBs in the absence of females. Therefore, freeze-fixed females were employed in experiments designed to test the effects of the tube and female waters in the absence of female behaviors.

Freeze-fixing involved removing the animal from its dish, placing it on paper towelling to absorb excess water, then placing the animal on a piece of aluminum foil resting on dry ice. The animal was frozen within 3-4 seconds, then placed immediately in the dish in which it was to be tested. Once in the water, the animal thawed within 3-4 seconds and was placed inside the tube in the dish. Males were introduced into the dishes immediately.

To control for the possible effects of carbon dioxide on the chemistry of the females' exoskeletons, 20 receptive females were removed from their home dishes and excess water was absorbed on paper towelling. Then they were placed on a piece of aluminum foil in a stream of carbon dioxide for five seconds, replaced in their tubes, and the frequency of IPBs observed.

The next set of experiments was designed to test the effects of the female's tube, water, and movements on eliciting IPBs. In the first three experiments receptive females were freeze-fixed and then placed in another animal's tube (receptive females', non-receptive females' or males'; 20 tests per experiment). The freeze-fixed females were held in place during the observations. The second three experiments were identical to the first, except that the freeze-fixed females were moved about within the tubes during the observations (Table II). The third and fourth sets of three experiments were identical to the first and second sets, respectively, except that before each test the dishes' waters were discarded, the dishes rinsed gently twice, and then filled with fresh sea water (six experiments, 120 tests all together: Table III).

To test the ability of different types of individuals' bodies to stimulate IPBs, 20 of each type of individual were freeze-fixed, then placed in males' dishes (three experiments).

Finally, to test the possibility that water-borne pheromones stimulate IPBs, but that the pheromones are only secreted by receptive females upon the male's encountering them, another experiment was conducted. Freeze-fixed receptive females were placed in other receptive females' tubes in which IPBs had been expressed seconds before (but the interacting pair removed). Then males were introduced and the expression of IPBs noted.

RESULTS

Observations of Living Animals

Effects of the Receptive Female's Location.

The number of males that expressed IPBs when they encountered females on top of their tubes was significantly less than the number that expressed them when they encountered females inside them (1 of 20 vs 19 Of 20 times, respectively; Fisher exact probability test, $p<0.001$). Thus cooccupancy of the tube is important in eliciting IPBs.

Expression of IPBs to Different Types of Individuals in their Home Dishes.

Males expressed significantly different frequencies of IPBs to the different types of animals in their home dishes (Table I.A; $X = 27.3$, $p<0.001$). Males expressed more IPBs to receptive females than to non-receptive females and males combined (16 of 20 vs 7 of 40 times, respectively; $X = 22.0$, $p<0.001$) and significantly more to both types of females combined than to males (23 of 40 vs 0 of 20 times, respectively; Fisher exact probability test, $p<0.001$). Thus, IPBs were not expressed to males, and were expressed to receptive females more often than to non-receptive females.

Expression of IPBs to Receptive Females in Different Types of Individuals' Dishes.

Significantly more IPBs were expressed to receptive females in other receptive females' as opposed to non-receptive females' and males' dishes combined (Table I.B; 36 vs 28 of 40 times, respectively, Fisher exact probability test $p=0.03$). This suggests that receptive females' tubes influence the males' expression of IPBs.

Observations of Freeze-Fixed Animals

Effects of Carbon Dioxide

Males expressed IPBs to females after they were frozen on dry ice and placed in tubes (see Tables II and III). The differences in the males' responses to freeze-fixed as opposed to unaltered animals (described below) was not due to the animals' exposure to carbon dioxide, however. Males behaved about the same way to CO2 treated and untreated females (19 of 20 vs 17 of 20 times, respectively; Fisher exact probability test, $p=0.302$).

Effects of Ambient Water on the Expression of IPBs.

The frequencies of IPBs expressed in ambient waters (Table II) were not significantly different from the frequencies expressed in fresh sea water (Table III: 43 of 120 vs 56 of 120, respectively; $X =2.91$, $p>0.05$). This suggests that the ambient water of different types of individuals does not influence the expression of IPBs. It is of special interest that there was no significant difference between the frequency of IPBs expressed to <u>receptive females</u> in their own water and in fresh sea water (20 of 40 vs 22 of 40 times, respectively; $X =0.201$ $p>0.05$).

In addition, the data suggest that females do not secrete a pheromone on encountering a male. Thirteen of 20 males expressed IPBs to freeze-fixed females placed in receptive females' dishes in which heterosexual encounters had just occurred. The same number was expressed in receptive females' dishes in which there had been no previous encounters (Table III).

Effects of the Tube on the Expression of IPBs.

Males expressed IPBs at a greater rate when presented with receptive females in the tubes of other receptive females than in the tubes of non-receptive females or males (Tables II and III combined: 42 of 80 vs 57 of 160 times, respectively; $X = 6.27$, $p<0.05$). This corroborate the results of observations of unaltered animals described above; namely, that males express IPBs more often in receptive females' as opposed to other _M. gryllotalpa_ individuals' tubes. Thus, the data suggest that receptive females mark their tubes to indicate their reproductive stage.

The Effects of Movement on the Expression of IPBs.

Another determinant of the males' expression of IPBs was the females' movements. When females were held in place there were significantly fewer IPBs expressed than when they were moved (Tables II and III combined: 34 of 120 vs 65 of 120 times, respectively; $X = 16.5$, $p<0.001$).

The Effects of Receptive Females' Bodies on the Expression of IPBs.

There was a significant difference in the frequencies of IPBs expressed to freeze-fixed individuals placed in males' dishes (Table IV: $X = 9.05$, $p<0.05$). Males expressed significantly more IPBs to receptive and non-receptive females combined than to males (17 of 40 vs 1 0f 20 times, respectively; Fisher exact probability test, $p<0.01$), but there was no significant difference between either the receptive females and males combined vs the non-receptive females (9 of 40 vs 9 of 20 times, respectively; X =3.21, p>0.05) or between the non-receptive females and males combined vs the receptive females (10 of 40 vs 8 of 20 times, respectively; X =1.43, p>0.05). The results show that after they are freeze-fixed: 1) females' bodies elicit more IPBs than do males'; but 2) receptive females' bodies do not stimulate more IPBs than do non-receptive females' bodies.

The Effects of Receptive Females' Viability on the Expression of IPBs.

Males expressed more IPBs to receptive than to non-receptive females when they were alive (Table IV: 15 of 20 vs 7 of 20 times, respectively; X =6.46, p<0.05). Fixed, moved receptive females elicited fewer IPBs than did living receptive females (Tables II and III combined vs Table I A and B combined: 65 of 120 vs 80 of 100 times, respectively; X =16.2, p<0.001). Further, when receptive females were freeze-fixed, their ability to elicit IPBs was *reduced* to the level of the ability of non-receptive females to elicit this behavior, regardless of whether the latter were fixed or alive (Table IV: 8 of 20, 9 of 20 and 7 of 20 times, respectively; X =0.417, p>0.05). These observations suggest that receptive females are the source of some additional stimulus which is eliminated by fixing them.

DISCUSSION

The most salient conclusion of the present study is that although receptive females' water-borne secretions are effective attractants for male *Microdeutopus gryllotalpa* , they do not influence courtship behaviors either alone (Borowsky, 1984) or in combination with other factors (present study). Instead, the males' courtship behaviors are influenced by at least five factors: 1) the female's presence inside its tube; 2) the nature of the receptive female's tube; 3) the female's movements; 4) the female's body; and

5) some additional, as yet unidentified stimulus.

The female plus her tube combined are required to stimulate male IPBs (Borowsky, 1984). But males expressed IPBs most often when the female was encountered inside as opposed to on top of its tube. Therefore it is the female's and tube's orientation, in addition to their mere presence, that stimulates courtship.

One explanation for the requirement that the female be inside its tube is that shelter may take precedence over heterosexual proximity for receptive females. The sexes come together by the male's travelling to the female's tube rather than either both sexes travelling to find each other or the female's travelling to the males. In addition, if females are evicted from their tubes they enter the nearest tube possible, and remain there (Borowsky, 1983). Thus, females encountered outside a tube will probably leave the site of the encounter in spite of the presence of a competent male. Under those conditions it is futile for the male to express courtship.

The second factor which influences the expression of IPBs is the receptive female's tube. Tubes are constructed by gluing bits of available debris together with secretions extruded in threads from pores in the tips of two pairs of walking legs (Bousfield, 1973). Since animals constantly rework their tubes, females could alter the structure of their tubes in a characteristic manner to advertise their receptive state. They might alter the nature of the threadlike secretions and/or other constituents. To my knowledge this is the first report of site marking to denote sexual receptivity in a crustacean. However, it occurs in other arthropods, for example the moth Pectinophora gossypiella (Colwell, et al , 1978).

The third influence on the males' expression of IPBs is the female's general movements. It is not surprising that males express IPBs to moving fixed females more often than to non-moving females even though the movements do not faithfully reproduce those of living animals. Movements indicate that the female is alive.

The fourth stimulus is the females' bodies. Males expressed IPBs more often to females' bodies (with no difference in fequency between receptive and non-receptive individuals) than to males' bodies. The specific stimulus on females' bodies could be either morphological and/or chemical. There are several sexually dimorphic characters in this species (Bousfield, 1973) and contact pheromones on the females' exoskeleta have been suggested for the amphipods Gammarus pulex and Gammarus fossarum by Hammoud et al , 1975. The nature of the fifth factor was not revealed by the present study. Its identification should prove of interest.

The number of factors that influence the males' IPBs is as interesting as is their nature. Their affects seem to be additive; the greater the number of factors present, the greater the probability that the male will express IPBs. This may be seen by organizing the data in a hierarchy in which the factors are removed one at a time. This results in the stepwise reduction of IPB frequencies (see Table V). Males will express courtship behaviors most often when they encounter living receptive females in the latters' tubes. However, all of these factors need not be in place for the male to express courtship; in fact IPBs and tube-sharing can occur early in the female's reproductive cycle, even when she exhibits behaviors designed to dissuade males from tube-sharing (Borowsky, 1983). The shorter the time to the molt, however, the more factors will be provided by the female, and the more likely an entering male will express courtship behaviors.

Thus, the results of observations on the first two of the three stages of the males' reproductive behaviors show that each is controlled by a different stimulus or set of stimuli and that water-borne secretions are only important in the first. To complete the determination of the information content of the attractant, investigations on the nature of the stimulus(i) for the final, and most important behavioral stage, copulation, are currently underway.

ACKNOWLEDGEMENTS

This research was aided by contract #N00014-82-C-0482 from the Office of Naval Research. I thank R. Borowsky for a critical review of the manuscript, and G. Ruggieri for general advice and support.

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Table I. Number of Males (of 20) That Express IPBs to Unaltered Animals

A. Previously untested animals observed in home dishes

Receptive females	16
Non-receptive females	7
Males	0

B. Pre-tested receptive females placed in other animals' dishes

Receptive females' dishes (after CO_2 treatment)	19
Receptive females' dishes	17
Non-receptive females' dishes	13
Males' dishes	15

Table II. Number of Males (of 20) That Express IPBs to Freeze-Fixed Receptive Females Placed in Different Types of Individuals' Home Dishes

Type of dish	Female held in place	Female moved	Total
Receptive female	7	13	20
Non-receptive female	2	8	10
Male	5	8	13
Total	14	29	43

Table III. Number of Males (of 20) That Express IPBs to Freeze-Fixed Receptive Females Placed in Different Types of Individuals' Dishes Containing Fresh Sea Water

Type of dish	Female held in place	Female moved	Total
Receptive female	9	13	22
Non-receptive female	7	13	20
Male	4	10	14
Total	20	36	56

Table IV. Number of Males (of 20) That Express IPBs to Different Types of Unaltered vs. Freeze-Fixed, Moved Individuals Placed in Males' Dishes

	Treatment	
Type of individual	Freeze-fixed and moved	Unaltered
Receptive female	8	15
Non-receptive female	9	7
Male	1	0

Table V. Changes in PIB Frequencies as the Number of Stimuli are Reduced, One at a Time.

Experiment	Stimulus removed	Number of males that express IPBs	Number of males that do not express IPBs	Statistical measure of differences	Source of data
1) Living, receptive females in receptive females' dishes		52	8		Table I. A & B
	Receptive female's tube				
2) Living, receptive females in non-receptive females' and males' dishes		28	12	$X_1^2 = 4.16 > p < 0.05$	Table I. B
	Receptive female's viability				
3) Freeze-fixed, receptive females moved in non-receptive females' and males' dishes		39	41	$X_1^2 = 4.88 > p < 0.05$	Table II & III
	Receptive female's movement				
4) Freeze-fixed, receptive females held in place in non-receptive females' and males' dishes		18	62	$X_1^2 = 12.01 > p < 0.01$	Table II & III

In Preparation III

THE RESPONSES OF MALES AND FEMALES TO EACH OTHER'S SECRETIONS IN THE AMPHIPOD CRUSTACEAN *GAMMARUS PALUSTRIS*

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ABSTRACT

A choice-test apparatus designed to mimic field conditions was employed to test for the presence of water-borne attractants in the amphipod crustacean *Gammarus palustris* . It was found that both males and females were attracted to secretions from all conspecifics, but not to the secretions of a sympatric congener. But when given the choice of secretions from different types of conspecifics, males behaved differently than females. Males were attracted more often to receptive females' and females were attracted more often to males' secretions. In the field, then, it is likely that all conspecifics travel toward each other, then sort themselves into competent heterosexual couples. The results suggest that this apparatus can be employed in future studies to determine the chemical nature of these pheromones.

Key Words--water-borne attractants, crustacean, *gammarus palustris*

INTRODUCTION

Males and females of temperate marine species generally do not communicate with each other via acoustic or visual cues. Water flowing over submerged objects makes sound uncertain, and cloudy waters reduce the effectiveness of visual cues over all but relatively short distances. Instead, many species employ water-borne pheromones, which circumvent acoustic and visual noise. But details about the mechanism of these pheromones' actions are scanty (for example see Salmon, 1983). The experiments reported here were undertaken to supply some basic details for one species, the amphipod crustacean *Gammarus palustris* .

In most crustaceans, the effects of females' secretions on males have been investigated, the idea being that when females are receptive they secrete substances which attract conspecific males, and/or stimulate their reproductive behaviors (Dunham, 1978). But, although individuals of both sexes are mobile, and it is as important to the receptive female to find a competent male as it is for the male to find a female, the possibility that male pheromones exist has been largely ignored. For that reason, the effects of different types of conspecifics' secretions on both males and females were investigated in *Gammarus palustris* .

MATERIALS AND METHODS

All animals were collected from the high tide mark of the intertidal zone at Jamaica Bay, Gateway National Recreation Area, Brooklyn, New York. A female was considered "receptive" if it was currently precopulating (being carried about by a male) (Borowsky, 1984), and non-receptive if it was both 1) not in precopulation and 2) carrying eggs at an early stage of development (eggs at the early stages contain a relatively large amount of dark brown yolk which is easily seen without the aid of a microscope). Receptive females were separated from their attached males by gently pulling the pair apart. This treatment had no apparent affect on either animal, for precopulation would immediately ensue if, after separation, they were replaced together in a dish of sea water. Subsequent survivorship was also unaffected.

The choice-test apparatus shown in Figure 1 was employed as follows: water was introduced into the apparatus in section A of each filter box at a rate of 200 ml/hour. The water then flowed into parts B of the filter boxes through a plastic mesh screen (C). The water then flowed out of each filter box through a 17.5 cm length of 1.2 cm diameter tubing which led to one arm of a glass Y-tube (D). The streams of water from the filter boxes united at the fork of the Y and continued their flow through an additional 17.5 cm length of tubing into the bottom of the seven liter plastic tank (E). Water exited the system through a hole near the top of the tank (F).

Forty individuals whose secretions were to be tested were introduced into one of the filter boxes' A sections. Ulva lactuca thalli (the most common marine alga at the collection site) were placed in the A sections and rocks were placed in the B sections to mimic field conditions. Water was allowed to flow through the system for several hours before an experiment was conducted, to allow the concentrations of whatever secretions might be present to equilibrate in the appropriate sections of the apparatus.

A test was begun by placing 20 males or non-receptive females in the tank. Animals could pass freely from the tank to the Y-tube and then to either of the B sections. They could also return or change direction at any time. They could not enter the A chambers, however, because of the plastic mesh partition. During an experiment, water passed over the test animals in the A section, thus carrying their secretions into the Y-tube, and finally into the tank, where they might influence the travelling animals' behavior. Twenty-four hours later the apparatus was disassembled, and the numbers of travelling animals in each of the B sections as well as the number in the tank were counted. The apparatus was washed in hot tap water and allowed to air-dry between tests.

Four tests were conducted for each experiment for the males, but eight tests were conducted for the females because they were less active than the males. Alternate filter boxes were used for each test.

First, the effects of light vs dark on the males' responses to receptive females' secretions were tested. One experiment was conducted in the light, the other in the dark. After determining that males were only responsive in the dark, all further tests were conducted in the dark (see Results section).

Next, the males' responses to secretions from conspecific non-receptive females, conspecific males, or receptive Gammarus mucronatus females were tested. G. mucronatus is a closely related species which is found on the same beaches as G. palustris (Borowsky, 1980). The next two experiments were conducted to test the relative attractiveness of the three types of conspecific adults to males. In these tests 20 individuals of one type were placed in one filter box, and 20 of another type were placed in the other.

The experimental procedures employed to test the females' responses were identical to those employed to test the males', except: 1) females were placed in the tanks; 2) only non-receptive females were tested, because receptive females are typically carried about by males and remain relatively quiescent when separated from them and 3) eight, rather than four tests were conducted for each experiment.

Hypotheses were tested using the χ^2 and binomial statistics. The overall significances of the experiments were obtained by combining the probabilities from separate replicates using the method of Fisher (1947). Individual probabilities for each replicate were obtained from the binomial distribution (two-tailed test) and -2 times the sum of their natural logarithms were taken as X with eight degrees of freedom for males' and 16 for females' experiments (referred to as the "aggregate χ^2 ").

RESULTS

There was no significant difference between the number of males that travelled to the females' as opposed to the empty filter boxes when the tests were conducted in the light (Table 1 A: aggregate $\chi^2=15.204$, $p>0.05$). But males travelled significantly more often to the females' filter boxes when the tests were conducted in the dark (Table 1 B.1: aggregate $\chi^2=30.914$, $p<0.001$). Thus, males were more responsive to receptive female attractants in the dark than in the light.

Males were attracted significantly often to both types of conspecifics but not to receptive _G. mucronatus_ females (Table 1 B.2,3,and 4: aggregate χ^2s=29.677, 35.615, and 9.964, respectively, ps<0.001 for the first two experiments, and >0.05 for the third). This shows that there is a species-specific substance secreted by all types of adults which attracts males.

The results of the next experiments show that males were attracted significantly more often to receptive females than to non-receptive females, but that they were attracted to non-receptive females and to males about equally (Table 3 A: aggregate χ^2 s=24.974, $p<0.01$, and 7.161, $p>0.05$ respectively).

Females travelled to conspecifics' filter boxes significantly more often than to empty filter boxes, but travelled about as often to competent male _G. mucronatus_ ' as to empty filter boxes (Table 2: receptive female, non-receptive female and male conspecifics, aggregate χ^2s=37.81, 49.09, and 36.97, respectively, all ps<0.01; male _G. mucronatus_ aggregate $\chi^2=13.73$, $p>0.05$). Thus, female _G. palustris_ are also attracted to all conspecifics.

But females travelled more often to males as opposed to other conspecifics. There was no significant difference between the number of females that travelled to receptive vs non-receptive females' sides (Table 3 B.1: aggregate $\chi^2=16.16$, $p>0.05$), but females travelled to males' sides significantly more often than to non-receptive females' sides (Table 3 B.2: aggregate $\chi^2=30.01$, $p<0.01$).

DISCUSSION

The results of these experiments have shed some light on the way pheromones act in Gammarus palustris . First, males are more responsive to attractants in the dark than in the light. This suggests that males are more active at night than during the day (an activity pattern typical of aquatic crustacea (Dunham, 1983). But since most animals are quiescent when they are exposed to the air in the field, their period of activity may be very brief. It may be confined to the four hour period of submersion at their high tide mark habitat which occurs during the night. Second, a species-specific water-borne attractant to which both sexes respond exists in G. palustris ; and third, males prefer receptive females' and females prefer males' attractants over other conspecifics'. These observations show that there are qualitative differences between the two sexes' secretions.

It is not surprising that G. palustris individuals do not respond to secretions from G. mucronatus . At least seven species of gammarid amphipods are permanent residents of the collection site during the warmer months (personal observation). It would be a waste of energy to travel to individuals of other species. Further, G. palustris ' preferred habitat is in a relatively narrow band in the intertidal zone. The secretion of a species-specific attractant may facilitate the location of adults in their niche.

The present study reveals no information about the effects of the attractants on initiating pair formation and mating behaviors. Investigations into these questions are currently underway. However, it is highly probable that the frequencies of these behaviors are enhanced by the existence of these attractants, because individuals of the opposite sex are more likely to travel toward and therefore encounter each other than other types of individuals.

It should prove fruitful to test for male attractants in other species as well. For example, although receptive female lobsters (*Homarus americanus*) secrete a sex attractant (Atema and Engstrom, 1971), they travel to males' home sites under field conditions (Atema *et al* ,1979). It seems possible that the females' movements are guided by male attractants.

Finally, the results pave the way for future, in depth studies of these pheromones. In particular, the results suggest that the apparatus can be employed to determine their chemical nature.

ACKNOWLEDGEMENTS

Support for this research came from ONR contract number N00014-82-C-0482, and is gratefully acknowledged. I thank R. Borowsky for a critical review of the manuscript, and Y. Mohamed for invaluable assistance in the laboratory and in the field.

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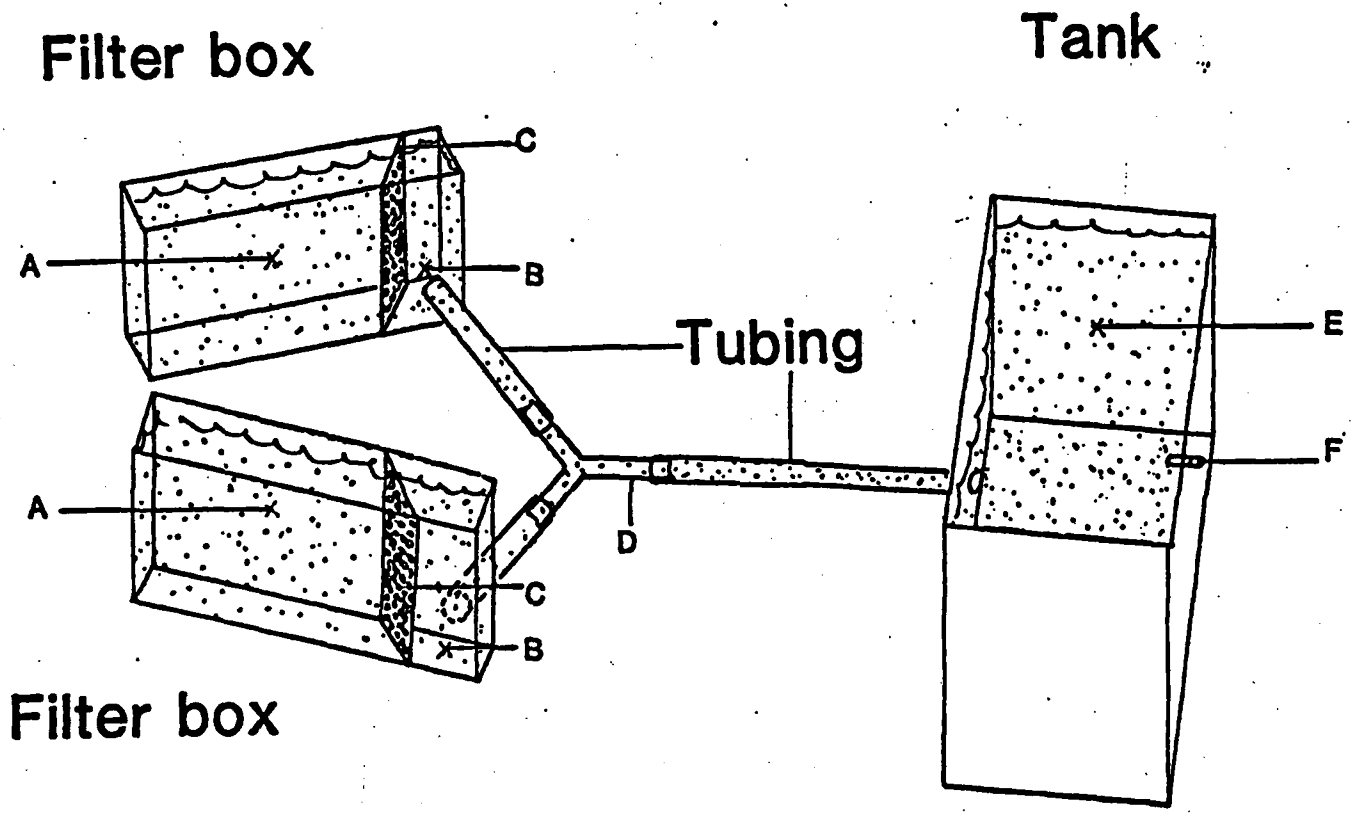
Fig. 1. Apparatus employed for testing Gammarus palustris individuals' responses to other animals' water-borne secretions. Animals whose secretions were tested were placed in the A sections of the filter boxes, and individuals whose responses were to be tested were placed in the Tank (E). The latter could leave the tank and travel to a Y-tube (D), where they could choose to travel to either filter box. Censuses of the number of animals in the B sections of the filter boxes revealed individuals' preferences for different types of animals' secretions. C =plastic mesh screen, F = tube through which water exited from the apparatus.



➡ Direction of Water Flow ➡

Table 1 Means ($\bar{x}$) and standard errors (se) of numbers of males found in different containers after tests

		Filter boxes with secretions from:		Tanks:
A. In the light		Receptive females	No animals	
	$\bar{x}$ (se)	5.7 (0.5)	8.5 (1.0)	5.5 (1.2)
B. In the dark				
1.		Receptive females	No animals	
	$\bar{x}$ (se)	13.8 (0.6)	4.3 (0.9)	2.8 (0.8)
2.		Non-Receptive females	No animals	
	$\bar{x}$ (se)	10.3 (0.6)	4.0 (1.0)	5.3 (0.2)
3.		Males	No animals	
	$\bar{x}$ (se)	13.3 (0.7)	2.3 (1.1)	4.8 (1.0)
4.		Receptive female *Gammarus mucronatus*	No animals	
	$\bar{x}$ (se)	7.5 (0.8)	4.5 (0.9)	7.8 (0.8)

Table 2 Means ($\bar{x}$) and standard errors (se) of numbers of non-receptive females found in different containers after tests

	Filter boxes with secretions from:		Tanks:
1.	Receptive females	No animals	
$\bar{x}$ (se)	7.1 (0.4)	5.0 (0.8)	8.3 (0.4)
2.	Non-receptive females	No animals	
$\bar{x}$ (se)	10.3 (0.6)	3.8 (0.4)	6.9 (0.9)
3.	Males	No animals	
$\bar{x}$ (se)	9.0 (0.4)	3.9 (0.4)	7.3 (0.6)
4.	Competent male *Gammarus mucronatus*	No animals	
$\bar{x}$ (se)	5.5 (0.4)	5.5 (0.5)	9.6 (0.5)

Table 3 Means ($\bar{x}$) and standard errors (se) of numbers of animals found in different containers after tests for preferences for different types of conspecifics

		Filter boxes with secretions from:		Tanks:
A. Males	1.	Receptive females	Non-receptive females	
	$\bar{x}$ (se)	10.8 (0.4)	3.8 (0.8)	5.3 (0.3)
	2.	Non-receptive females	Males	
	$\bar{x}$ (se)	8.3 (0.7)	8.8 (0.5)	3.0 (0.4)
B. Females	1.	Receptive females	Non-receptive females	
	$\bar{x}$ (se)	5.4 (0.4)	4.5 (0.3)	10.1 (0.5)
	2.	Non-receptive females	Males	
	$\bar{x}$ (se)	3.6 (0.4)	7.1 (0.6)	8.5 (0.6)

In Preparation IV

The Reproductive Behaviors of the Amphipod Crustacean Gammarus palustris and Some Insights Into the Nature of Their Stimuli

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Abstract. This paper describes the reproductive behaviors of both sexes of the amphipod crustacean Gammarus palustris in detail and sheds light on the mechanism by which the female conveys its current reproductive condition to the male, and elicits the males' appropriate responses. Males and females are guided to each other by water-borne attractants. Heterosexual contacts are facilitated by visual cues. After contact, males treat other males, non-receptive, receptive and newly molted females differently; they generally drop the first two types of individuals, begin to carry the third type, and copulate with the fourth. They employ behavioral cues and cues associated with the animals' exoskeleta to distinguish among the different types of individuals. The data point to the existence of contact pheromones on the exoskeleta which stimulate the males' principal behaviors.

The reproductive behaviors of many aquatic crustaceans have been described in detail (for example, the stomatopod Gonodactylus bredini (Dingle and Caldwell 1972), and the lobsters, (reviewed by Atema and Cobb, 1980)). However, the nature(s) of the stimuli that elicit these behaviors are not completely understood. In this paper I describe the reproductive behaviors of another crustacean and provide some insights into their stimuli.

Gammarus palustris is a resident of the high tide mark of salt marshes along the western Atlantic seaboard. It travels freely in its preferred habitat when covered by water but rests quiescent under rocks and debris when exposed to the air (which occurs for about two eight hour periods every 24 hours). Females produce several broods in succession, but require insemination just before each ovulation, because there is no sperm storage. Typically, a male picks up a female that is due to ovulate in a few days and carries her about until the female molts (precopulation; Borowsky, 1984). (The length of the intermolt period varies with temperature; at 17°C it is about 13 days, and at 21°C it is 7 days). Immediately after the molt, copulation occurs, and the pair separates. Ovulation occurs within 30 minutes of copulation (unpublished observations). The female then remains alone until a few days before her next molt, when precopulation is reinitiated. Thus, the animals' behaviors may be divided into three successive stages: 1) a male and female come in contact with each other; 2) precopulation occurs; and 3) molt and copulation occur.

The female must somehow inform the male of her reproductive state, because the male's behavior is determined by it. It was reasoned that there are three broad categories of potential sources of such information: 1) the female's behavior; 2) its morphology; and 3) pheromones. The present study was designed to test the effectiveness of each of these factors as stimuli. The results suggest that each of them contributes to the male's appropriate responses.

Materials and Methods

All animals were collected at low tide from the high tide mark at two localities at Jamaica Bay, New York: the Cross Bay Boulevard Bridge, Gateway

National Recreation Area, Queens, and Sea Breeze Marina, Flatbush Avenue, Brooklyn. They were maintained in the laboratory at 18°C in water taken from their collecting localities. Four types of adults were observed: newly molted females, receptive females, non-receptive females, and males. Newly molted females were those which had molted within the previous 24 hours, receptive females were currently precopulating, and non-receptive females were not precopulating and had young embryos in their brood pouches (embryos in early stages of development appear as black spheres, because of the relatively great amount of unconsumed yolk they contain (personal observation)). To ensure their competency, only males that were currently precopulating were employed in these observations. Just prior to an observation or to blinding (see below) a test male was separated from the female it was carrying by gently pulling the couple apart. This had no ill effects on either individual, for if they were placed together again, precopulation was immediately reinitiated, and survivorship was unaffected.

Experiment I was conducted to determine whether receptive females' water-borne secretions affect the males' key reproductive behaviors. Forty males, 40 non-receptive females, 40 receptive females, and 40 newly molted females were isolated in individual dishes for at least 24 hours. Twenty of each group of forty individuals were placed in dishes containing fresh sea water and the other 20 were placed in waters in which receptive females had been maintained for at least 24 hours. Then a male was introduced into each dish and the occurrence of precopulations and copulations were noted. Since no differences in the animals' behaviors were observed in the two types of water, all subsequent experiments were conducted in fresh sea water.

Each of the following four experiments consisted of observations of males interacting with 1) other males (20 replicates) 2) non-receptive females (20 replicates) 3) receptive females (20 replicates) and 4) newly molted females (20 replicates) (80 tests per experiment). Each test involved placing one male and one other individual in a 10.5 cm diameter glass culture dish containing fresh sea water from the collection site plus about 0.5 g chopped algae (Ulva lactuca). The other individuals had been isolated for 24 hours before the test. The behavior of both animals in experiment II, but of only the males in experiments III-V was observed. Observations continued until either 1) precopulation occurred; 2) copulation occurred; or 3) three contacts followed by separations had occurred. All individuals were tested only once, and then discarded.

The following experiments were performed:

II) to permit the description of typical reproductive behaviors of both sexes, and to serve as controls for subsequent experiments, both the male and the other individual were unaltered

III) to investigate the importance of visual cues as stimuli for the males' behaviors, the male was blinded and the other individual was unaltered

IV) to investigate the importance of the exoskeleton the male was blinded and the other individual was freeze-fixed

V) to determine whether the exoskeletal cues were morphological or chemical, blinded males were presented with freeze-fixed individuals which had been stored for 24 hours in fresh sea water at 1°C.

Males were blinded by blotting them dry on paper towels, then using a fine brush to cover their eyes with metallic colored model airplane paint. The paint dried within 30 seconds, forming an opaque shield. Then the males were placed in fresh sea water for at least an hour before testing to recover from the effects of the procedure. Males treated in this way were apparently not harmed, for they generally survived past their next molts.

Freeze-fixing animals eliminated their behaviors while minimizing morphological or chemical changes. Animals were freeze-fixed by blotting them dry on paper towels, then placing them on a piece of aluminum foil which, in turn, was placed directly on a piece of dry ice. Thus, they were not exposed directly to the dry ice or gaseous carbon dioxide. Animals were frozen in between ten and 30 seconds. Then they were replaced in their dishes, where they defrosted within five to ten seconds (as evidenced by the return of their original colors and the extension of their bodies as they warmed up).

Animals were not manipulated during observations in experiments II and III, but in experiments IV and V the freeze-fixed individual was touched to the blinded males' chelae ~~claws~~; otherwise contacts might never have occurred.

The significances of differences were determined with the χ^2 when less than 20% of the classes were under five. Fisher's exact probability test was employed when the χ^2 was inappropriate (as suggested by Siegel, 1956).

Results

Experiment I. Unaltered Individuals Observed in Receptive Females' vs Fresh Sea Waters

Water-borne secretions did not influence the expression of precopulations or copulations. There was no significant difference in the expression of these behaviors to the same types of individuals, whether they were placed in receptive female or fresh sea waters (Table I). Further, receptive female waters did not stimulate males to treat males, non-receptive females or newly molted females as receptive females.

Experiment II. Unaltered Males vs Unaltered Individuals

Male behaviors : The males' behaviors were more obvious and more elaborate than the females'. No noteworthy behaviors occurred before the male touched another individual. On contact, however, the male either 1) did not express additional behaviors or 2) grasped the other individual with its chelae and drew it rapidly toward itself ("grabbing "). Then the male either 1) dropped the individual or 2) palpated it vigorously. "Palpation" involved the male's touching of the other individual repeatedly with many limbs simultaneously. Males palpated with their second antennae, chelae, mouthparts and walking limbs. The other individual was manipulated so that its anterio-posterior axis was perpendicular to the male's. The palpated individual was touched over most of its dorsal surface during the five or ten seconds that palpation alone was expressed. When palpation lasted longer, an additional behavior, "brushing", was often expressed. The males scraped their most posterior limbs (uropods) against the other individual in an anterior to posterior direction (relative to the male). After palpation, the male either 1) dropped the other individual 2) initiated precopulation, or 3) copulated. The latter two behaviors were only expressed to females. "Precopulation" involved the male's reorienting the female so that the two bodies' axes were parallel to each other, then carrying the female with its chelae (figured in Borowsky, 1984). Occasionally, males alternated bouts of palpation with bouts of precopulation, and then copulated. Thus, precopulation was scored only if it lasted longer than five minutes. "Copulation" involved the male's holding a female with the female's body perpendicular to its own, and pressing ventral posterior limbs (the pleopods) repeatedly into the female's brood pouch. Each bout lasted about five seconds, and there were usually three bouts.

Three major conclusions may be drawn from observations of unaltered males' behaviors to unaltered individuals (Table II). First, males grabbed and palpated other males significantly less often than they did females of all types (grabs; males vs all females combined, 6 of 20 vs 48 of 60, respectively; $\chi^2_1=17.094$, $p<0.001$: palpations; males vs all females combined, 2 of 20 vs 48 of 60 times, respectively; Fisher exact probability test, $p<0.001$). Second, precopulations occurred almost exclusively with receptive as opposed to other females (14 of 20 vs 1 of 40 times, respectively; Fisher exact probability test, $p<0.001$). And third, copulations only occurred with newly molted females.

Female Behaviors : Females never exhibited the reproductive behaviors

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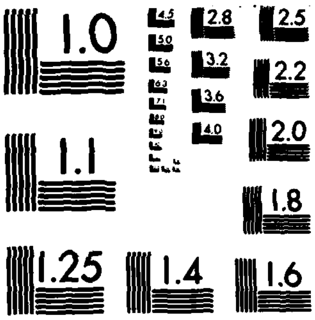

MICROCOPY RESOLUTION TEST CHART
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typically expressed by males (grabbing, palpation, brushing, precopulation or copulation; described above). However, females did exhibit significant, albeit subtle, behaviors. At rest, females' bodies were held in a slight ventral curve (Figure 1, above). When touched by a male, they either swam rapidly away or lay still during the males' manipulations. If they did the latter, they generally assumed one of three positions. Females in the "posterior curl" posture kept their bodies straight from the head to the seventh segment. The posterior portion of the body was curled ventrally (Figure 1, below). Females in a "full curl" curved their bodies ventrally until their heads touched their most posterior segments (Figure 2, above). Females in "angulation" bent their bodies sharply ventrally at two points (the third and tenth segments), holding their anterior limbs pressed forward, and their posterior limbs pressed back against their bodies (Figure 2, below).

These behaviors were correlated with the females' reproductive state. First, there was a significant difference among the three classes of females in the numbers that swam rapidly away from males after being touched (Table III: non-receptive, receptive, and newly molted females, 11, 13, and 5 of 20 times, respectively; $\chi^2_2 = 6.941$, $p<0.05$). Fewer newly molted females swam away than non-receptive or receptive females did (data in Table III: non-receptive and receptive females combined vs. newly molted females, $\chi^2_1 = 6.541$, $p<0.05$; non-receptive and newly molted females combined vs receptive females, $\chi^2_1 = 3.337$, $p>0.05$; receptive and newly molted females combined vs non-receptive females, $\chi^2_1 = 0.534$, $p>0.05$). Thus, newly molted females were more passive than other females, but receptive females were not more passive than non-receptive females.

Second, once in the males' grasp, the type of position assumed by the female was correlated with its reproductive state. Non-receptive females exhibited the posterior curl more frequently, and receptive females exhibited the full curl more often than did the other types of females combined (posterior curls: non-receptive vs other females combined, 9 of 20 vs 6 of 40 times, respectively; $\chi^2_1=6.400$, $p<0.05$: full curls: receptive vs other females combined, 13 of 20 vs 7 of 40 times, respectively; $\chi^2_1=13.538$, $p<0.01$). And only newly molted females exhibited angulation, which preceded every copulation observed.

Experiment III. Blinded Males vs. Unaltered Individuals

When males were blinded, they expressed no behaviors to other males (Table IV). This is in contrast to the behavior of non-blinded males who grabbed 6 and palpated 2 of 20 other males (Table III). Blinded males also grabbed fewer females than did the controls (Tables IV and II: 38 of 60 vs 48 of 60 times, respectively; $\chi^2_1=4.104$, $p<0.05$). This suggests that males employed visual cues, at least in part, to initiate contact with other individuals.

But, other than grabs, blinded males behaved about the same way to females as unaltered males did. There was no significant difference in the number of palpations expressed by blinded and non-blinded males (Tables IV vs II respectively: non-receptive females, 12 vs 13 of 20 times; $\chi^2_1=0.107$, $p>0.05$: receptive females, 14 vs 17 of 20 times; Fisher exact probability test, $p=0.23$: and newly molted females, 13 vs 18 of 20 times; Fisher exact probability test, $p=0.06$). There was also no significant difference in the number of brushes expressed by blinded and non-blinded males (to non-receptive females, 5 vs 2 of 20 times, respectively; Fisher exact probability test, $p=0.204$; to receptive females, 12 vs 10 of 20 times, respectively; $\chi^2_1=0.404$, $p>0.05$; to newly molted females, 9 vs 12 of 20 times, respectively; $\chi^2_1=0.902$, $p>0.05$). In addition, there were no significant differences in precopulations (Fisher exact probability tests, blinded vs non-blinded males, respectively; to non-receptive females, 2 vs 0 of 20 times, $p=0.24$; to receptive females, 14 vs 16 of 20 times, $p=0.36$; to newly molted females, 0 vs 1 of 20 times,

$p=0.50$). And finally, blinded males only copulated with newly molted females, and copulated as often when they were blinded as when they were unaltered (14 vs 17 of 20 times, respectively; Fisher exact probability test, $p=0.24$). Thus, once they were grasped, males could distinguish among the three types of females as accurately when they were blinded as when they were unaltered. The results of this experiment suggest that males employ visual cues to initiate contacts with other individuals, but use information obtained after contact to distinguish among them.

Experiment IV. Blinded Males vs Freeze-Fixed Individuals

As mentioned in the Materials and Methods section, freeze-fixed individuals had to be touched to blinded males, or contacts might never have occurred. This 1) supports the conclusion from experiment III that visual cues facilitate contacts and 2) suggests that other individuals influenced the occurrence of contacts with males. When the other individuals were unaltered no male was grabbed. In contrast, the majority of females were grabbed. In fact, females tended to swim to the blinded males and remain next to them, thus facilitating contacts, but males generally ignored blinded males. The data suggest that contacts are facilitated by the females' behaviors.

Blinded males behaved about the same way to non-receptive and newly molted females whether they were unaltered or freeze-fixed (statistics comparing the data on Tables IV and V on Table VI). In contrast, they precopulated with fewer freeze-fixed than unaltered receptive females. Thus, freeze-fixing receptive females reduced their ability to stimulate precopulations. However, even though they were freeze-fixed, blinded males could still accurately distinguish among the three types of females.

Experiment V. Blinded Males vs Freeze-Fixed Individuals Stored for 24 Hours

Before Testing

Storing reduced freeze-fixed females' abilities to stimulate the males' reproductive behaviors (see Table VIII for statistics of comparisons between experiments IV and V: Tables V and VII, respectively). There was no significant difference between the males' treatment of males and non-receptive females whether they were tested immediately or tested 24 hours after freeze-fixing. And receptive and newly molted females were palpated about the same number of times. But males precopulated with significantly fewer receptive females and copulated with significantly fewer newly molted females when they were stored than when they were tested immediately. Thus, storage resulted in a significant reduction in the frequency of precopulations and copulations. The most logical explanation for these results is that some chemical or chemicals on receptive and newly molted females' exoskeleta lost their potency after 24 hours.

Discussion

The First Stage of Reproductive Behavior

In this stage males and females 1) travel toward, and 2) contact each other. Their travels are probably guided by the water-borne attractants they secrete. Males and females are attracted to the secretions of both sexes. In addition, females prefer males' and males prefer receptive females' secretions (Borowsky, in prep).

Contact is stimulated by visual cues. Blinding males eliminated their ability to initiate contacts. However, there is no evidence for visual discrimination between the sexes before contact, because unaltered males touched all individuals of both sexes.

Immediately after contact, males grabbed other individuals. The function of this behavior is probably to lock onto the other individual and position it properly for palpation. They grabbed females more often than males, which shows that they distinguished between them after contact. But the present study provides no information about the cues for this discrimination.

The Second Stage of Reproductive Behavior

In this stage precopulation is initiated and continued until the female molts. Whether the pair separates or begins to precopulate is probably decided during the period when males palpate (and sometimes brush) other individuals.

In contrast to stage one, there is no evidence for the existence of either visual or water-borne chemical stimuli for this stage. Blinded males behaved the same way to receptive females as unaltered males did. And about the same number of precopulations occurred in fresh sea as in receptive females' waters.

Some factor associated with the female's exoskeleton is implicated as the stimulus for the onset of precopulation. It seems likely that during palpation males are investigating the nature of the other individual's exoskeleton. Palpation involves repeated contact of another individual's exoskeleton with many of the males' limbs. The majority of these limbs have chemosensory functions in other crustaceans (the antennae of most species perceive chemical substances, and Dahl et al , 1970, has established this in the amphipod Gammarus duebenii : the chelae of the crayfish Cambarus are chemosensory; Hodgson, 1958: the walking legs of the lobster Homarus americanus are, Derby and Atema, 1982: and so are the maxillipeds of the same species; Devine and Atema, 1982).

Prolonged palpations were often accompanied by brushing. This behavior may serve to explore the other individual's exoskeleton and/or as a signal to the female to cease behaviors which inhibit precopulations or copulations. The "beating" described for Gammarus duebenii males by Hartnoll and Smith (1980) may be what has been called "brushing" here.

The female's behavior may influence the onset of precopulation as well. First, when touched, females can either swim rapidly away from the male, which inhibits further reproductive exchanges, or they can lie quiescent and permit the males' manipulations. Second, females may influence the males' behaviors by the positions they assume during the males' palpations.

In Gammarus precopulation always occurs by the males' inserting the anterior hook of their chelae between the dorsal segments of the female's exoskeleton where they overlap (Borowsky, 1984). Non-receptive females assumed the posterior curl more often than did other types of females during palpation. This may prevent, or at least make more difficult, the males' insertion of their hooks between those segments, because the overlapping portions are held tightly together when the female is in this position (Figure 1, below). In contrast, receptive females assumed a full curl more often than other females did. In this postion the situation is reversed; the segments are separated at the points of overlap, which may facilitate the correct positioning of the males' hooks (Figure 2, above).

Freeze-fixing receptive females reduced the frequency but did not eliminate the occurrence of precopulations. One explanation for this is that both the females' behaviors and their exoskeleta influenced the males' behaviors. If behavior was the only stimulus, males would have treated all three types of freeze-fixed females in the same way. On the other hand, if the exoskeleton was the only stimulus, the frequency of the males' behaviors to freeze-fixed females should have been as great as to unaltered females. An alternative, but not mutually exclusive, explanation for the results is that the females' behavior did not influence the males' behaviors at all. Instead, freeze-fixing females caused a reduction in the potency of the exoskeletal stimulus.

The Third Stage of Reproductive Behaviors

The stimuli for copulation seem to be similar to those for the previous stage. Support for the importance of behavior as a stimulus is: 1) newly molted females were more passive than other types of females; 2) angulation was only expressed by newly molted females; and 3) this behavior preceded every copulation with unaltered females. It is interesting that newly molted females were more passive than other females. The timing of the onset of precopulation is not of critical importance. It is not even a prerequisite for the female's successful production of a brood; most males did not precopulate with newly molted females, but sought to copulate immediately. In contrast, the timing of copulation is critical, for it must occur after the female's molt, but before the female's new exoskeleton hardens sufficiently to prevent ovulation (Kaestner, 1970). Thus, it is important for newly molted females to permit the males' attentions; and that is probably why they remain quiescent after contacts. Although males expressed copulation to newly molted females that did not assume this posture (the freeze-fixed females), it is unlikely that semen was introduced into the brood pouch. Since fertilization can only be effected during a brief interval, and since it occurs either in the oviducts or in the brood pouch (Kaestner, 1970) it is important to place semen directly in the brood pouch. The female's angulation posture undoubtedly facilitates this (Figure 2, below).

Support for the importance of the exoskeleton as a stimulus for copulation is that blinded males copulated with freeze-fixed animals, but only with newly molted females. Again, there was no difference in the frequencies of copulations that occurred in receptive females' as opposed to fresh sea waters.

Evidence for the Existence of Contact Pheromones in Gammarus palustris

When animals were freeze-fixed and tested immediately, blinded males expressed precopulations and copulations to the appropriate individuals, albeit in reduced frequencies. But when the freeze-fixed individuals were stored at 1°C for 24 hours, males no longer responded to them. It is unlikely that there were morphological changes in the calcified exoskeleta during that period. Arthopods' exoskeleta generally remain unchanged between molts. It is far more likely that some chemical lost its potency during the 24 hour storage period. Thus the data point to the existence of contact pheromones in G. palustris . However, the final proof awaits the transferrence of receptivity from receptive to non-receptive females or males by chemical methods. Efforts toward this end have been reported by Hammoud et al (1975) for Gammarus pulex , and Hartnoll and Smith (1980) for G. duebenii . Firm conclusions are impossible at this time, however, because the former authors reported that they could, and the latter that they could not transfer the females' receptivity by chemical methods.

Finally, tactile behaviors are typically observed before mating in other crustaceans (Salmon, 1983). It may prove fruitful to investigate the existence of contact pheromones in other species as well.

Conclusion

The most significant conclusion of observations of male Gammarus palustris ' reproductive behavior is that it is governed by many distinct stimuli whose effects are additive. Individuals of this species are relatively mobile, and live in a harsh and uncertain environment. The existence of a suite of stimuli may enhance the chances of reproductive success. Undoubtedly, when all the stimuli are in place, pair formation and mating are expedited. But should one, or even several, stimuli be missing, it is likely that a viable brood will be produced anyway.

Acknowledgments

I thank Y. Mohamed for unstinting assistance in the laboratory and in the field. This work was done with the aid of ONR contract N00014-82-C-0482. Figures modified from Plate III, Bousfield, 1973. Copyright 1973 by the National Museum of Canada. Used by permission of the publisher, Cornell University Press.

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Table I. Number (of Twenty) of Precopulations and Copulations Expressed by Unaltered Males to Individuals in Fresh Sea vs. Receptive Female Waters.

		Precopulations	Copulations
A. With males in:	Fresh sea water	0	0
	Receptive female water	0	0
B. With non-receptive females in:			
	Fresh sea water	0	0
	Receptive female water	0	0
C. With receptive females in:			
	Fresh sea water	20	0
	Receptive female water	19	0
D. With newly molted females in:			
	Fresh sea water	4	16
	Receptive female water	1	18

Table II. Number (of Twenty) of Behaviors Expressed by Unaltered Males to Other Unaltered Individuals

Behavior	Type of individual			
	Male	Non-receptive female	Receptive female	Newly molted female
Grabbing	6	12	18	18
Palpation	2	13	17	18
Brushing	0	2	10	12
Pre-Copulation	0	0	14	1
Copulation	0	0	0	17

Table III. Number (of Twenty) of Behaviors Expressed by Different Types of Unaltered Females to Unaltered Males

Behavior	Type of female		
	Non-receptive	Receptive	Newly molted
Swim away rapidly	11	13	5
Posterior curl	9	2	4
Full curl	2	13	5
Angulation	0	0	17
Precopulation	0	14	1
Copulation	0	0	17

Table IV. Number (of Twenty) of Behaviors Expressed by Blinded Males to Unaltered Individuals

Behavior	Type of individual			
	Male	Non-receptive female	Receptive female	Newly molted female
Grabbing	0	12	11	15
Palpation	0	12	14	13
Brushing	0	5	12	9
Pre-copulation	0	2	16	0
Copulation	0	0	0	14

Table V. Number (of Twenty) of Behaviors Expressed by Blinded Males to Freeze-Fixed Individuals Tested Immediately

Behavior	Type of individual			
	Male	Non-receptive female	Receptive female	Newly molted female
Palpation	9	10	13	18
Brushing	1	9	9	15
Pre-copulation	0	1	8	4
Copulation	0	0	1	13

Table VI. Two-Tailed Chi-Square (X^2) or Fisher Exact Probability Tests (F) of the Significance of Differences in the Expression of Behaviors of Blinded Males to Unaltered vs. Freeze-Fixed Females. N.D. = No data.

	Non-receptive female		Receptive female		Newly molted female	
	test	P	test	P	test	P
Palpation	$X_1^2=0.902$	>0.05	$X_1^2=0.114$	≯0.05	F	0.64
Brushing	$X_1^2=1.76$	>0.05	$X_1^2=0.902$	>0.05	$X_1^2=3.75$	>0.05
Precopulation	F	0.50	F	0.02	F	0.10
Copulation	ND		F	0.50	$X_1^2=0.114$	≯0.05

Table VII. Number (of Twenty) of Behaviors Expressed by Blinded Males to Freeze-Fixed Individuals Stored for Twenty Four Hours Before Testing

Behavior	Type of individual			
	Male	Non-receptive female	Receptive female	Newly molted female
Palpation	4	12	18	17
Brushing	0	3	4	7
Pre-copulation	0	0	0	1
Copulation	0	0	0	1

Table VIII. Two-Tailed Chi-Square (X^2) or Fisher Exact Probability Tests (F) of the Significance of Differences in the Frequencies of Behaviors Expressed by Blinded Males to Individuals That Were Freeze-Fixed and Stored vs Freeze-Fixed and Tested Immediately. N.D. = No data.

	Males		Non-receptive females		Receptive females		Newly molted females	
	Test	P	Test	P	Test	P	Test	P
Palpation	F	0.18	$X_1^2=0.404$	>0.05	F	0.06	F	0.50
Brushing	F	0.50	F	0.04	F	0.09	$X_1^2=6.46$	<0.05
Precopulation	ND		F	0.50	F	0.002	F	0.17
Copulation	ND		ND		F	0.50	F	0.02

Figure 1. Two postures of Female *Gammarus palustris*

Above:At rest. Body is held in a slight ventral curve.

Below: The "Posterior Curl Position". The anterior part of the body is stretched to form a straight line, while the posterior part is curled sharply ventrally, with the uropods held under the body. This position is often assumed by non-receptive females that are grabbed by males.

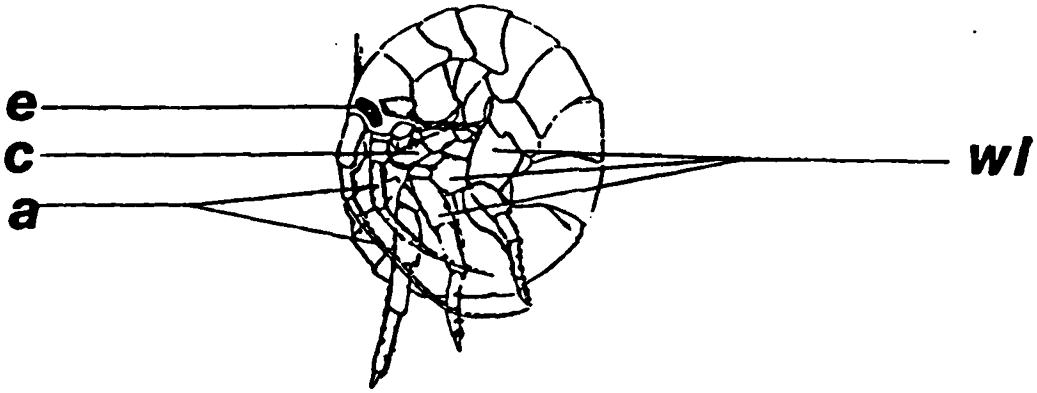
a = antennae; c = chelae; e = eye; wl = walking legs; u = uropods

Figure 2. Two Postures of Female Gammarus palustris

Above: The "Full Curl" position. The entire body is curved ventrally into a compact ball. (The uropods are covered by the other limbs). This position is often assumed by receptive females when grabbed by males and precedes the onset of precopulation.

Below: The "Angulation" position. The body is bent sharply ventrally at two points, and held in a straight line between those points. In addition, the walking limbs and chelae (here hidden by other limbs) are held away from the area of the ventral brood pouch. This position is always assumed by newly molted females after being grabbed by males and immediately before copulation.

a = antennae; bp = brood pouch; c = chelae; e = eye; wl = walking legs; u = uropods.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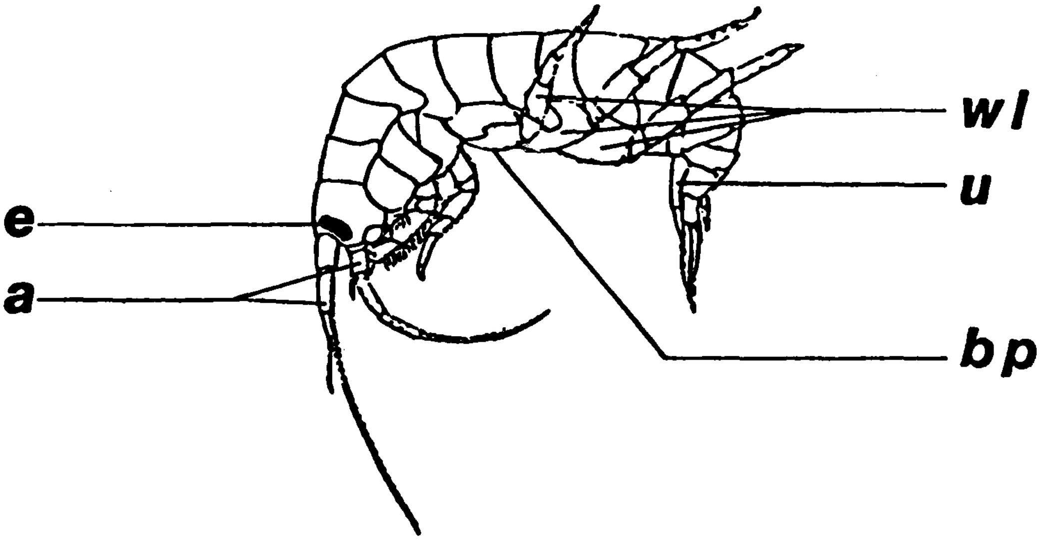
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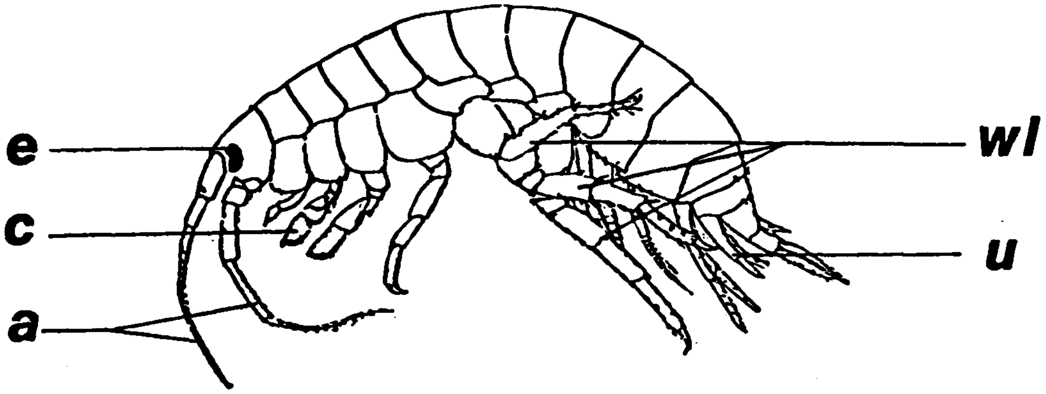
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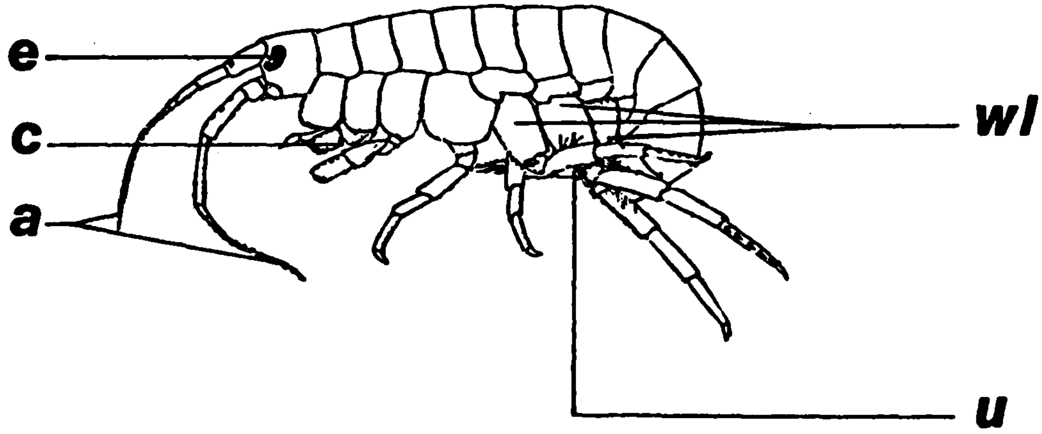
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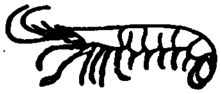
from: Ripples 9(3): 7. 1984

NOTES FROM A RESEARCH SCIENTIST:



Dr. Betty Borowsky, a behavioral physiologist at the Osborn Laboratories of Marine Science at the New York Aquarium, offers a peek into her research world.

Many species of marine animals and plants affix themselves to the surfaces of ships and stationary man-made installations. These species are called "fouling organisms" because when sufficient numbers are present (which always happens with time) they interfere with the efficiency of submerged ships and machines. The Navy spends millions of dollars a year scraping off these organisms -- the only method of controlling fouling which is effective at this time.

Some of the principal fouling organisms are the 1/4 inch long amphiod crustaceans. These animals resemble tiny shrimp. They secrete a sticky, thread-like substance which they use to tack bits of debris together to form residential tubes on hard surfaces. I have been studying the life-history of these species. One key feature of their life-histories is that females tend to remain inside their tubes while males travel between tubes seeking females receptive to reproduction. One aspect of my current research concerns determining how the male finds a female, and how he learns whether or not she is receptive. Early results suggest that receptive females produce a water-borne chemical messenger (called a pheromone) which both attracts the male and informs him of the female's receptivity. I am collecting data to substantiate (or discount) these preliminary results.

If a pheromone is demonstrated unequivocally, I will isolate and characterize it. Then the pheromone can be synthesized and commercially employed in a trap similar to the "roach motel". Instead of males being attracted to man-made surfaces, they will be attracted to the trap, thus preventing fertilizations and the rapid multiplications of individuals on the surfaces. This should be less costly than current methods of control.

From: American Zoologist 23. 1983

THE EXISTENCE OF A WATER-BORNE MALE ATTRACTANT SECRETED BY RECEPTIVE FEMALES OF THE AMPHIPOD CRUSTACEAN *MICRODEUTOPUS GRYLLOTALPA* (COSTA). B. Borowsky, Osborn Laboratories of Marine Sciences, Brooklyn, New York 11224

Males of the tube-building amphipod *M. gryllotalpa* cruise from tube to tube, testing the nature of each occupant until they find a receptive female. They also share the receptive female's tube until copulation occurs. It was hypothesized that a water-borne chemical attractant secreted by receptive females might serve to guide males specifically to their tubes. A choice-test apparatus, designed to mimic field conditions, was employed to test this hypothesis. The results demonstrated that receptive females do secrete a male attractant, but males and non-receptive females do not. Other experiments demonstrated that the attractant remains potent for at least 1.9 hours, but does not, by itself, stimulate the males' courtship behavior.

From: American Zoologist 23. 1983

AMYLASE IN THE AMPHIPOD GAMMARUS PALUSTRIS : A CHEMOSENSORY HYPOTHESIS TO ACCOUNT FOR HETEROZYGOTE DEFICIENCY AND DIFFERENTIAL FOOD CHOICE.

R. Borowsky*, B. Borowsky**, and Haleh Milani* *Dept. Biol., N. Y. Univ., **Osborn Labs., N. Y. Zool. Soc., New York City.

G. palustris is polymorphic for salivary amylase, and populations in the NYC area have two common alleles. Repeated collections demonstrate a consistent pattern of heterozygote deficiency characteristic of population subdivision rather than selection against the heterozygote. Computer simulations show that the observed distribution can be generated by *non-random mating if homozygotes choose* different subniches. Experiments suggest that genotypes with the slow allele tend to choose Enteromorpha over Ulva for cover and food, more often than do fast homozygotes. Christensen (1977, Hereditas 87:21) demonstrated both food choice based on amylase genotype and heterozygote deficiency in the isopod Asellus aquaticus . Our results suggest that this may be a general phenomenon, at least in the peracarida. We hypothesize that salivary amylase may play a chemosensory role and condition behavior by controlling the nature of the oligosaccharide pool produced by the hydrolysis of substrate.

From: American Zoologist 22: 909. 1982

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POSITION OF THE GNATHOPODS OF SOME MALE GAMMARID AMPHIPODS DURING PRECOPULATORY BEHAVIOR. B. Borowsky. Osborn Laboratories of Marine Sciences, Brooklyn, NY 11224

Epibenthic gammarid amphipods typically carry a female about for a few days prior to the time the female is due to ovulate. This guarantees the presence of a male during the brief period when copulation and fertilization can be effected. Among the gammarids, the males' gnathopods are generally larger than the females'. In addition, in males, one pair of gnathopods is generally larger than the other. It has been assumed that in species which precopulate, the male carries the female with the larger of the pair. However, a survey of five species, from three genera, has shown that the smaller pair is used. In addition, the use of these gnathopods is highly stereotyped, and generic-specific. Previous observations on benthic gammarid males have shown that they use the larger gnathopods to gain access to a female. Observations on one epibenthic species, Gammarus palustris, have shown that males use the larger pair of gnathopods to dislodge other males from the females they are carrying. Thus, the function of large gnathopods in male gammarids may be universal; to gain access to a female and prevent her loss to other males.

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