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Mating behaviour and spermatophore transfer in the shrimp *Heptacarpus pictus* (Stimpson) (Decapoda: Caridea: Hippolytidae)

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Introduction

Events occurring during mating of caridean shrimp have been described by Needler (1931) (Pandalidae), Nouvel and Nouvel (1937) (Alpheidae, Palaemonidae), Nouvel (1939) (Crangonidae), Höglund (1943) (Palaemonidae), and Wickler and Seibt (1970) (Gnathophyllidae) with observations by Burkenroad (1947) (Palaemonidae), Forster (1951) (Palaemonidae), Carlisle (1959) (Pandalidae), Ling (1967) (Palaemonidae) and Hoffman (1973) (Pandalidae). General features of moulting, mating, and spawning of these carideans are similar to those observed in the hippolytid *Heptacarpus pictus*.

The first two pleopods of most male decapod crustaceans are modified in some manner from the remaining pairs and in those species which have been investigated it has been established that these first pleopods serve as gonopods. Endopods of the anterior two pairs in caridean shrimp differ somewhat from the remaining pairs of pleopods. The endopod of the first pair usually differs in shape, size, and setation from that of posterior pairs. In some carid families (e.g., Crangonidae), it is reduced to a simple leaf, while in others (e.g., Pandalidae), it is stout and complex in form and setation (Berkeley, 1930; Hoffman, 1972). The medial edge of the inner ramus of the second pair of pleopods bears a spinous process, the appendix masculina, in addition to the appendix interna found on all the remaining pleopods.

While it is often assumed that the appendix masculina and the modified endopod of the first pleopod function as copulatory processes, their actual role in sperm transfer has never been demonstrated (Balss, 1944). Needler (1931) believed that the spines of the appendix masculina helped to guide sperm to the body of female *Pandalus*. However, Nouvel (1939) and Burkenroad (1947) discounted their contribution to spermatophore deposition in *Crangon* and *Palaemonetes* respectively. Mating acts are usually quite brief in this decapod group; pleopods and genital orifices of the male are hidden from the view of the observer. Elucidation of the important process of sperm transfer has therefore not progressed beyond these few observations.

This report will discuss mating behaviour and the mechanics of spermatophore formation and transfer in *H. pictus*. Observations on life history and reproductive events associated with mating are included.

Methods

H. pictus used in the study were collected by dipnet from tidepools at Bird Rock, La Jolla, California and maintained in seawater systems at the

Scripps Institution of Oceanography. Brooding females with enlarged ovaries showing through the carapace were individually isolated and used in laboratory matings after hatching of embryos and the subsequent post-hatching moult. Females were isolated from contact with males for a short time after introduction into small aquaria so that they could acclimate to the new surroundings. Matings were observed directly and also recorded with an 8 mm cine camera.

Ablation experiments were conducted on males anaesthetized by chilling. These were held in place ventral side up on a wax-filled dissecting dish with crossed insect pins placed over thorax and abdomen. Appropriate pleopod rami were removed with jewellers forceps. Experimental males recovered within minutes but were not used in matings for 2–3 days afterwards.

Life history

Heptacarpus pictus occurs from Monterey, California (Rathbun, 1904) to at least as far south as the San Benito Islands, Baja California. The depth distribution extends from the shallow subtidal up to the lower intertidal, which, in southern California, is the region of the *Ulva-Colpolmenia-Corallina* algal association.

Mature males of this small species range from 1–3 mm carapace length, while brooding females vary from 2·5–4·5 mm carapace length—the mean size varies seasonally (carapace length is defined as the mid-dorsal distance from the posterior edges of the eye orbits to the posterior edge of the carapace). The reproductive season occurs during the winter and spring months, extending into the summer and ending for most of the population in August. Large (older) females are found in berry in August and September, but are quite rare.

During the last two months of the summer, most of the mature females lose the elements of the 'breeding dress', including the lengthened coxae of the pleopods; the posteriorly directed flange on the basipodites of the pleopods; the compressed and widened basipodite of the first pleopod forming a slightly curved plate; the pronounced enlargement of the epimeres (pleural plates); and the ovigerous setae on the pleopods. The last two characters appear suddenly at the first reproductive moult (non-brooding females with ripe ovaries) while the other characters develop gradually in a series of moults throughout the fall.

Males that are mature at the end of the summer retain secondary sexual characters throughout the fall, although the vasa deferentia are no longer filled with sperm. Males which are still immature at the end of the summer acquire sexual characteristics gradually during this period. Energy used in reproduction during the winter, spring and early summer appears to be channelled into growth during the late summer and fall months. The greatest increase in body size of the population occurs during this latter period.

Size-frequency data taken irregularly in 1970 and 1971 and observations taken during collecting from La Jolla tidepools provides the following picture of population recruitment and growth. Brooding females appear in late December with nearly all individuals in the population sexually mature by spring (fig. 1(A)). Juveniles appear in increased numbers throughout the summer months while large females become scarce. The maximum life span appears to be not more than a year and a half. Junveniles settling early in the

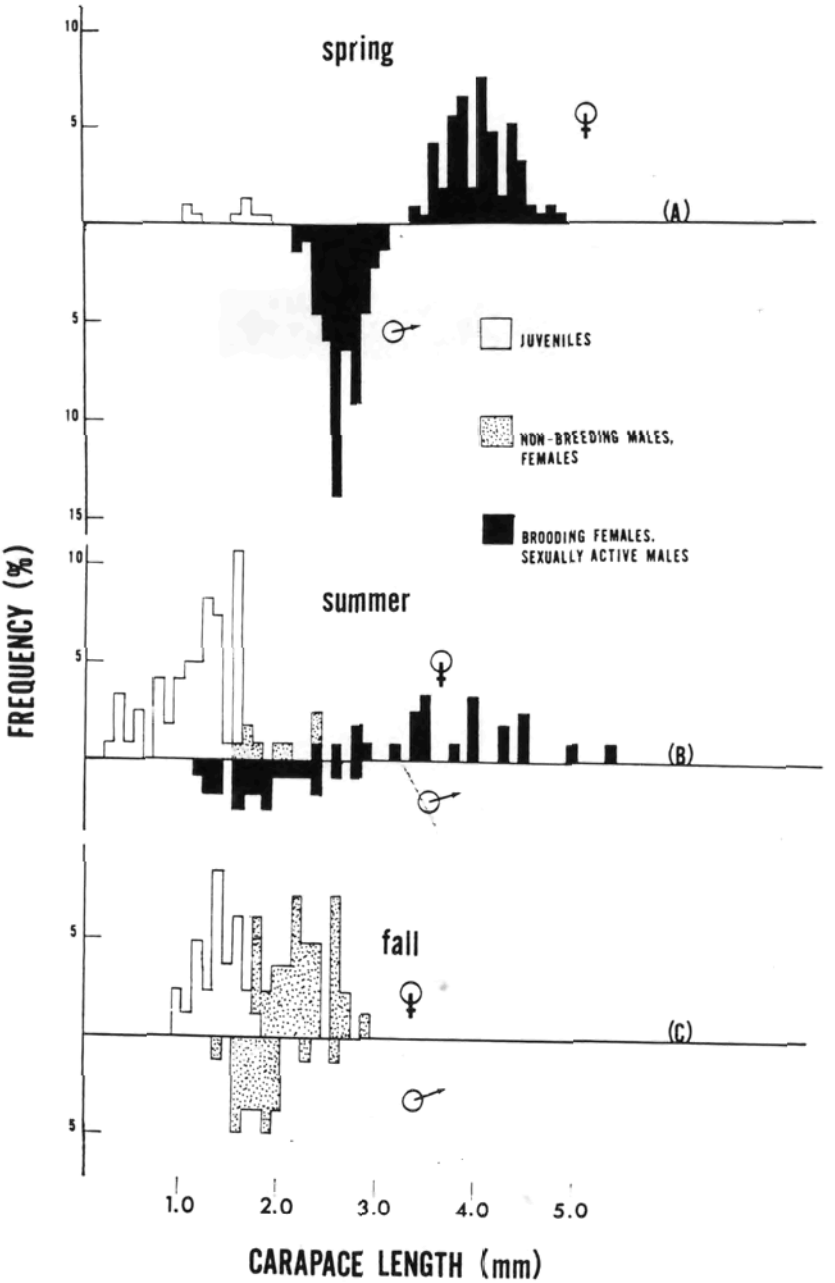

FIG. 1. Typical size-frequency distributions of *Heptacarpus pictus* collected from La Jolla, California. (A) Spring (combined similar samples of April 16, 18, 28, 29, 30, 1970; $N=217$); (B) Summer (8–10 July 1971; $N=121$); (C) Fall (17–18 October 1970; $N=82$)

breeding season (spring) reproduce during their first year, as indicated by the appearance of small males and small berried females during the summer (fig. 1(B))—these individuals go through the non-reproductive period of growth in the fall and reproduce again the following winter and spring, leaving the

population during the next summer. Juveniles arriving in the late summer do not grow large enough to mature the first year, grow during the fall and also begin to reproduce during the winter—these individuals also do not survive through the following fall (fig. 2).

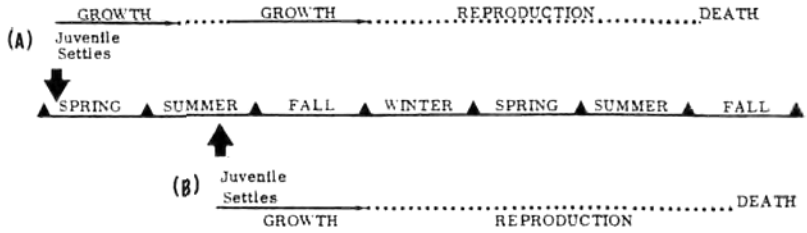

FIG. 2. Schematic representation of the juvenile and adult life history of *Heptacarpus pictus*. (A) an individual settling out of the plankton in early spring; (B) an individual settling in late summer.

Large (older) individuals become rare and disappear from fall samples (fig. 1(c)). It may be that there is a physiological life span for this species, but it seems more likely that the number of mature individuals gradually declines through the spring and summer months due to constant exposure to predation. There are several species of fish (e.g. the woolly sculpin, *Clinocottus analis* (Girard); the California clingfish, *Gobiesox rhessodon* (Smith)) abundant in the tidepools which readily prey on *Heptacarpus* in the laboratory.

Mating

Premating activities of the female

Females with egg-filled ('ripe') ovaries that have recently moulted are receptive to mating and attractive to males. Since *H. pictus* is a multiple brooder, the female carries developing embryos as the ovary increases in size. Hatching of larvae occurs shortly before moulting (fig. 3(A)). Besides renewing the exoskeleton, molting removes attached egg membranes of hatched larvae and the bodies of the few unhatched embryos. Females are attractive to males for only a few days after this moult.

Male mating behaviour

Mating behaviour of the male can be divided into seven events, taking place within 10–30 sec:

(1) *Contact:* Males introduced into an aquarium containing a receptive female behave normally until the female is touched, usually with the outstretched antennal flagellum (fig. 4(A)). Males can walk close to such a female repeatedly but show no sign of recognition until physical contact is made. Thus, there appears to be no searching or display behaviour of any kind by the male. Males apparently recognize attractive females by contact chemoreception; the response is evoked by any part of the female's body.

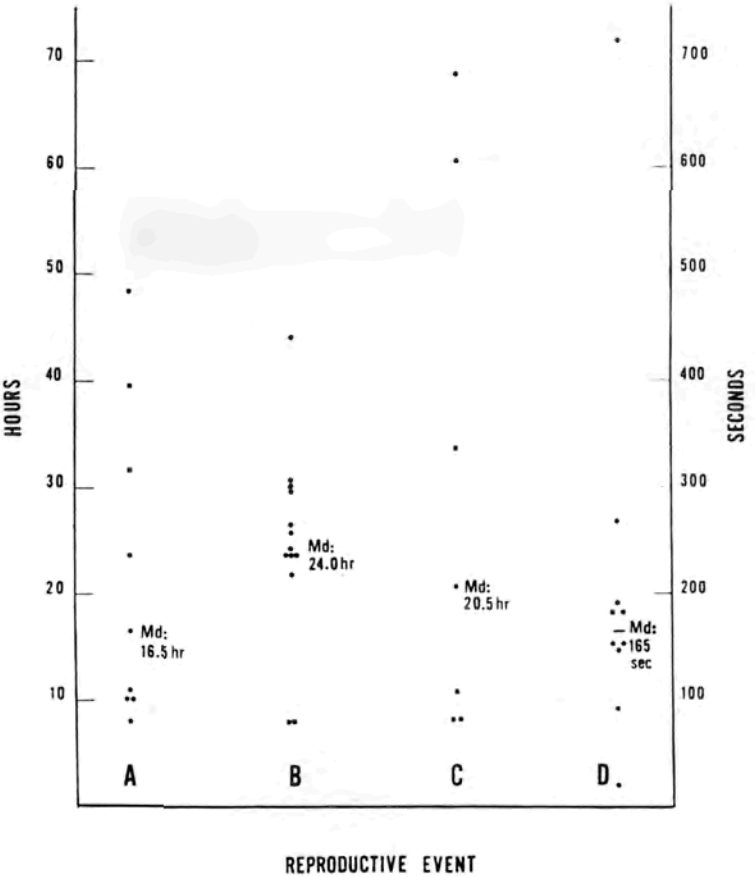

FIG. 3. Reproductive events associated with mating and spawning in female *Heptacarpus pictus*. Time intervals between: (A) Hatching and moulting; (B) Post-hatching moult and spawning in male-deprived females; (C) Spawning and discarding of unfertilized eggs; (D) Mating and spawning.*

Upon contact, change in behaviour is obvious and abrupt; the male attempts to seize the female with its pereopods, especially the walking legs.

Elicitation of a male mating response by contact and the lack of precopulatory behaviour has been noted in the carideans *Palaemon squilla* (L.) (Höglund, 1943); *Palaemonetes vulgaris* (Say) (Burkenroad, 1947); *Palaemon serratus* (Pennant) (Forster, 1951); *Pandalus borealis* Kroyer (Carlisle, 1959). Carlisle (1962) comments on the apparent lack of a distance pheromone in caridean communication and believes that a non-diffusible substance on the surface of the female exoskeleton is the sex pheromone. However, a distance pheromone important in individual recognition has been demonstrated by Seibt (1973) in *Hymenocera picta* Dana (Gnathophyllidae), a species in which stable pair bonds are formed between males and females.

*For measurement of A, B, and C, isolated females were checked at intervals to see if the event had occurred. If an event had not taken place at observation t_x, but had occurred at observation t_y, the event was considered to have been completed midway between t_x and t_y giving the points on the graph. D was observed directly.

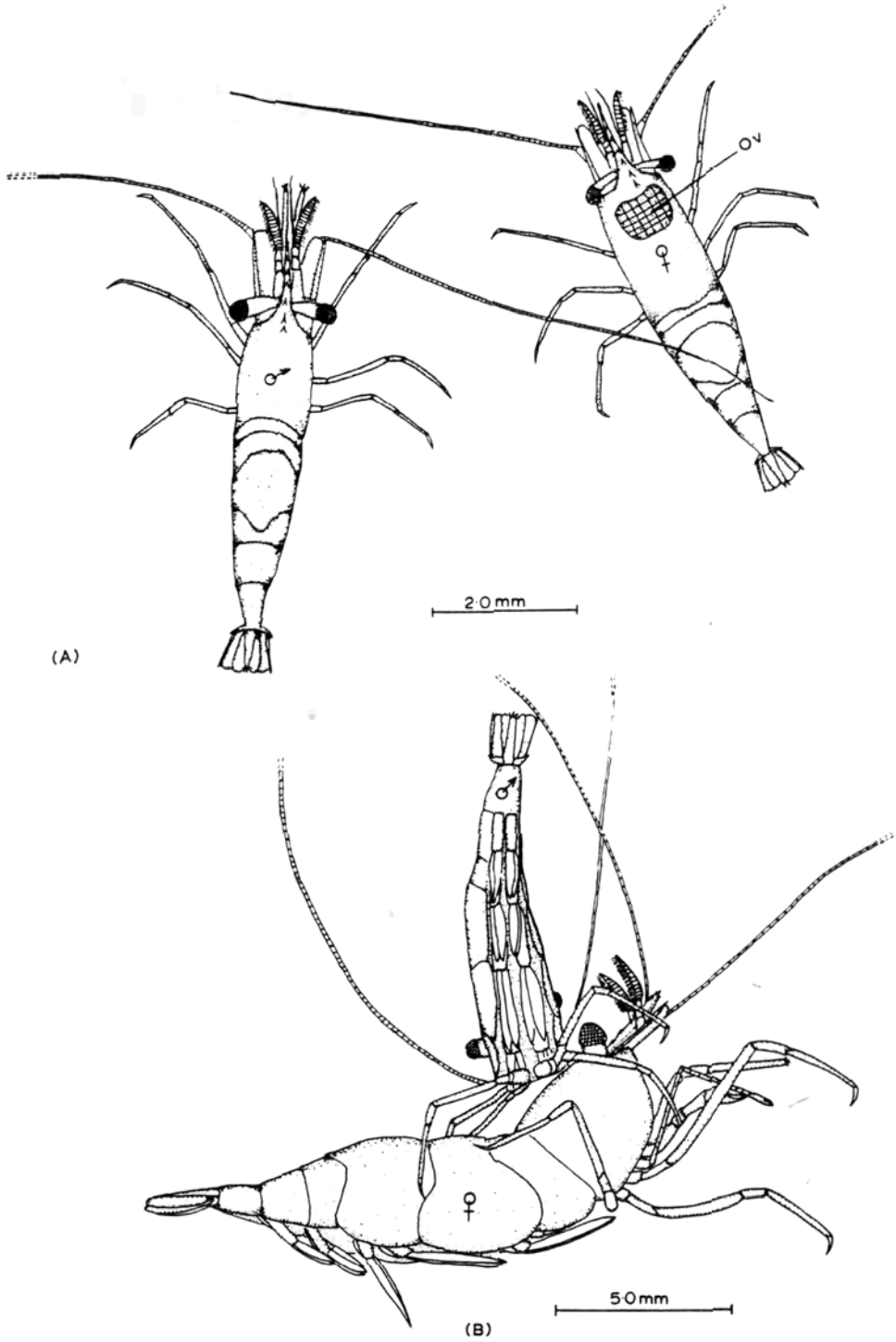

FIG. 4. Mating in *Heptacarpus pictus*. (A) Initial contact of the female by the male's antennal flagellum; (B) Male climbing onto the female (abbreviations used in the figures are found at the end of the text).

(2) *Climb:* After contact, the male attempts to crawl up to the dorsal midline of the female (fig. 4(B)). If the contact has been made at the anterior of the female, there is usually a brief flurry of rapid blows or strikes by the pereopods and third maxillipeds between the male and female. Although appearing initially to be some sort of recognition or pre-copulatory behaviour, this activity is probably a residual defensive behaviour seen in non-mating individuals. It also appears to prevent entanglement of the many appendages

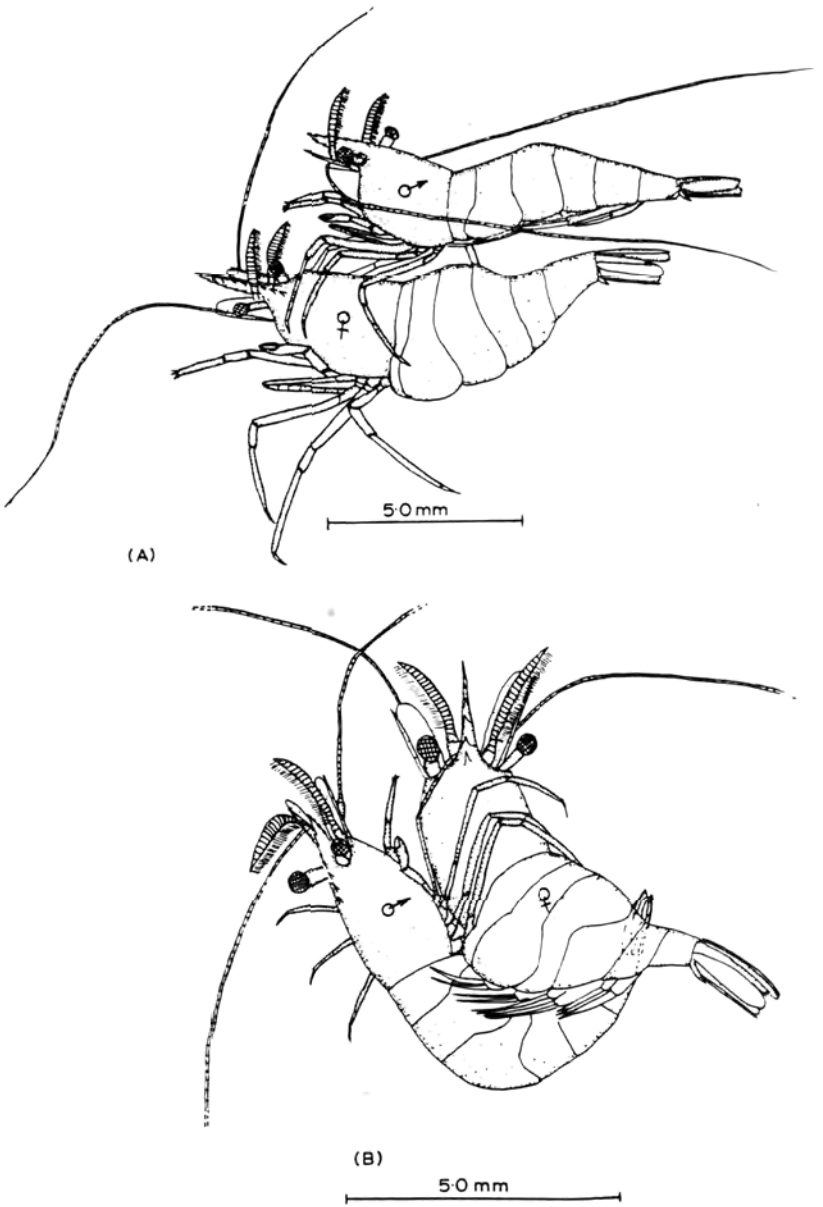

FIG. 5. Mating in *Heptacarpus pictus*. (A) Male above the female in the *straddle* position; (B) Male shifting into the *dip* position beneath the female.

of the mating partners as the male climbs up the female's cephalothorax. No such behaviour is shown when the male approaches the female from behind.

(3) *Straddle:* Clutching the female with his walking legs, the male is astride the female's dorsal midline with the anterior ends of both animals facing the same direction (fig. 5(A)). This component of mating behaviour appears to allow the female time to settle down and accept the presence of the male before the next event. A male is usually successful in mating if he has attained this position. Rejection by the female takes place most often in the contact and climb phases.

(4) *Mount:* The male swings his body to either side of the female so that his abdomen is down along her anterior abdominal region. He adjusts his grip on the female in order to secure this position.

(5) *Dip:* The male then swings under the female, positioning the thoraco-abdominal junction beneath and perpendicular to the female's first abdominal sternite (fig. 5(B), 6).

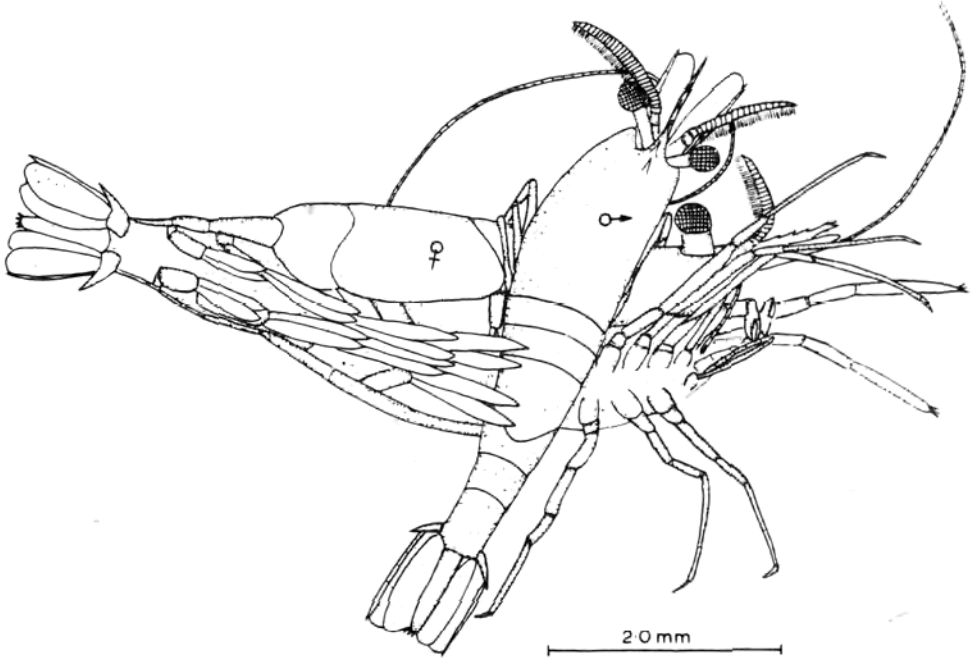

FIG. 6. Male in the *dip* position during copulation; viewed from below the female.

(6) *Pleopod beat*: The male briefly beats the pleopods when in the *dip* position, and it is at this time that the spermatophore is emitted and transferred.

(7) *Disengagement:* Separation of the male and female occurs just after the *pleopod beat*. The female may disengage by jumping backward, stimulating the same behaviour by the male. Males may swim off or sometimes simply climb off a passive female, although the latter method is least common.

Female mating behaviour

The normal reaction of *H. pictus* to another member of its species is avoidance. Shrimp in tidepools or in the laboratory will space out at antennal length when crowded except when feeding. Intraspecific behavioural interactions are chiefly confined to mating behaviour. Thus, one of the necessary components of female behaviour during mating is passivity and acceptance of the male's physical presence. Attractive females generally are not completely passive. They will strike at and attempt to knock the male off their body with the walking legs as he attempts to attain the *straddle* position (fig. 5(A)). Rejection of the male, the chief cause of unsuccessful matings, is due either to this defensive behaviour towards the male or flight during the *contact* or *climb* phases. *Pleopod lowering* is the only active participation in mating by the females. The platelike first pleopods of the female are normally held against the first abdominal sternite where the spermatophore must be deposited. If the female does not lower her pleopods before the male dips beneath her, the spermatophore will be laid on the posterior sides of the pleopods and mating will be unsuccessful. Uncovering of the spermatophore-receiving area has been observed in *Palaemon squilla* (Höglund, 1943) and in *Hymenocera picta* (Wickler and Seibt, 1970).

Post-mating behaviour of the female

Cleaning: After copulating, females immediately begin to clean the posterior thoracic sterna, the bases of the walking legs, and especially the abdominal sterna and pleopods. Bouts of brushing and picking with the second chelipeds are alternated with brief flurries of pleopod undulation. Höglund (1943) has pointed out that since the females have so recently moulted, the prespawning grooming is probably more related to arrangement of setae involved in spawning than in removing particulate debris. Ingle and Thomas (1974) have described the extensive (36 hour) preening of the pleopods and abdomen by prespawning crayfish *Austropotamobius pallipes* (Lereboullet). These crayfish do not moult before mating and the cleaning must both remove debris and arrange setae.

Break-up and spread of the spermatophore about the area where it is deposited is another consequence of the cleaning behaviour. Spermatophores on females killed immediately after mating are neatly arranged on the first abdominal sternite, but when females are allowed to engage in post-mating grooming, the spermatophore is shredded and spread to the anterior surfaces of the endopods of pleopods 1. Eggs must pass between the slowly moving endopods and the abdominal sternite during spawning. Thus, sperm on both surfaces insures fertilization.

Spawning: Egg-laying takes place within a few minutes after mating (fig. 3(D)). Nouvel and Nouvel (1937) also found that spawning occurred 'immediately' after mating in hippolytids, while Nouvel (1939) found that in *Crangon crangon* this process took place immediately in small females but after a 24 hour delay in large females. Between 2 and 3½ hours usually elapses after mating in *Palaemon squilla* (Höglund, 1943), and about 1–2 hours in *Hymenocera picta* (Wickler and Seibt, 1970). Initiation of

egg laying in *Heptacarpus pictus* is marked by the pronounced antero-posterior rocking movements of the walking legs. The female's abdomen is also stretched and depressed from its usual humped posture, in a position very similar to that figured for *Palaemon* by Höglund (1943). Two observations on the duration of oviposition were 20 and 30 min.

Female *H. Pictus* deprived of male contact will spawn within a few days of the reproductive moult (fig. 3(B)), but will retain the unfertilized eggs for only a short time (fig. 3(C)). This is a general phenomenon among carideans, noted several times by the workers cited above. The alpheid *Athanas nitescens* (Leach) is the only caridean studied where spawning does not occur upon male deprivation (Nouvel and Nouvel, 1937). In *H. pictus*, the unfertilized eggs are removed by the second chelipeds in normal cleaning behaviour. Continued delay in spawning by a female until a male is encountered might seem to be a useful adaptation but in nature there is probably no selection for this characteristic. Males are generally available to females: palaemonids, hip-

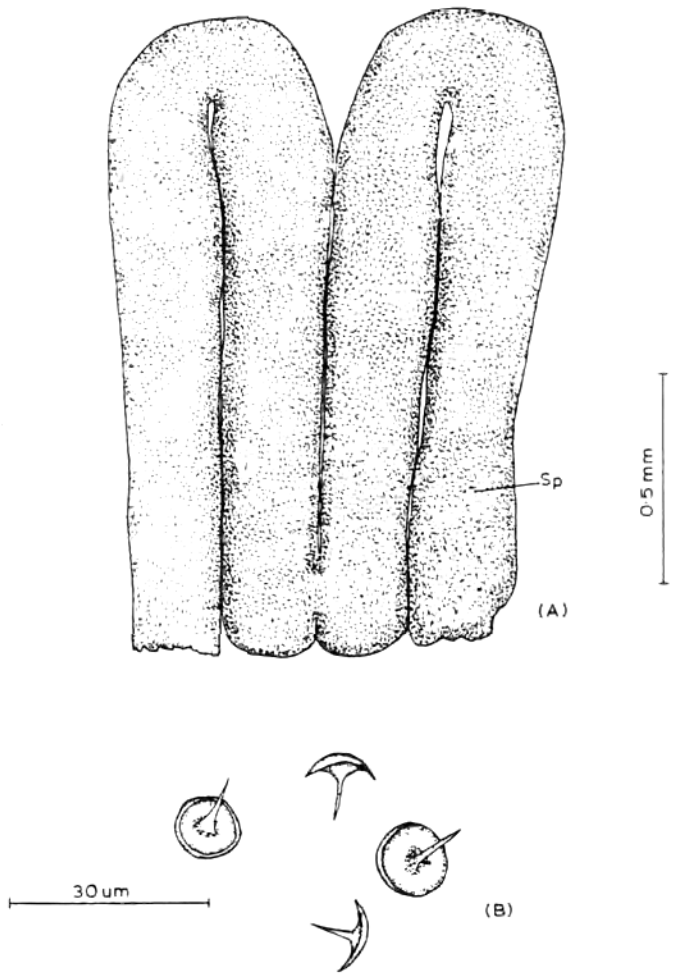

FIG. 7. (A) Spermatophore emitted by male *Heptacarpus pictus*; (B) Spermatozoa.

polytids, and pandalids are generally highly aggregated, while in other groups (e.g., Alpheidae) males and females occur in pairs.

Spermatophore structure and formation

Morphology of the spermatophore

Sperm packets of this species are composed of two ' U ' shaped cords joined together along one arm of each ' U ' (fig. 7(A)). Each cord is composed of an opaque gelatinous material in which the thumbtack-shaped sperm are embedded (fig. 7(B)). Spermatophores are very adhesive and will cling to objects that are contacted. Hoffman (1972) states that the acinar cells of the testis (*Pandalus platyceros* Brandt) produce a mucus secretion. Maintenance of the immotile sperm in the ducts of the testis and evacuation are believed to be the function of this material, i.e. similar to the prostatic fluids of mammals. The matrix of the spermatophore of *H. pictus* appears to be composed of a similar material.

Spermatophores have been reported between the bases of the walking legs in females of *Pandalus danae* (Needler, 1931), *Palaemon squilla* (Höglund, 1943), *Palaemonetes vulgaris* (Burkenroad, 1947) and *Hymenocera picta* (Wickler and Seibt, 1970). Nouvel (1939) found sperm on *Crangon crangon* females on the underside of the thoraco-abdominal junction. Spermatophores are located beneath the first abdominal sternite of *H. pictus* females after a successful copulation. Arms of the spermatophore are at right angles to the sagittal axis of the female (fig. 8). There are no structures on the smooth and mem-

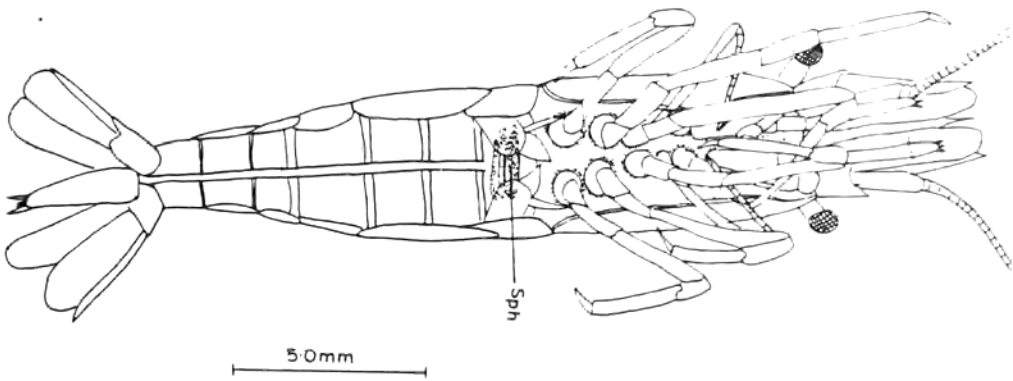

FIG. 8. Ventral view of a female showing a spermatophore in typical position beneath the first pleopods on the first abdominal sternite (pleopods 2–5 removed).

branous sternite to receive the spermatophore, and it is held in place by the glutinous nature of the sperm-bearing material.

Male genital structures

Genital openings of the male are located on a posteriorly directed spur on the posterior side of the coxa of the last walking leg (fig. 9(A)). Each opening is covered by a curtain-like membranous valve. Inside the coxa, the vas deferens, descending from the dorsally located testis, dilates into the

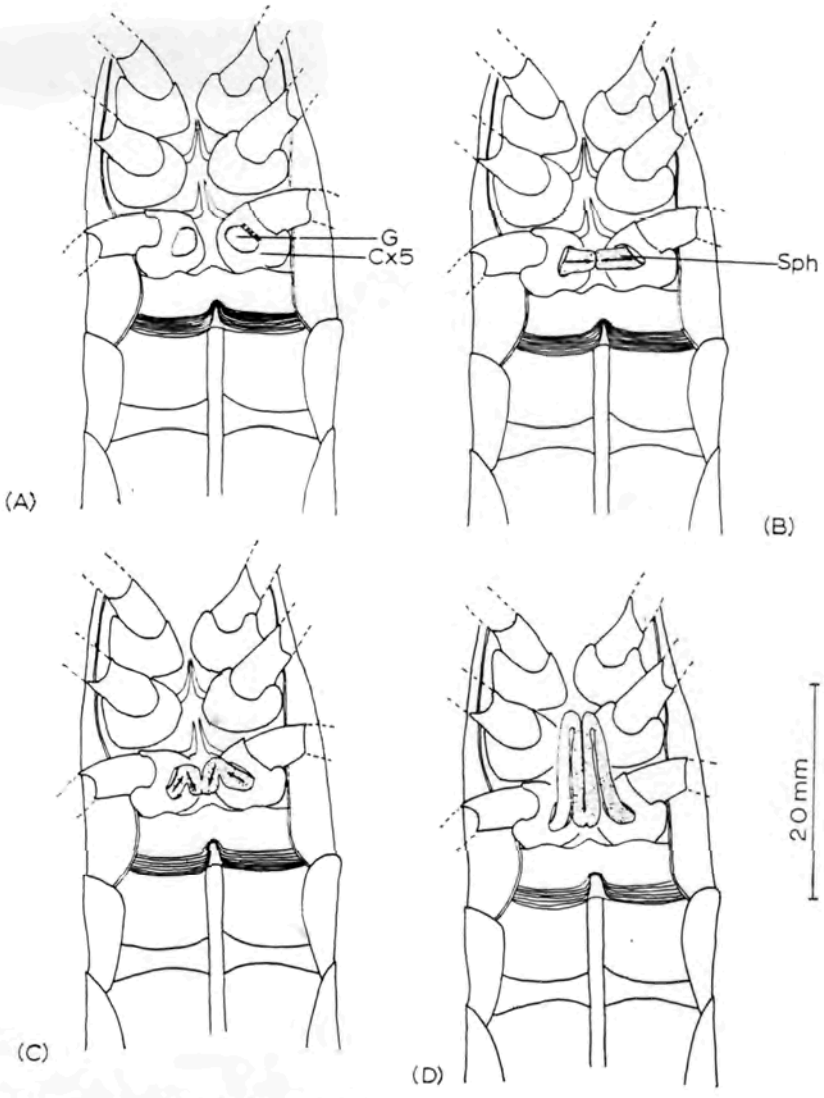

FIG. 9. (A) Thoraco-abdominal region of the male, ventral view; (B–D) Spermatophore emission and formation; the spermatophore is actually forming in a ventral (out of the page) direction.

ejaculatory duct (fig. 10). A sac-like evagination of the distal portion of the ejaculatory duct, the ejaculatory bulb, is confluent with the lumen of the duct. The volume of the ejaculatory duct is increased by this evagination, i.e., it serves as an additional space for storage of spermatophoric material until ejaculation. A coat of striated circular muscle bands surrounds the ejaculatory duct and bulb; contraction of this muscle causes the emission of the spermatophoric cords.

Spermatophore formation

Spermatophores were sometimes emitted during the ablation of the copulatory structures. Pressure applied to the posterior thoracic area caused the

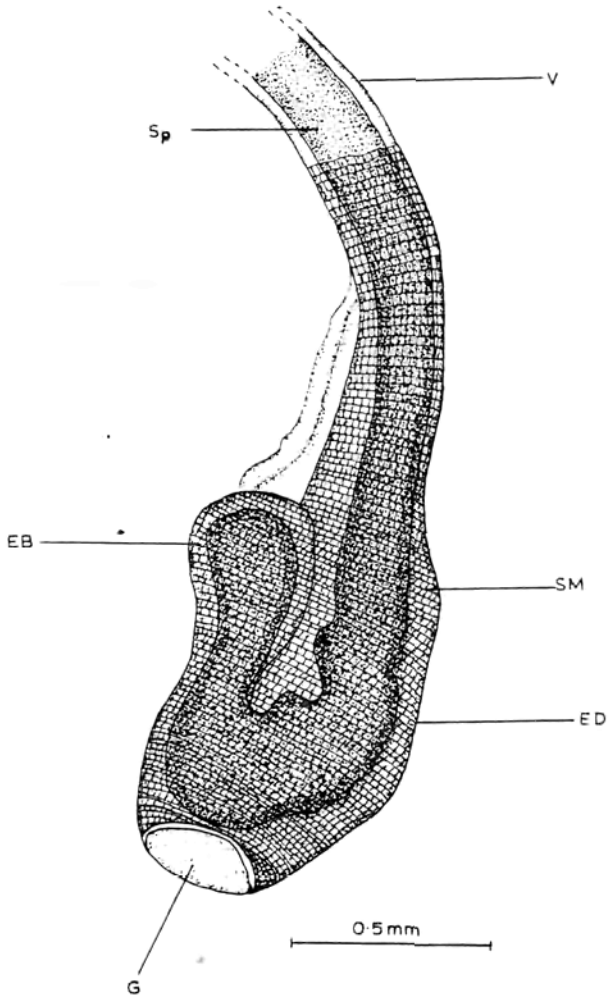

FIG. 10. Distal end of the sperm duct.

cords of a spermatophore to be extruded from the genital openings. Sperm packets of the form found on the female have thus been observed just after formation. From the structure of the spermatophore and the above observations, the following mode of formation is proposed: the sphincter muscles of the ejaculatory duct contract, squeezing a cord of spermatophore material out, and the cords push open the valves covering the genital openings in the process. Advancing cords from each side meet; each cord experiences pressure on one end from the contracting sphincter and on the other from the force of the opposite cord. Each cord is thus forced to bend into a loop away from the body (fig. 9(B)–(D)).

Gordon (1935) has figured what she considered to be the spermatophores of the palaemonid *Ewyrhynchus wrzesniowskii* Miers. They are short cords attached to the genital openings of males and are of various shapes, fused in different ways. Gordon did not observe any on the females, and implied that

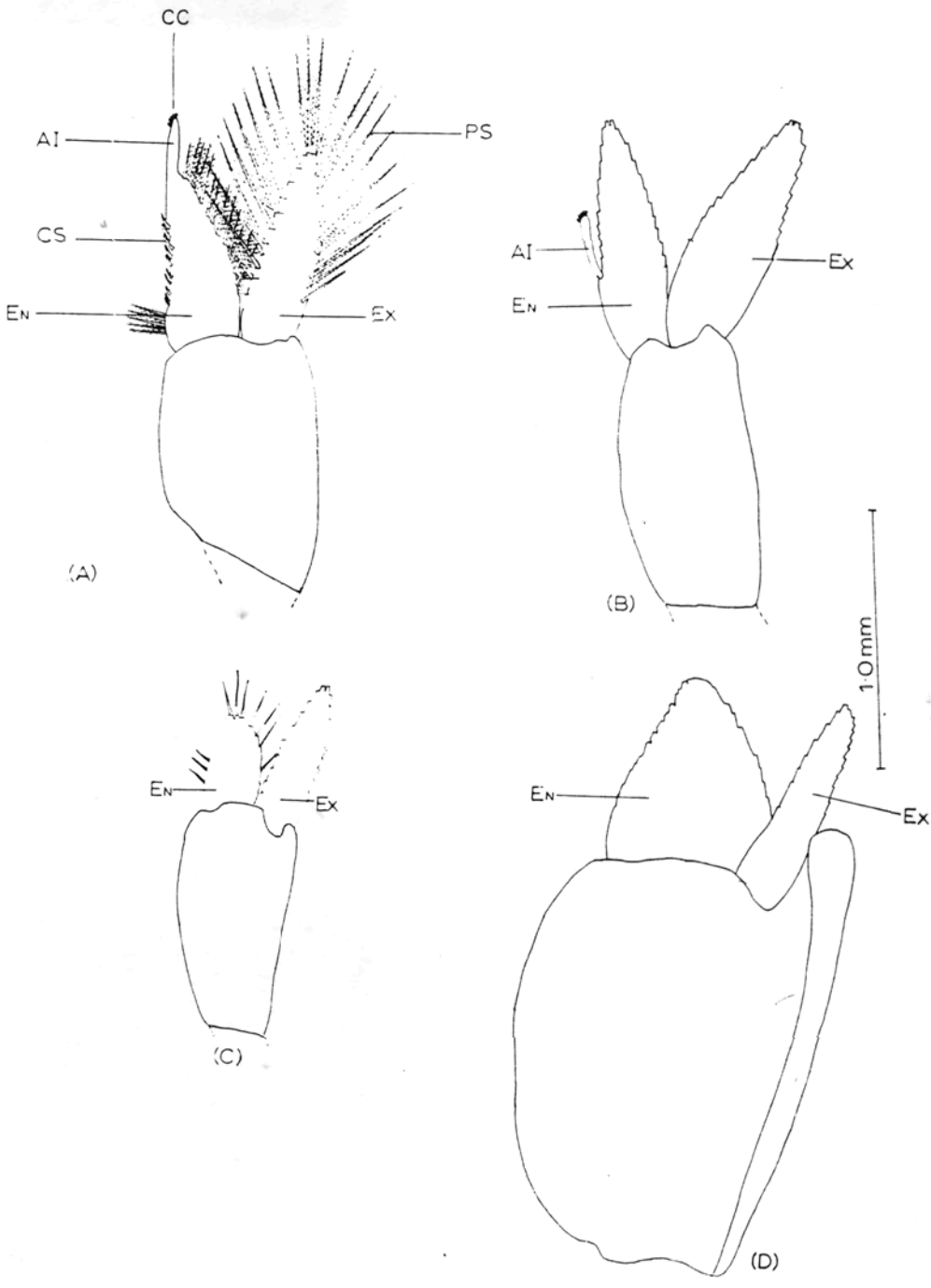

FIG. 11. (A) Pleopod 1 of a mature male; (B) Pleopod 3 of a mature male; (C) Pleopod 1 of a juvenile; (D) Pleopod 1 of an adult female. All right, anterior views; setae figured only in (A) and on the endopod of (B).

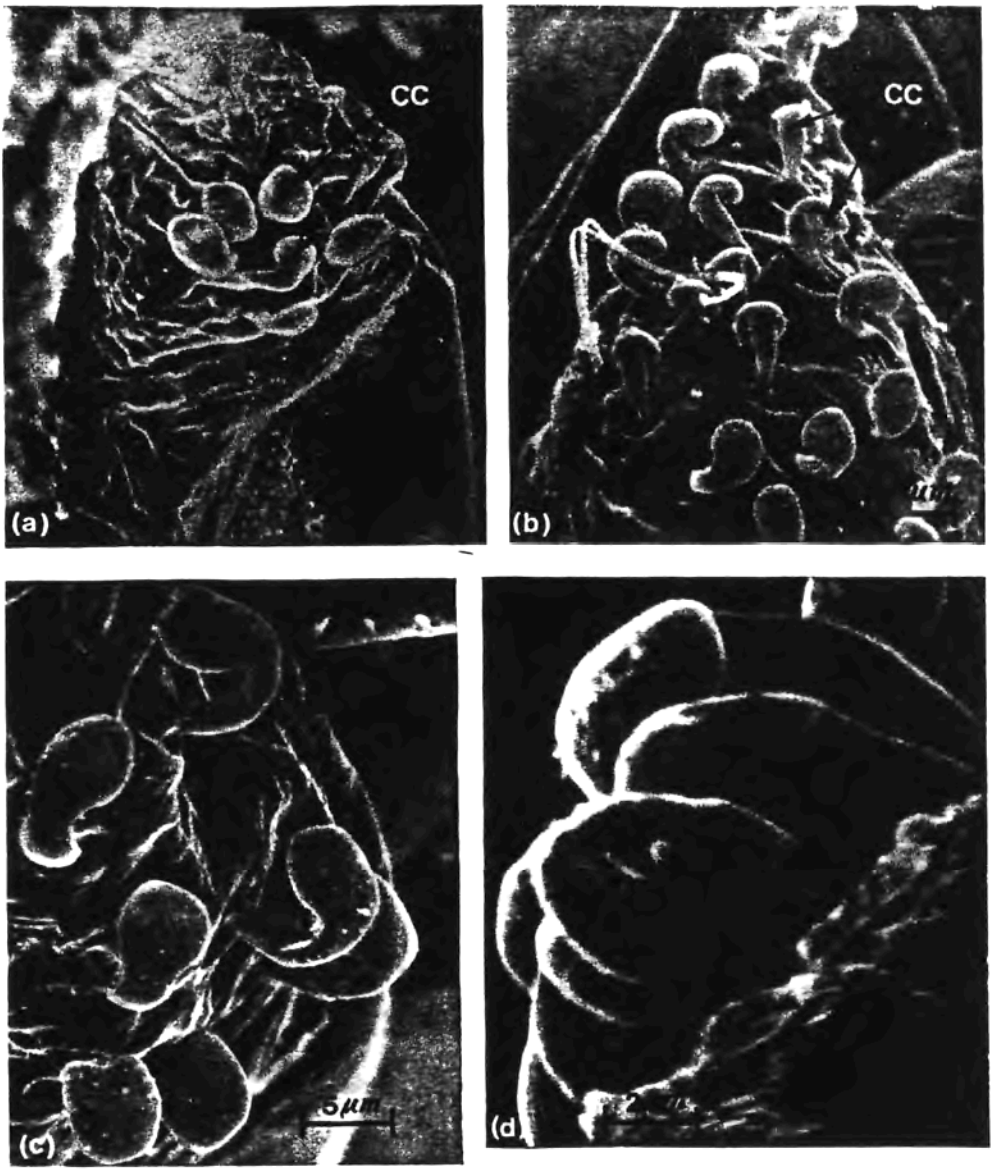

FIG. 12. Appendices internae on anterior pleopods of mature males. (A) medial tip of the endopod, pleopod; (B) tip of appendix interna, pleopod 2; (C) close-up of (B); (D) cincinuli.

these 'spermatophores' might be carried by the male for a time. A more probable explanation is that these structures are incomplete and imperfectly formed spermatophores emitted due to handling and preservation. Her figures indicate, however that spermatophore formation in this species must be similar to that of *Heptacarpus*. In other decapods (e.g., pagurids and brachyurans), structured spermatophores are preformed in the sperm ducts (Balss, 1943); while in carideans, they are formed upon extrusion.

Modifications of pleopods 1 and 2

Pleopod 1

Endopods of the first pleopods of mature males differ in size, shape and setation from the posterior pairs. They are smaller, more slender, and their distal ends narrow abruptly into a digitate process bearing coupling hooks or cincinuli (fig. 11(A), (B)). Inner rami of this pair are hooked together in life by the distal processes. Cincinuli on these structures are identical to those of the appendices internae of the other pleopods (fig. 12). Appendices internae are short stalks on the proximo-medial edge of the endopods of pleopods 2–5 which hook up the pleopods of a pair and apparently enhance their synchronous swimming movements. Thus, the digitate process on the endopods of the first pleopods in males are also appendices internae, moved distally and fused with the endopod. This process is often present in other male carideans, and is usually assumed to be a copulatory structure (e.g. Hoffman, 1972). However, its structure and function show that it is homologous with the appendix interna of more posterior pleopods. Moreover, some carideans (Rhynchocinetidae) clearly show an unfused appendix interna on the first pleopods (males only). The distal position of this process and its presence on only sexually mature males will be discussed in a later section.

Unlike rami of posterior pleopods, the endopods of the first pair are nearly devoid of plumose setae along the medial edge. Short, anteriorly curved setae equipped with fine setules replace swimming setae in this location.

This pair of rami in juveniles are simple leaves with few setae (fig. 11(C)). No appendix interna is present, and these rami must contribute little to swimming. In contrast, those of adult females (fig. 11(D)) are short and broad, with both plumose and ovigerous setae, the former used in producing the egg-bearing current during spawning, the latter in egg attachment to the pleopods. Juveniles and females never show an appendix interna on these first pleopods, although this structure is as well developed on the other pleopods as it is in males.

Pleopod 2

Sexually mature (sperm-producing) males can be identified by the presence of a spinous process, the appendix masculina, on the medial edge of the endopod of pleopod 2 (fig. 13). The process arises, along with the appendix interna, from a depression in the endopod (fig. 14(A)). Composed of a short, cylindrical stalk, the appendix bears a circlet or crown of long setae on its distal end (fig. 14(B)). Each seta shows a well-developed articulation basally, and bears rows of needle-like setules which are finely serrate themselves (fig. 14(C)). The appendix projects anteriorly between the endopods of pleopods 1 (fig. 14 (D)). Appendices masculinae are developed to some extent on this pleopod in most male carideans.

Experiments

Due to the small size of the copulatory structures, the positioning of the mating partners, and the brief duration of copulation, the functioning of pleopod modifications in males cannot be directly observed. Besides inferences drawn from morphology, anomalies in spermatophore deposition due to ablation of male sexual processes were used to ascertain the mechanics of

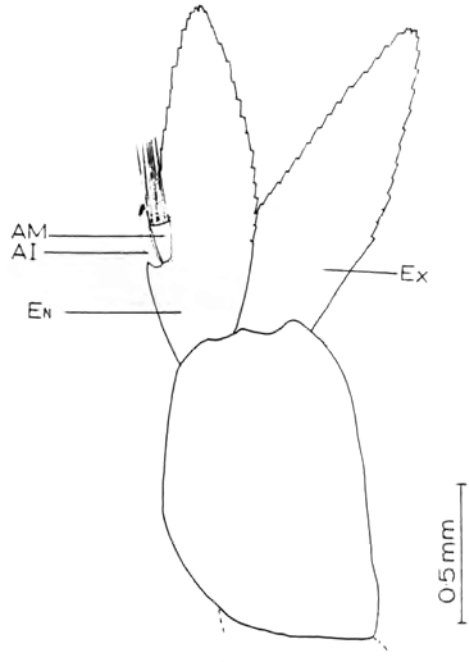

FIG. 13. Anterior view of the right, second pleopod of a mature male.

spermatophore transfer. Experimental procedures were of two kinds. In the first set, pleopods or pleopodal rami presumably possessing copulatory function were ablated individually or in pairs. Males and females were dip-netted out of the mating chamber immediately after disengagement from mating, chilled out of water until anaesthetized, then sacrificed by preservation in seawater formalin. This procedure was used to prevent the female from dislodging or deforming a misplaced spermatophore after mating. Experimental males in the second set of experiments had all copulatory rami removed, while those of normal males were untouched. Protocol of these matings allowed the female to remain undisturbed after copulating until the onset of spawning or the elapse of 20 min, whichever came first. Behaviour of mating partners during and after mating was observed and recorded on tape.

Results

Experiments, first set

Presence or absence, and position and shape, of the spermatophore were the criteria used to determine whether spermatophore deposition was successful or otherwise in the first set of experiments. Spermatophore transfers were classified as successful if the spermatophores were in place on the first abdominal sternite, oriented normally (fig. 8), and were not grossly misshaped or missing sections of cord. Comparisons among the three treatments (no ablation, one or a pair of copulatory structures ablated) are shown in table 1. Non-ablated individuals showed the fewest unsuccessful transfers; however,

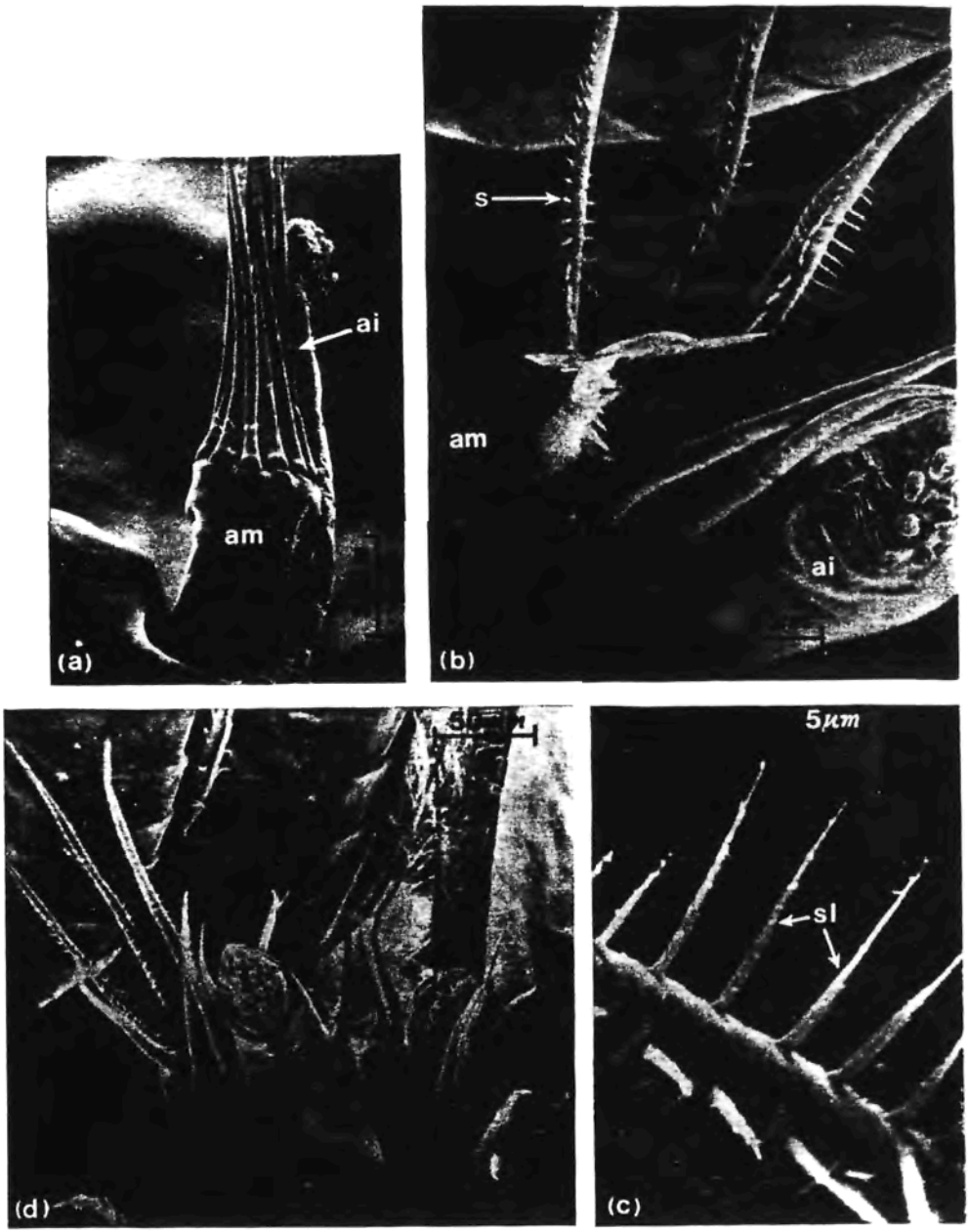

FIG. 14. Appendix masculina of mature males (A) arising from the endopod, pleopod 2 (B) view from above; (C) portion of a seta from the appendix masculina; (D) anterior view between the endopods of pleopods 1 and 2.

there is clearly no statistically significant difference between normal matings and those by individuals with three copulatory rami present. When normal males are compared with individuals without a pair of rami, however, differences in the success of sperm transfer are significant at the 10% level. Furthermore, unsuccessful transfers were usually due to absence of the spermatophore

Table 1

Comparison of success in spermatophore transfer between normal and experimental males in ablation experiments (first set)

Treatment	% Unsuccessful Transfers
Normal males: all copulatory rami present	4 of 12 = 33 %
Treatment 1 males: 3 of 4 copulatory rami present	8 of 18 = 44 %
Treatment 2 males: one pair of copulatory rami only	8 of 10 = 80 %

Null hypothesis (H_0): No correlation between treatment and success of spermatophore transfer

	unsuccessful	successful
normal	4 (a)	8 (b)
treatment 1	8 (c)	10 (d)

Result: $\chi_1^2 = 0{\cdot}05$; $p(H_0) \begin{matrix} > 0{\cdot}75 \\ < 0{\cdot}95 \end{matrix}$

	unsuccessful	successful
normal	4	8
treatment 2	8	2

Result: $\chi_1^2 = 3{\cdot}09$; $p(H_0) \begin{matrix} > 0{\cdot}05 \\ < 0{\cdot}10 \end{matrix}$

For the 2×2 contingency table, $\chi_1^2 = \dfrac{(|ad - bc| - N\ 2)^2 N}{(a+b)\ (c-d)\ (a-c)\ (b-d)}$

(Tate and Clelland, 1957)

in the first two treatments; but half of the unsuccessful transfers in the group without a pair of copulatory rami were due to anomalies in spermatophore form.

Absence of copulatory rami did not appear to affect the mating behaviour of experimental males, which completed all phases of copulation, including *pleopod beat*. Experimental males with one copulatory process removed were statistically as successful in spermatophore transfer as normal males, while males with a pair of rami removed showed a much lower degree of sperm deposition. However, both experimental groups went through the chilling and ablation procedures. Thus, operational procedures had no noticeable affect on mating behaviour and sperm deposition. Presence or absence of copulatory rami was the important factor in transfer success.

Experiments, second set

Experimental males in the second set of experiments had both sets of copulatory rami ablated. A successful mating in these tests was defined as the condition when a spermatophore was deposited on the first abdominal sternite of the female and survived on that location to egg-laying or the passage of twenty minutes. Orientation and shape of the sperm packet were not used in this case because the female considerably destroys its form during cleaning activities. Females also had the option of rejecting a spermatophore,

presumably because of incorrect placement, and some were observed to do so during cleaning activities.

It is possible that females might recognize experimental males as aberrant individuals and not display the proper behaviour during copulation. However, there was no indication that females behaved any differently with experimental than with normal males. Sequences of mating events recorded on tape showed no difference between non-experimental and experimental matings; experimental males spent no greater time in copulating than normal individuals (fig. 15). Thus, differences in spermatophore transfer between treatments are not a result of aberrant mating behaviour by experimental animals, but are due instead to the absence of functioning copulatory rami.

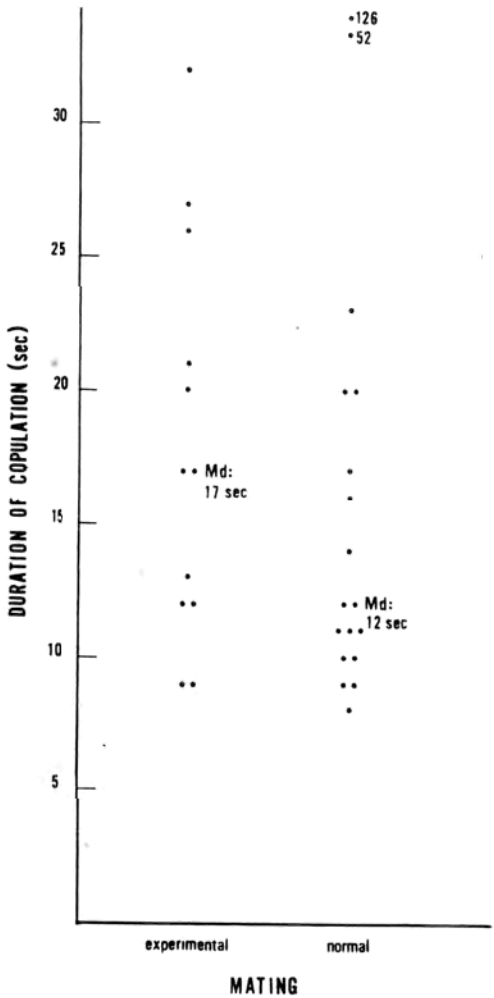

FIG. 15. Comparison of copulating times between experimental and normal males. A rank sum test was used to test the hypothesis that the median copulating times were not different between the two groups; the hypothesis was accepted—$p(H_0) > 0{\cdot}25, < 0{\cdot}50$.

Results from these matings show a greater number of spawnings in normally mated females than in experimental animals (table 2). This is due to the

Table 2

Spawning of experimental and normal females within 20 min of copulation

Condition	Normal	Experimental
Spawning, no spermatophore present	2	2
Spawning, spermatophore in position	11	1
No spawning, no spermatophore present	3	7
Spermatophore present, no spawning	0	1

Table 3

Unsuccessful matings in normal and experimental males in ablation experiments, second set

Treatment	% Unsuccessful Transfers
Normal males: all copulatory rami present	5 of 16 = 31 %
Experimental males: both pair of copulatory rami removed	10 of 12 = 83 %

H_0: No correlation between treatment and success of spermatophore transfer

	unsuccessful	successful
normal	5	11
experimental	10	2

Result: $\chi_1^2 = 5{\cdot}53$; $p(H_0) \begin{matrix} > 0{\cdot}01 \\ < 0{\cdot}025 \end{matrix}$

greater percentage of successful transfer and retention in non-experimental matings, although a female will sometimes spawn without having a spermatophore after contact with a male. Comparison of normal males and experimental animals shows that the former had a significantly greater number of successful transfers (table 3). These results clearly show that the copulatory rami play an important role in the placement of a sperm packet on the abdominal sternite of the female.

Mechanics of spermatophore transfer

Observations on male and female morphology, behaviour during copulation, emission of the spermatophore, and results from the ablation experiments suggest the following sequence of events during spermatophore passage to the female (fig. 16): when the male is in the *dip* position beneath the female, the spermatophore is produced (fig. 16(A)) and is caught on the antero-medial edges of the inner rami of pleopods 1 (fig. 16(B)). I have observed this in the process of ablating copulatory rami from males. Plumose setae, if present, would become entangled in the spermatophore, hence their absence and

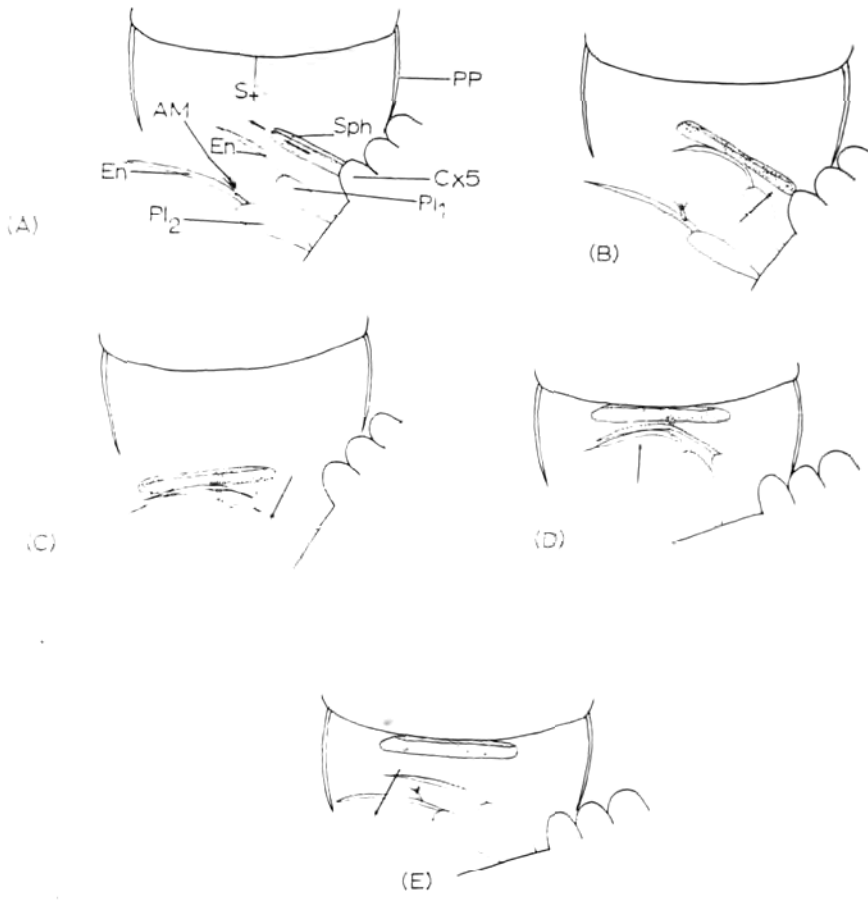

FIG. 16. Proposed method of spermatophore transfer during copulation (*pleopod beat*). A cross-section of the male and female, at the level of the first abdominal segment of the female, is figured. See text for explanation.

replacement by short anteriorly curved setae. These setae probably hold the spermatophore off the rami proper; the sperm packet observed was lightly but definitely caught on the anterior edges of the inner rami. Next, the first pleopods swing back against the second; then, both swing up against the sternite of the female (fig. 16(C),(D)). Appendices masculinae projecting between the endopods of pleopods 1 penetrate the spermatophore, pushing it off the first endopods. When the glutinous spermatophore strikes the sternite, it preferentially adheres to it because there is a broad surface for attachment. The appendices only touch a very small area of the spermatophore and, therefore, the spermatophore remains glued to the sternite as the pleopods swing back (fig. 16(E)). If the spermatophore were not held off from the endopods as they neared the sternite of the female, it would be pressed between the anterior surfaces of the endopods and the sternite, decreasing the chance that it would remain on the latter and increasing the chances of fouling with the male endopods.

Alternative hypothesis of the mode of sperm transfer should be discussed. If genital orifices were directly applied to the sternum of the female, the spermatophore could be squeezed out along it. However, cinematography

of matings show that the bases of the male's fifth legs are not close to the female's sternite. Intimate contact with the male's genital orifices is prevented by the large expanded abdominal pleurae of the female. Artificial placement of dead males and females into mating positions confirm that the female's pleurae form such a barrier. The female's abdominal sternite is too far above the edges of the pleurae to be reached by direct contact.

Another possibility is that the spermatophore is emitted freely and is forced up toward the female's body by the force of contraction of the male's sphincter. However, it is difficult to imagine the spermatophore being applied as precisely as it is in this way. Pleopods are beating at this time and a spermatophore travelling free in the water would likely be knocked and tumbled about, with a high probability of its striking and adhering to the wrong area of the female's abdomen.

Presence of an appendix interna on the first pleopod of mature males but not on juveniles or females can be accounted for by the proposed method of spermatophore transfer. Spermatophores received by first pleopods not beating in synchrony would be unable to meet the female's body evenly and not adhere uniformly. Possibilities of fouling with the endopods of the first pleopods would be great. Appendices internae of the first pleopods of males are located distally, while on other pleopods, they are proximally placed. Processes bridging the endopods of pleopods 1 proximally would block the appendices masculinae from protruding between them and prevent the spinous processes from contacting the spermatophore. Possession of the appendices internae of pleopods 1 by mature males only and the reduction of plumose swimming setae on the endopods indicates the importance of these modifications in copulation.

Discussion

Nouvel (1939) states that the appendices masculinae of *Crangon crangon* males are not involved in sperm transfer; rather, the genital orifices of the male oppose the area of the female where the sperm packet is found. This method of spermatophore deposition is made possible by the macruran facies of *Crangon* and the concomitant method of spawning. *Crangon* is a dorsoventrally compressed shrimp without the hump in the abdomen characteristic of more natant carideans. Females spawn by lying on their sides with the abdomen flexed against the thorax (Meyer, 1934, cited in Höglund, 1943). The broad expanded abdominal pleurae important in the spawning of natant shrimp such as palaemonids, hippolytids and pandalids are absent in crangonids due to the differences in spawning procedure. Apposition of the male genital orifices to the female is possible. The endopod of the first pleopod of an adult male *Crangon* is a simple reduced leaf without an appendix interna. Pleopods of the burrowing crangonids are splayed out laterally and lack appendices internae. Synchronous interplay between the anterior pleopods in sperm transfer is thus not possible. The more typically caridoid pandalids have highly developed copulatory rami similar to that of *Heptacarpus* and a similar method of transfer is probable in this group.

Modifications of the pleopods for copulation are slight in carideans compared to the petasmae of penaeids and the complex first two pair in nephropideans and brachyurans. In advanced brachyurans, fertilization is internal while

in penaeids, nephropideans and primitive brachyurans, it is external, but the spermatophore is often deposited in a ventral thoracic groove or pouch (thelyca of penaeids, annulus ventralis of some nephropideans, spermathecae of primitive brachyurans). Carideans simply plaster the spermatophore on some location beneath the female that will be in the path of the eggs as they pass back from the bases of the first walking legs to the pleopods for attachment. Two females of the 12 experimental matings (second set) in this study had spermatophores in the correct location. Thus, the copulatory structures of *Heptacarpus* are not absolutely essential for sperm transfer, but they assist in the passage of the spermatophore and increase the probability of its reaching the correct location on the female. Andrews (1910) has shown that ablation of a copulatory ramus will invariably prevent passage of sperm to the female (the crayfish *Oronectes limnosus* Rafinesque). Removal of one or the other pair of male copulatory structures in branchyurans would surely prevent passage of sperm into the female's genital openings. Complexity of the anterior pleopods of male decapods is thus correlated with increased protection of the sperm, culminating in internal fertilization in the Brachyura. Proximity of the anterior pleopods to the genital openings of the male has led to their interplay in sperm transfer, simple in the caridean and becoming more refined in the higher decapods.

Summary

Heptacarpus pictus, a small caridean shrimp inhabiting the low intertidal of southern and Baja California, breeds during the winter, spring, and summer months. Fall is a period of growth. Life span of an individual does not exceed 18 months, with fish predation as the most likely source of mortality.

Females are multiple brooders, carrying developing embryos concomitant with increase in ovary size. Hatching of larvae is followed by a moult, after which the female is attractive to males and receptive to copulation. A distance pheromone does not appear to be involved in attraction of males to females. Males apparently respond to a non-diffusible substance on the exoskeleton of newly moulted females.

Precopulatory behaviour is absent. Copulation can be divided into a series of relatively stereotyped events. Female rejection of the male or his spermatophore is the chief cause of unsuccessful matings.

Males deposit the spermatophore on the underside of the female's first abdominal segment. Sperm packets are formed upon extrusion from the male's genital openings, and are composed of a mucoid material in which sperm are mixed. The glutinous spermatophores adhere to the female's smooth abdominal sternite.

The endopods of pleopods 1 and 2 of the male are different in shape, size and setation from homologous rami of females and juveniles. Endopods of pleopod 1 possess a distally located appendix interna, absent in juveniles and females. An anteriorly projecting process, the appendix masculina, is located on the endopod of pleopod 2 in males. Experiments were performed which showed that these modifications insure proper deposition of spermatophores. Males which had the copulatory rami removed did not transfer spermatophores as successfully as normal males.

Transfer of the spermatophore from the male to the female is a result of the interplay of male pleopods 1 and 2 during copulation. The large expanded abdominal pleurae of females prevent the male's genital opening from contacting her abdominal sternite. Thus, the male's anterior pleopods have become modified to lift the emitted spermatophore from his genital orifices to the first abdominal sternite of the female.

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Abbreviations used in the figures

AI	Appendix Interna
AM	Appendix Masculina
CC	Cincinulli
CS	anteriorly curved seta
Cx5	coxa of the last walking leg
EB	Ejaculatory Bulb
ED	Ejaculatory Duct
En	Endopod
Ex	Exopod
G	Genital opening
Pl_1, Pl_2	pleopod 1, pleopod 2
PP	Pleural plate
PS	Plumose seta
SM	Striated muscle band
Sp	Spermatozoa
Sph	Spermatophore
St	Sternite
V	Vas Deferens
xxx	Attachment of genital valve to the coxa
Ov	Ripe Ovary

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