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ADAPTATIONS OF THE BRANCHIAL ECTOPARASITE *PROBOPYRUS PANDALICOLA* (ISOPODA: BOPYRIDAE) FOR SURVIVAL AND REPRODUCTION RELATED TO ECDYSIS OF THE HOST, *PALAEMONETES PUGIO* (CARIDEA: PALAEMONIDAE)

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ABSTRACT

Survival of the branchial ectoparasite *Probopyrus pandalicola* through ecdysis of the host, *Palaemonetes pugio*, was studied by observing infected shrimps before and after ecdysis and directly with time-lapse video. The parasite pair retained its position through 97.3% of 112 host ecdyses. This observation and positive correlations between parasite and host size support the hypothesis that bopyrid isopods remain on and grow together with their host. Video recordings of shrimp ecdysis showed that the female and male maintain their position rather than being cast off and reentering the host branchial chamber. Observations on exuviae from parasitized shrimps and from video recordings suggest that the female parasite avoids being discarded with the molt skin by immediately attaching to the newly exposed inner lining of the gill cover as the shrimp backs out of its exuviae.

Reproductive activities of the parasite relative to host molting were studied. Epicaridium larvae were released from the host branchial chamber several hours to 5 days before the host molt. Spawning by the parasite female took place within several hours (median = 12 h) after host ecdysis. Expulsion of exuvial fragments of female parasites from the host branchial chamber was observed in 2 video recordings prior to spawning. In several video recordings, the usually inactive male moved from its site on the female abdomen up to and inside the female marsupium after the host molt and before female spawning, presumably to inseminate the female. Females from which males had been removed failed to produce a brood after the next host molt, tentatively supporting the hypothesis that females must be inseminated prior to each spawning.

The bopyrid isopod *Probopyrus pandalicola* (Packard) (sensu lato, see Dale and Anderson, 1982; Markham, 1985) infects a variety of palaemonid shrimp species. Various aspects of its life history and life cycle have been investigated (Morris, 1948; Anderson, 1975, 1977, 1990; Anderson and Dale, 1981, 1989; Beck, 1979, 1980a, b). These studies reveal that the life cycle of this species follows the typical bopyridian pattern of hatching as an epicaridium larva, subsequent attachment to a copepodan intermediate host, metamorphosis to the microniscus larva, and development of the final infective stage, the cryptoniscus, which leaves the copepod in search of the final host, typically a decapod crustacean (Reinhard, 1949; O'Brien and Van Wyk, 1985). Evidence to date indicates that the first cryptoniscus to infect the host becomes the large female parasite while the second (or subsequent) cryptoniscus that arrives on the host develops into the dwarf male that remains attached to the female (Reinhard, 1949; Anderson, 1990).

High positive correlations between the size of female bopyrids and the size of their caridean shrimp hosts indicate that the host is usually infected early in life and that the parasite remains on and grows with the host [*Probopyrus pandalicola* on *Palaemonetes paludosus* (Gibbes), Beck, 1980a; *Pr. ringueleti* Verdi and Schuldt (=*Pr.* cf. *oviformis* Schuldt) on *Pa. argentinus* Nobili, Schuldt and Rodrigues Capítulo, 1985; *Pr. pandalicola* on *Palaemon ritteri* Holmes, Campos and Campos, 1989; *Pr. bithynis* Richardson on *Macrobrachium ohione* (Smith), Truesdale and Mermilliod, 1977; *Pseudione affinis* Sars on *Dichelopandalus bonnieri* (Caullery), *Hemiarthrus abdominalis* (Kröyer) on *Spirontocaris lilljeborgii* Danielssen, Pike, 1960]. In order to remain with the same host, the ectoparasitic bopyrid must survive the several ecdyses the host undergoes during its lifetime.

The male and female of *Probopyrus pandalicola* live in the branchial chamber of the host shrimp, *Palaemonetes pugio* Holthuis. However, since the branchial chamber is

lined with exoskeleton, the bopyrid pair is actually attached to and surrounded by host cuticle. Thus, these ectoparasites are in a position in which they could be cast off during host ecdysis, during which the cuticle of the branchial chamber is shed along with the rest of the old exoskeleton. Parasite behavior during the host molt has never been reported, and the mechanism by which the parasite pair maintains or regains its position in the branchial chamber of the host has not been investigated.

Ecdysis of the host is also critical to parasite reproduction. The female parasite uses the branchiostegite of the host to form the floor of its marsupium or brood chamber (Beck, 1980b). Molting by the host while the parasite is brooding embryos would result in their loss. Several studies mention or indicate that spawning, incubation, hatching, and liberation of larvae are completed between host ecdyses (Anderson, 1975; Beck, 1980a; Walker, 1984, unpublished dissertation). However, parasite reproduction was not the principal topic of these studies, and little specific information is available on hatching and liberation of parasite larvae, insemination of the female parasite, and subsequent spawning.

The activities of the dwarf male in regard to copulation with or insemination of the female are unknown. Hiraiwa (1936), working on the bopyrid *Epipenaeon japonica* Thielemann, showed that the female parasite had no seminal receptacle for sperm storage, unlike many isopods, and that the male lacked gonopods, as in all Bopyridae. He hypothesized, based on this morphological evidence, that the male never entered the brood chamber of the female to inseminate her, simply shedding sperm into the chamber when the female spawned. To date, there have been no direct observations on male activities related to mating in bopyrid isopods.

The objectives of this study were, using time-lapse video and other observations, (1) to directly record parasite behavior during the host molt and to determine the mechanism by which the parasite pair maintains its position through host ecdysis, (2) to make quantitative observations on the timing of female parasite spawning and larval release in relation to the host molt, and (3) to make observations on the dwarf male related to insemination of the female. In addition, observations on molting of the female parasite, obtained from video recordings of parasitized shrimps, are reported.

Materials and Methods

Specimens of *Palaemonetes pugio* infested with *Probopyrus pandalicola* were collected with long-handled dipnets in shallow water in marshes of *Spartina* at the following locations in Louisiana: (1) LUMCON marine laboratory at Cocodrie, (2) Rockefeller Wildlife Refuge, and (3) Cypremort Point. Other specimens used in morphological work were taken at South Padre Island, Texas. Specimens were prepared for scanning electron microscopy according to the methods in Bauer (1987). Living infected shrimps were maintained individually in perforated plastic cups in sea water at the same salinity (2–8 ppt) as the water from which they were collected and at a temperature of 18–25°C. Shrimps were fed flakes of aquarium fish food.

Total lengths of male and female parasites taken from a sample of infected shrimps were measured to the nearest 0.01 mm using a stereomicroscope with ocular micrometer. Males were measured from the anterior margin of the head to the posterior margin of the pleon. Total length of the asymmetrical female was measured along the longest line from the anterior end through the pleotelson. Carapace lengths of shrimps were measured along the line from the posterior edge of the eye orbit to the middorsal posterior edge of the carapace. Regressions of parasite size on host size were calculated using the least square method (Wilkinson, 1988).

In order to determine the sequence and timing of events of parasite reproduction relative to the host molt, the absence or presence and developmental stage of parasite embryos and larvae were observed in female parasites of individually isolated infected shrimps. Three stages of parasite embryo development that could be identified by unaided visual inspection of the embryo mass on the infected shrimp were defined and recognized: stage 1, newly fertilized egg, embryo mass appearing translucent, white when viewed through the gill cover of the host; stage 2, embryo reniform, tissue surrounding central yolk mass segmented, embryo mass yellow; and stage 3, body segmentation well developed but appendages not free, little or no yolk, pigmentation of tissue, embryo mass with tan color. The hatched embryo, or epicardium larva, was recognized by its free appendages and well-developed eye spots; a mass of epicardium larvae in the parasite marsupium appeared quite dark or black.

In the first set of observations, designed to describe the general timing and sequence of release of parasite larvae, host molting, and parasite spawning, shrimps ($N = 28$) with female parasites bearing stage 3 embryos or epicaridium larvae were maintained individually at ambient water temperature of 20–23°C. The presence or absence of shrimp exuviae was recorded daily, and the absence or presence of parasite larvae or embryos, and the development stage of the latter in the marsupium of parasite females was recorded every 1–2 days. Thirteen of these females were followed through 2 host ecdyses. In the second set of observations (water temperature 22–25°C), these data were recorded twice

per day for a new set of infected shrimps ($N = 36$) to more precisely determine time periods between events. For purposes of tabulating time periods between events, an event (e.g., host molting) was considered to have occurred at the time midway between the observation at which the event was recorded and the time of the previous observation.

Time-lapse video recordings were made on 11 infected shrimps to observe and study parasite activities within the gill chamber of the host, especially the release of parasite larvae, host molting, spawning of the parasite female, and movements of the male and female parasites. For each set of recordings, a single infected shrimp was placed in one of the chambers (4.5 cml × 3.5 cm w) of a clear plastic storage box. A plexiglass insert restricted the depth of the chamber to 2.0 cm in order to maintain the shrimp within focus of the camera. Perforations (3-mm diameter) in the back and sides of the chamber and in the plexiglass insert allowed for circulation of water (18–22°C) into the chamber. The storage box with observation chamber and shrimp was placed into a larger aquarium (30 cm × 10 cm × 16 cm), filled with continuously aerated ambient sea water, with the unperforated side of the observation chamber against 1 aquarium wall. Recordings were made with a video surveillance camera, sensitive to low light intensity, equipped with a 8.0- or a 12.5-mm lens equipped with extension tube rings to increase magnification. The camera was connected to a time-lapse video recorder, and recording were made at either the 12 h (10 pictures/s) or 24 h (5 pictures/s) recording speed. The complete sequence of release of parasite larvae, host molting, and female parasite spawning was recorded in 7 of the 11 infected shrimps filmed. Median observation time on these shrimps and their parasites was 86 h (49–172 h), and total time of all video observations was 950 h.

Male parasites were removed from the female parasites of 9 infected shrimps. A shrimp was held in a petri dish, and watchmakers forceps were used to gently lift up the shrimp branchiostegite and remove the male. These shrimps and 5 other untreated infected shrimps were maintained in individual containers as described above, and host molting and parasite spawnings were recorded. Females from 5 of the treated pairs were incubating embryos (4 in stage 1, 1 in stage 3), while all other females of treated and untreated parasite pairs were not incubating embryos when their host shrimps were captured.

Results

Variation of Parasite Size with Host Size

Total length (Y) of both female and male parasites was positively correlated with carapace length (X) of the host (Fig. 1). Regression equations calculated for the female and male parasite were $Y = 0.64(X) + 0.82$ (Pearson correlation coefficient $r = 0.84$, $N = 39$) and $Y = 0.19(X) + 0.44$ ($r = 0.83$, $N = 38$), respectively. The probability of the null hypothesis: no regression (slope of regression line = 0) was less than 0.0001 for both regressions.

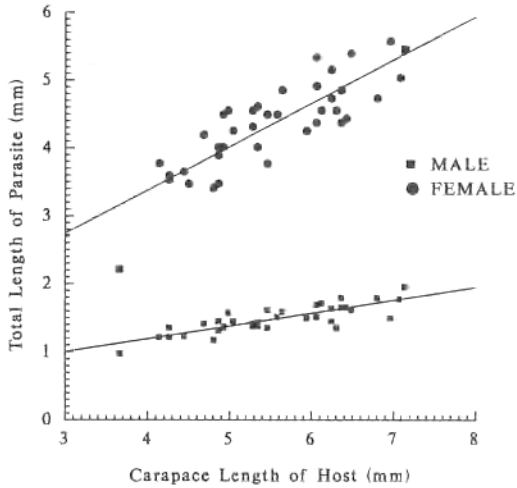

Fig. 1. Variation of total length of male and female *Probopyrus pandalicola* with carapace length of the host, *Palaemonetes pugio*.

Parasite Adaptations for Survival of Host Molting

The female of *Probopyrus pandalicola* fills most of and is tightly lodged within the branchial chamber of the host, producing a characteristic bulge in the host branchiostegite or gill cover (Fig. 2A). The ventral surface of the female is directed outward against the host branchiostegite, with the female's anterior end and mouthparts contacting its posterodorsal edge (Fig. 3). The male parasite is tightly wedged among the pleopods of the female (Figs. 2B, C; 3). In both the male and female, the recurved dactylus of each pereiopod fits into a deep socket on the propodus (Fig. 2D–F). Histological sections through the branchial chamber of an infected shrimp showed that the pereiopodal dactyli of the female pinch the thin inner cuticle of the branchiostegite for attachment. The details of attachment of male pereiopods to the female were not observed.

Video observations showed that female parasites do not noticeably move within the branchial chamber between sequential ecdyses of the host. The only female activity observed during host intermolt periods was ventilatory movements of its anterior oostegites. Males appeared totally inactive, never changing their position on the ventral surface of the female.

Details of molting in infected shrimps were revealed by video recordings (Fig. 4A–F). When the shrimp began to molt, it fell

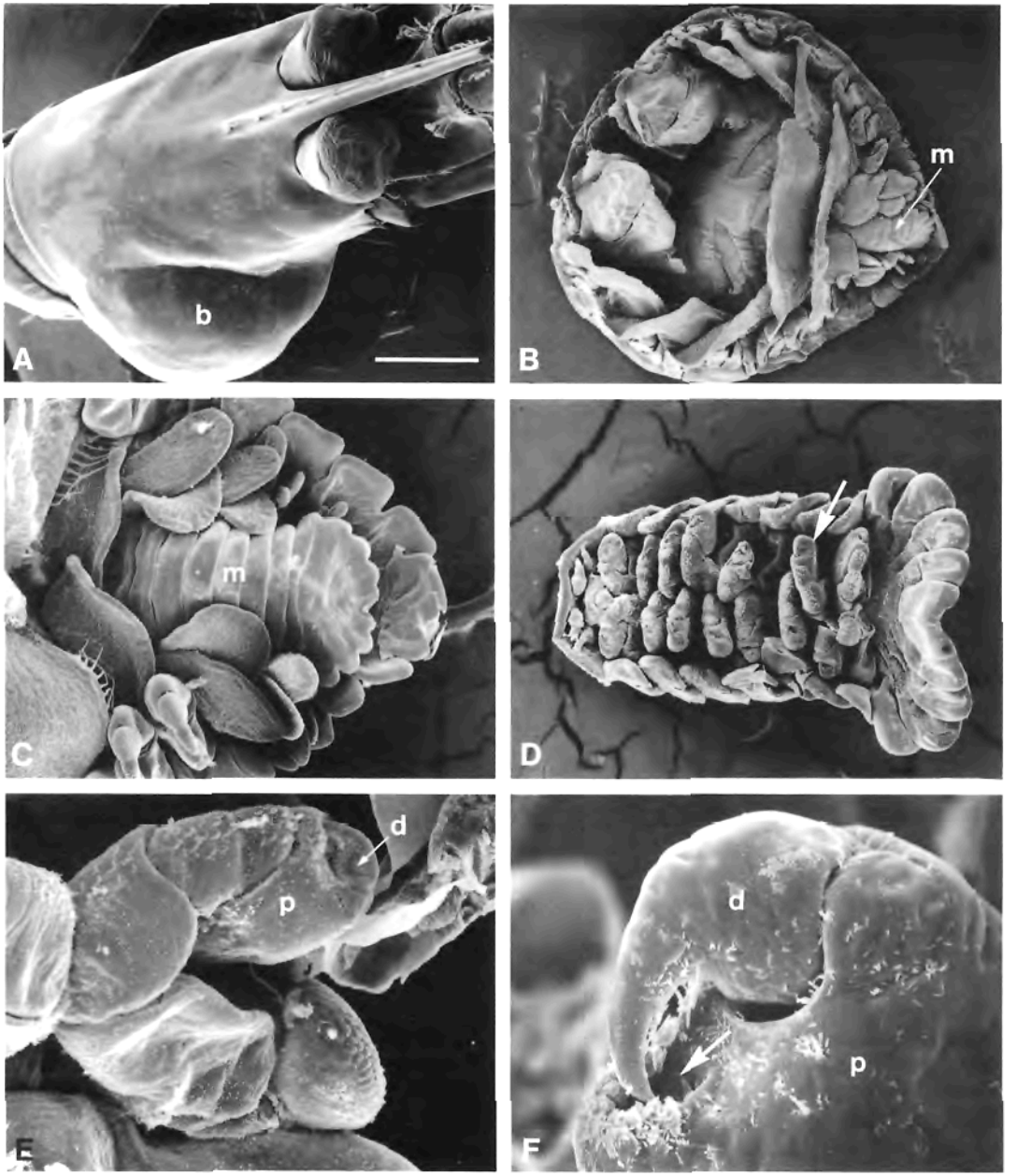

Fig. 2. Morphological features of male and female *Probopyrus pandalicola* from branchial chamber of host shrimp. A, dorsolateral view of infected *Palaemonetes pugio* showing distortion of branchiostegite (b) formed by presence of female bopyrid in gill chamber on right side of shrimp; B, ventral view of female bopyrid with empty marsupium showing male bopyrid (m) on abdomen of female; C, dorsal view of male (m) in usual site among pleopods of female; D, ventral view of male bopyrid showing pereiopod (arrow) featured in E, F; E, sixth pereiopod of male, d = dactylus, p = propodus; F, distal end of male pereiopod, showing dactylus (d) recurved into deep socket (arrow) on propodus (p). Scale bar in A = 1.2 mm in A, 0.8 mm in B, 0.3 mm in C, 0.2 mm in D, 55 μm in E, 15 μm in F.

on one side or on its dorsum (Fig. 4B, C). As the shrimp backed out of the exuviae, including that of the inner branchiostegite and gills, the parasite pair maintained its position in the host's branchial chamber (Fig. 4C–F). Just prior to and after ecdysis, the parasite female flexed its entire body ventrally several times within the branchial

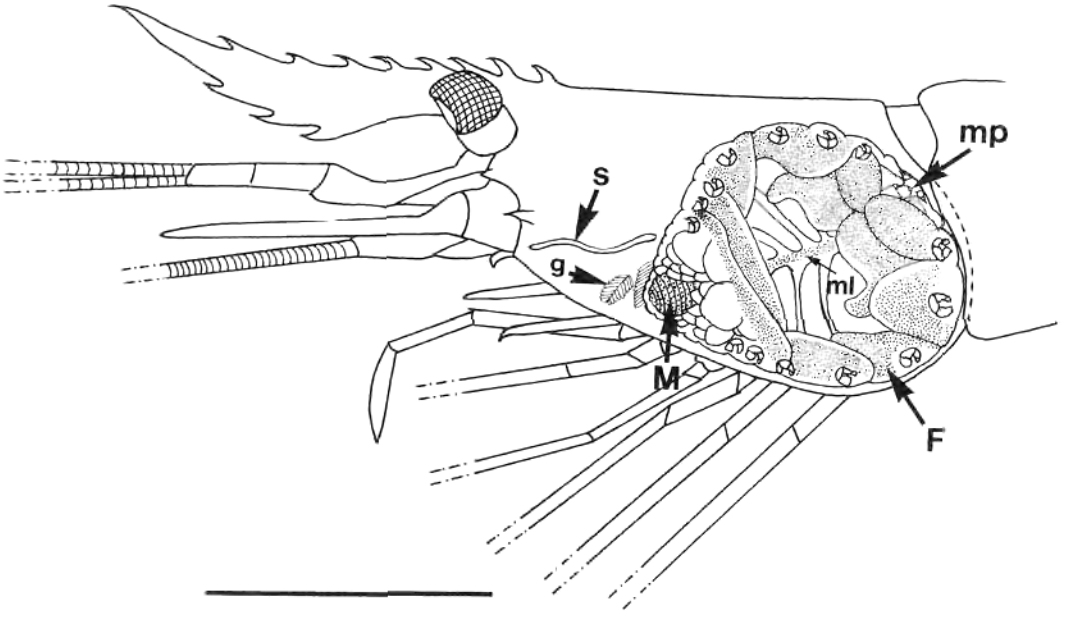

Fig. 3. Diagram showing position of male and female *Probopyrus pandalicola* inside left branchial chamber of *Palaemonetes pugio*. F, female bopyrid with empty marsupium, g = anterior gills of shrimp; M, male bopyrid, ml = melanophores distributed along ventral midline of female, mp = mouthparts of female, s = scaphognathite (gill bailer).

chamber. After the molt, the female and male occupied the same position in the host branchial chamber as before the molt.

During this study, 112 parasitized *Palaemonetes pugio*, isolated individually in the laboratory, molted. In 109 (97%) of these cases, the parasite pair survived the host molt, always remaining in the same branchial chamber as that occupied before the molt.

Observations were made on the condition of the cuticle lining the branchial chamber from the exuviae of 36 parasitized shrimps. In 16 exuviae, the soft and thin cuticular inner lining of the branchial chamber from the nonparasitized side of the shrimp was entire, still partially attached to the rest of the carapace (outer wall of branchiostegite) anteriorly as shown in Fig. 5. The cuticle of the inner branchiostegite and body wall was shredded or missing in all molt skins from the parasitized side of the shrimp and in 20 from the nonparasitized side.

Female Parasite Reproductive Activities Related to the Host Molt

Observations on presence, absence, and developmental stage of offspring in the marsupium of female parasites infecting shrimps maintained in the laboratory show the sequence and timing of larval release and parasite spawning relative to the host molt. In the first set of observations, female parasites initially incubating stage 3 embryos or hatched larvae (Fig. 6C, D) were observed at 1–2 day intervals before and after the host molt. The reproductive state of female parasites prior to and after the host molt is shown in Fig. 7. Three days before the host molt, most females observed were still brooding epicaridium larvae; a single female parasite had released the larvae the day previously. Larval release increased dramatically the day before the molt. On the day molt skins of host shrimps were observed, a majority of females had spawned a new brood of stage 1 embryos (Fig. 6A) when the observation was made (Fig. 7). Observations made one day after the host molt showed that most females had spawned, and by day 2 after the molt, all parasite females examined had spawned and a small percentage were brooding stage 2 embryos (Figs. 6B, 7).

More detailed information on the timing of larval release, host molting, and parasite female spawning was obtained from a sec-

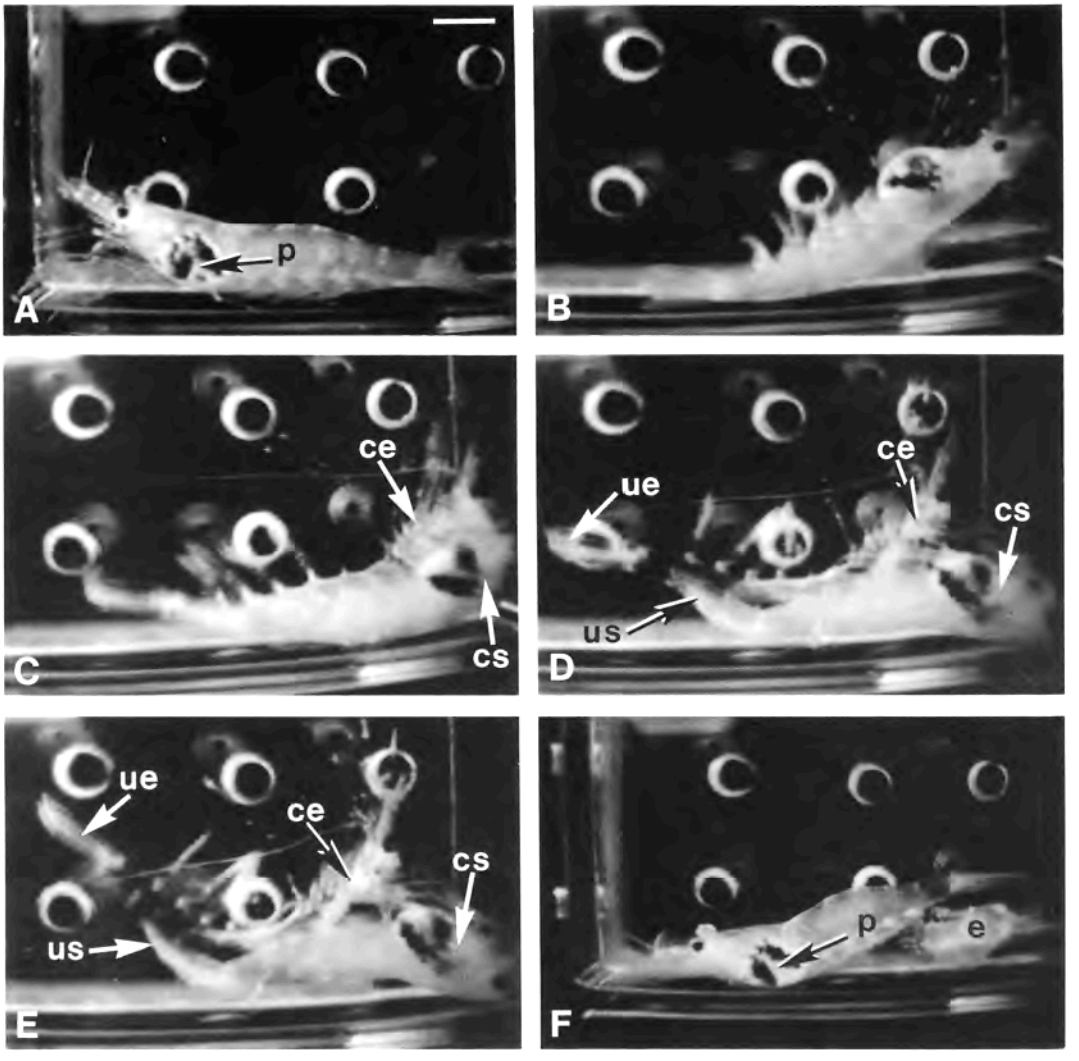

Fig. 4. Video images of molting sequence of *Palaemonetes pugio* parasitized by bopyrid, ce = exuviae of cephalothorax, cs = cephalothorax of molting shrimp, e = exuviae, p = female parasite with attached male, ue = exuviae of uropods, us = uropods of molting shrimp; A, shrimp just before ecdysis with parasite pair in left branchial chamber; B, shrimp falls on side as ecdysis begins; C–E, shrimp pulls out of exuviae, parasite pair maintains position in branchial chamber; F, parasite pair in position in branchial chamber just after ecdysis of shrimp. Scale bar in A for A–F = 5 mm.

ond set of observations on 36 other shrimps with female parasites carrying late stage embryos or epicaridium larvae in the marsupium. In these observations, reproductive condition of the female and presence/absence of a host molt skin were recorded twice daily. The median time from larval release to molting of the host was 42 h (minimum = 6 h, maximum = 120 h, $N = 36$), while the median period of host molting to spawning of the parasite was 12 h (minimum = 6 h, maximum = 24 h, $N = 33$).

Video recordings on another set of infected shrimps gave more exact times of time periods between larval release, host molting, and parasite spawning. The median time from larval release to host molt was 59.3 h (minimum = 28.8 h, maximum = 111.3 h, $N = 8$), while the median period from host molt to parasite spawning was 12.4 h (minimum = 8.0 h, maximum = 17.8 h, $N = 7$).

Video recordings also revealed details on liberation of larvae from the host branchial

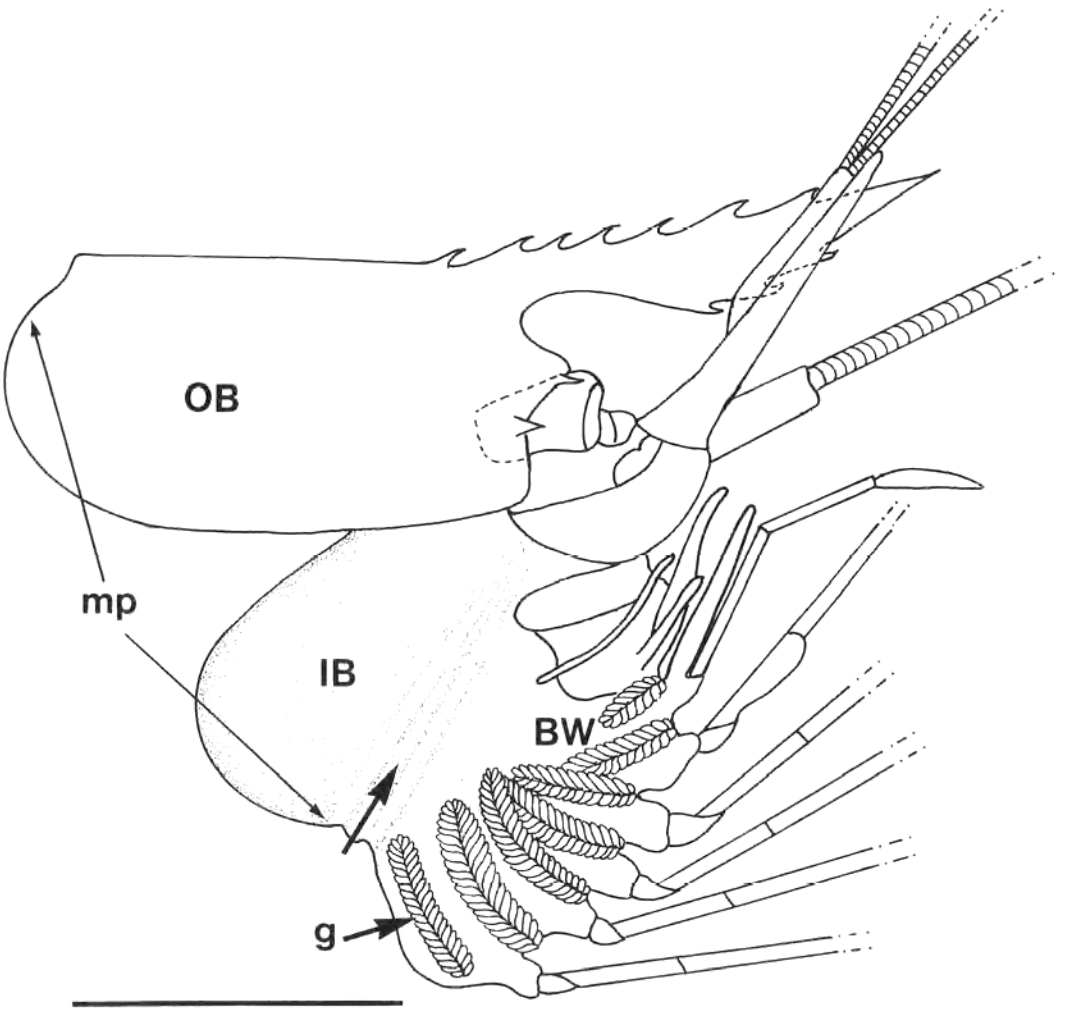

Fig. 5. Diagram of exuviae of cephalothorax of parasitized *Palaemonetes pugio* from nonparasitized side of shrimp, showing example of complete exuviae of branchial chamber. BW = cuticle of body wall, with gills, of branchial chamber, g = gills, IB = inner cuticle of branchiostegite, mp = region of branchiostegite where mouthparts of bopyrid female would be situated on parasitized side, OB = outer cuticle of branchiostegite; arrow indicating region forming roof of branchial chamber prior to molting. Scale bar = 5 mm.

chamber (Fig. 8A–F). A mass of epicaridium larvae in the parasite female marsupium appeared dark or black (Fig. 8A), while an empty marsupium was pale or white, with a narrow black band of melanophores visible along the ventral midline (Figs. 3, 8F). The release of larvae could be followed by exposure of the light ventral surface of the female as the darkly pigmented larvae left the branchial chamber (Fig. 8B–E). During larval release, the female parasite flexed its body dorsally, away from the host branchiostegite, which forms the floor of the parasite's marsupium. The larvae were thus freed and exposed to the respiratory stream of the host which swept them out of the anterior end of the branchial chamber. The release of larvae progressed from the posterior to the anterior part of the female's marsupium, that is, from anterior to posterior in the host's branchial chamber. In six recordings, the infected side of the shrimp faced the camera during the entire sequence of larval release. Median time from beginning to end of larval release was 22 min (minimum = 6 min, maximum = 72 min).

Spawning of a new parasite brood after host molting could be observed by the filling

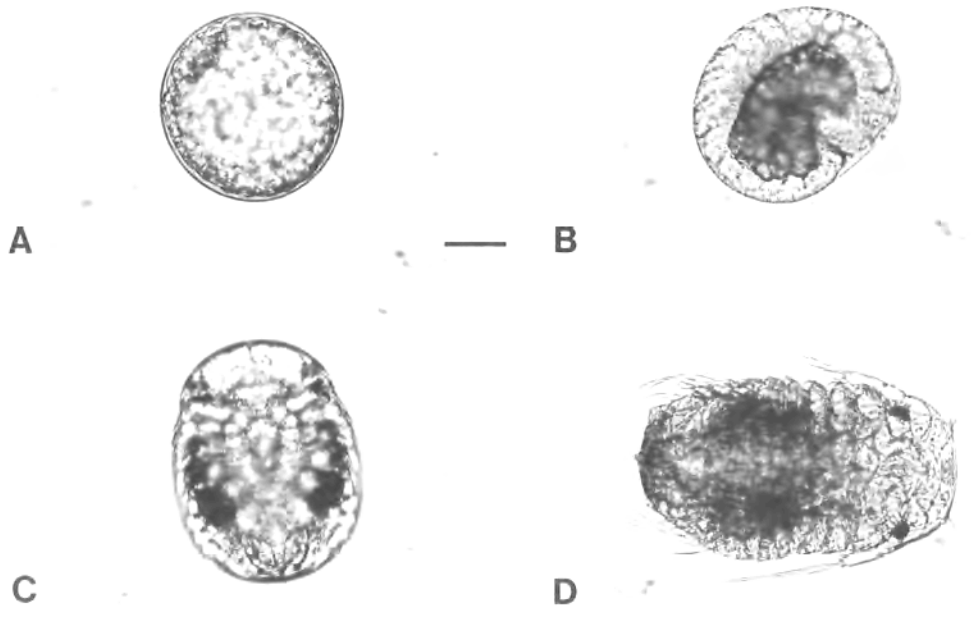

Fig. 6. Developmental stages recognized and used in following larval release from and spawning by female *Probopryus pandalicola*. A, B, C, embryo stages 1, 2, 3, respectively; D, epicaridium larva. Scale bar in A = 93 μm in A, 64 μm in B, 66 μm in C, 63 μm in D.

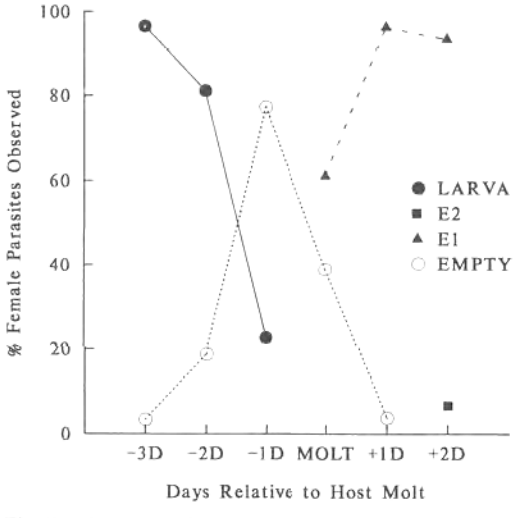

Fig. 7. Reproductive condition of females of *Probopryus pandalicola*, initially incubating stage 3 embryos or epicardium larvae, in days before and after molting of shrimp host. The number of females whose reproductive condition was recorded 3, 2, 1 days before host molting, on day of host molt, and 1, 2 days after host molting was 29, 32, 22, 36, 26, and 30, respectively. "E1, E2, larva, empty" in the figure legend = stage 1 embryos, stage 2 embryos, epicaridium larvae, and no embryos or larvae, respectively, in marsupium of female bopyrid.

of an empty marsupium (Fig. 9A–C), and the process was observed from beginning to end in three infected shrimps. In these three cases, the duration of spawning was 2, 10, and 22 min.

Observations on Ecdysis of Female Parasites

Evidence of molting of two female parasites was observed during the course of viewing the video recordings on parasitized shrimp. In both cases, fragments of female cuticle were observed exiting the anterior end of the branchial chamber of the host. In one observation, a small triangular piece of cuticle, apparently that of the female abdomen, was observed leaving the host branchial chamber 6.5 h after the host had molted. In the other observation, pieces of cuticle, again apparently from the abdomen of the female, exited in the host's excurrent respiratory stream 1.5 h after ecdysis of the host. The anterior portion of the female cuticle was observed leaving the host respiratory chamber 2.5 h later (Fig. 10A–D).

Observations on Activities of Male Parasites and Insemination

In 5 of 7 recordings of host molting and parasite female spawning, the male parasite

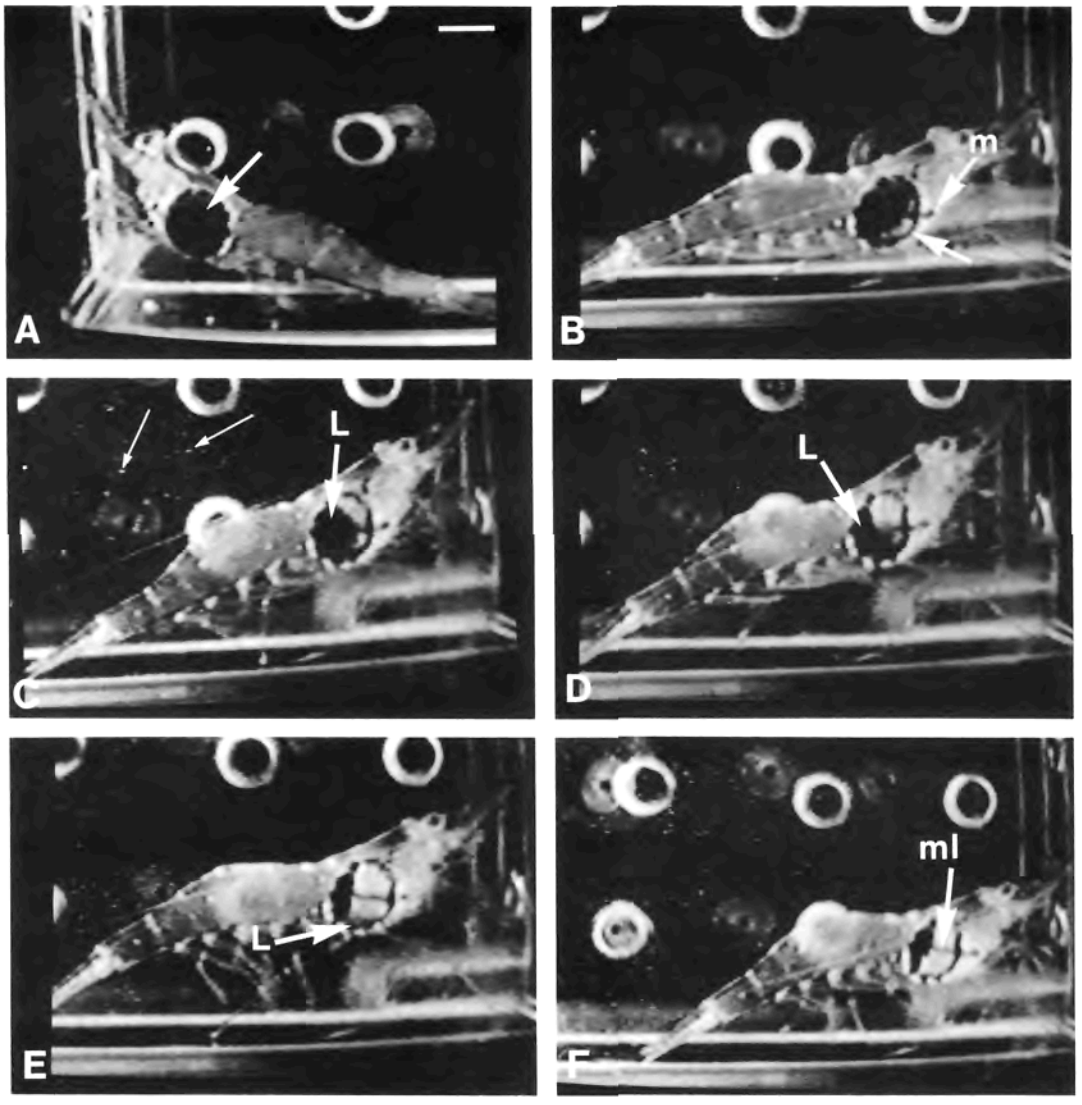

Fig. 8. Video images of release of epicaridium larvae from marsupium of female bopyrid. A, marsupium of female parasite full of epicaridium larvae, appearing as a dark mass (arrow); B, larval release begins, unlabeled arrow points out part of marsupium emptied of larvae, m = parasite male; C–E, further release of larvae from female, unlabeled arrows point out larvae swimming free in observation chamber; L, unreleased mass of larvae; F, larval release completed, ventral surface of female (roof of marsupium) completely empty, exposing melanophores (ml) along midventral line of female parasite. Scale bar in A for A–F = 5 mm.

moved from its usual site on the abdomen of the female after the shrimp had molted but prior to spawning of the female parasite (Fig. 11A–C). In all cases, the male was observed on the lateral surface of the female marsupium near the level of its fifth pereiopod (as in Fig. 11B). In one case, the male was recorded inside the female marsupium (Fig. 11C), actively moving and turning.

None of 9 parasite females from which males had been removed spawned after molting of the host, which occurred from 3–20 (median = 10) days after male removal. All females of five infected shrimps collected and maintained together with the treated shrimps spawned after molting of their hosts, which occurred 8–21 (median = 16) days after male removal.

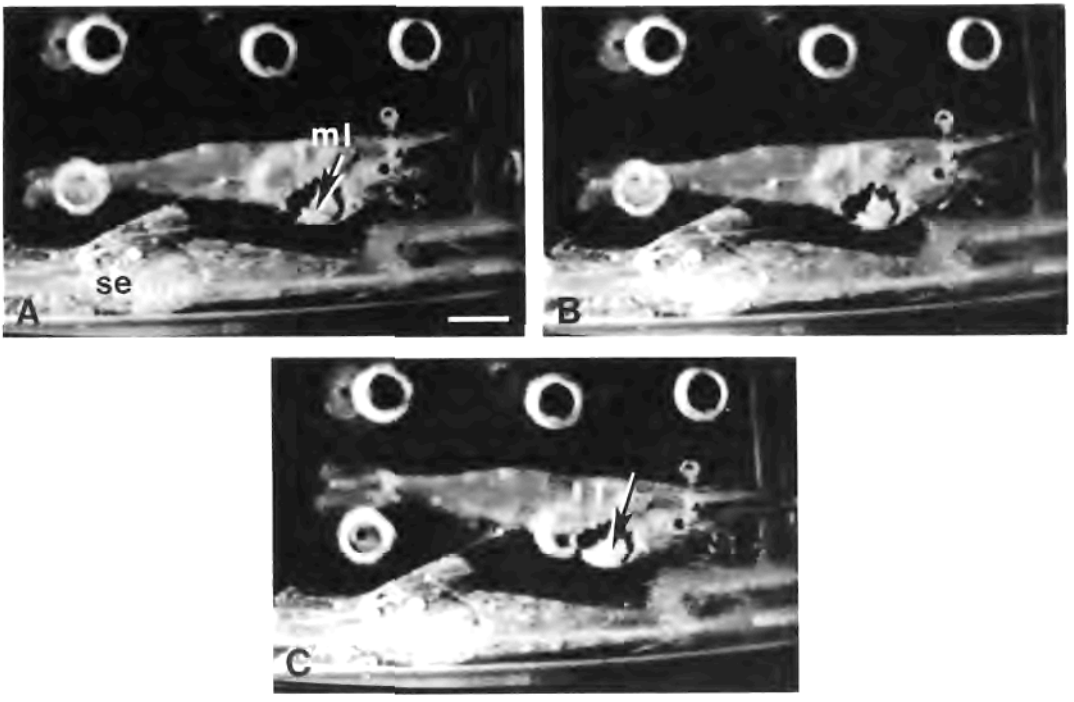

Fig. 9. Video images of spawning of eggs into marsupium of female *Probopryus pandalicola*. A, newly molted shrimp with female parasite prior to spawning, ml = line of melanophores on ventral surface of female exposed, indicating empty marsupium, se = exuviae of shrimp; B, mass of newly spawned eggs partially filling marsupium (line of melanophores on ventral surface of female parasite partially covered); C, spawning completed, marsupium of female filled with white mass of eggs, line of melanophores not visible (arrow). Scale bar in A for A–C, 5 mm.

Discussion

Indirect evidence from previous studies indicated that female bopyrid parasites infect their decapod crustacean hosts early in life and that the same parasite and host live and grow together thereafter. In several studies (cited in the Introduction, this study) a significant positive correlation between female parasite length and host length has been found. In addition, in this study, we found that the size of the dwarf male of *Probopryus pandalicola* also varies positively with host size, indicating that it, too, might remain with the same host (and female parasite) throughout its lifetime. These observations suggest that the male and female of *Pr. pandalicola,* which reside in the branchial chamber of the shrimp *Palaemonetes pugio,* must survive ecdysis of the host. Some workers have indicated (Anderson, 1977, 1990; Walker, 1984, unpublished dissertation for *Pr. pandalicola*) or reported directly (Caroli, 1927, for *Bopyrus squillarum* Latreille; Van Wyk, 1982, on *Aporobopyrus muguensis* Shiino) that the parasite is retained after a host molt. In this study, we observed the parasite in place in the host branchial chamber before and after molting of over 100 infected shrimps, including 13 shrimps in which the parasite pair survived at least two successive host ecdyses. Observations with time-lapse video directly verified the process of parasite survival of the molt in several infected shrimps.

However, in our observations a low percentage (2.7%) of parasite pairs did not survive host ecdysis. Van Wyk (1982), in the course of following ecdyses in the porcellanid crab *Pachycheles rudis* Stimpson parasitized by *Aporobopyrus muguensis,* observed a discarded parasite on the floor of the container after ecdysis of the crab. Anderson (1990) indicated that young infective stages of *Probopyrus pandalicola* can be cast off during host molting. Indirect evidence that branchial bopyrid parasites may suffer mortality while on the host comes from ob-

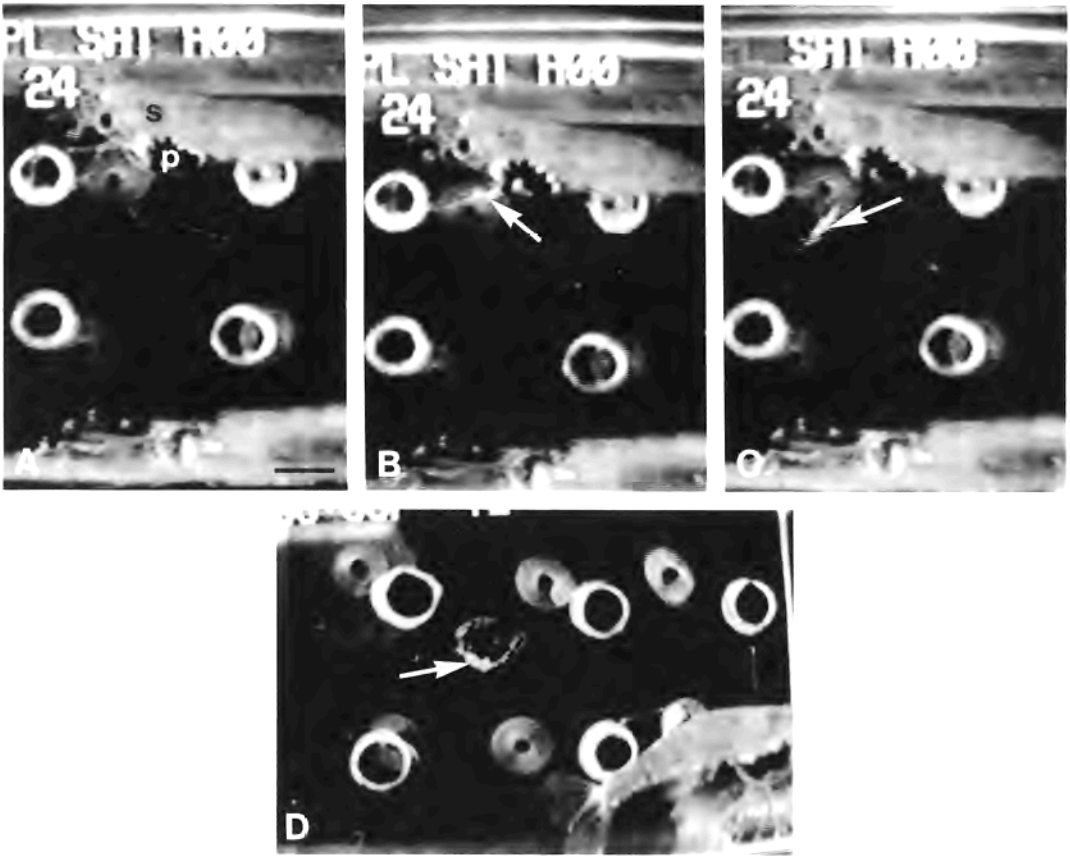

Fig. 10. Exuviae of female parasite exiting branchial chamber of the host. A, infected shrimp (upper part of figure) prior to observation of parasite exuviae, p = female parasite, s = shrimp cephalothorax (shrimp's anterior end faces to the left); B, C, side view of exuviae (arrows) leaving and falling from host branchial chamber; D, frontal view of exuviae of female parasite; arrow pointing to anterior oostegites of exuviae. Scale bar in A for A–D, 5 mm.

servations that the swollen branchiostegite of infected hosts keeps its shape through subsequent ecdyses even if the parasite has been lost or removed (Van Wyk, 1982; Schuldt and Ituarte, 1985; personal observation on *Macrobrachium ohione* infected with *Probopyrus* spp.). Deparasitized hosts have been identified by swollen but empty branchial chambers by Hiraiwa (1936), Pike (1960), and Beck (1980a). Although the source of parasite mortality can not be determined by simple observation of a deparasitized branchial chamber, molting of the host is certainly a possible cause, given the above observations on known parasite expulsion due to molting and the fact that the cuticle which surrounds the parasite pair is cast off with the host molt.

Our video observations show that the female parasite (and attached male) do not leave and reenter the branchial chamber of the host during and after its molt. Rather, the female parasite retains its position within the gill chamber. Examination of exuviae of infected shrimps and video observations on molting suggest the following hypothesis on how the female avoids expulsion from the branchial chamber. During ecdysis, the thin inner cuticle of the branchiostegite, to which the parasite female clings, becomes torn or separated from the stiff outer cuticle. As the shrimp flexes and convulses, moving backwards out of the old exoskeleton, the new inner cuticle of the branchiostegite is exposed. It appears that the female immediately clings to the new cuticle, exposed first posterodorsally in the branchial chamber near the anterior end of the parasite. As

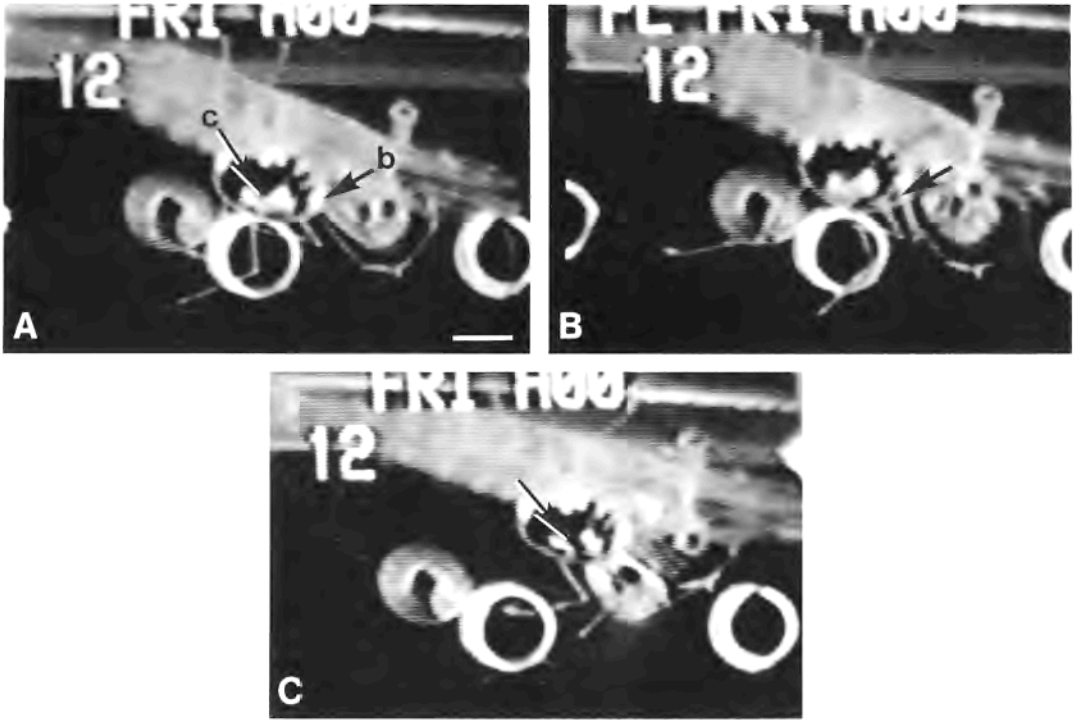

Fig. 11. Movement of male parasite into female marsupium after molting of host and before spawning of female. A, female parasite prior to movement of male; male is in usual position on abdomen of female but out of sight because of dorsolateral view of shrimp; b, c, arrows indicating positions in which male appears in B and C. B, male (arrow) crawling up left side of female but lateral to darkly pigmented oostegites; C, male (arrow) inside marsupium of female parasite. Scale bar in A for A–C = 2.5 mm.

the molting shrimp continues to move out of its molt skin, more new branchiostegal cuticle is exposed which the female can grasp with its posterior pereiopods. The female, attached to the newly exposed inner wall of the branchiostegite, is passively carried along in the branchial chamber of the host through the torn and broken thin cuticle of the exuviae. The molted inner cuticular lining of the gill chamber of the infected side of the shrimp was always badly shredded or missing, perhaps in part due to activities of the female parasite. We observed that, moments before the molt, the female flexed the body several times, perhaps aiding the natural shredding and tearing of the soon-to-be-molted inner lining of the branchial chamber. However, the same movements were also observed just after the molt, possibly related to adjustment and firm attachment of the female inside the new branchial chamber.

Molting of the host is also a critical point in the reproduction of *Probopyrus pandalicola*. Walker (1984, unpublished dissertation), without giving details, reported that the parasite epicaridium larvae hatched and were released before the molt of the shrimp and a new spawning occurred after the molt. Beck (1980a) also reported that the cycle of spawning to larval release occurred during the host intermolt. This study confirms the sequence of larval release prior to and spawning shortly after host molting. We found that larval release can occur as long as 5 days before and as briefly as several hours prior to the molt. Female parasites produce successive broods (Beck, 1980a), and we found that spawning of a new brood took place within several hours after host ecdysis.

After the shrimp molts, at least two important events in parasite reproduction occur in the several hours before female spawning. One is molting of the female parasite, which Walker (1984, unpublished dissertation) reported in *Probopyrus pandalicola*. In video recordings, we observed

expulsion of exuviae of 2 female parasites within a few hours after host molting and prior to parasite spawning. The exuvial fragments of the female parasites are small and quickly swept away from the shrimp by water circulation and shrimp activities. In addition, the parasitized side of the host faces away from the camera about half the time during video observations. Thus, lack of observation of exuviae of the female parasite in the other 5 video recordings of recently molted shrimps is perhaps understandable. It is likely that molting of the female parasite after host ecdysis and before spawning of a new brood is a characteristic feature of the reproductive cycle of *Pr. pandalicola*.

In addition, insemination of the female by the male appears to occur prior to each female spawning. Video recordings demonstrated that males move, after host molting but prior to female spawning, from their position on the female abdomen to inside the female marsupium or in some instances up to the level of the female's fifth pereiopods (where female gonopores are located). We never observed any other movements or activities by males.

Removal of male parasites from females in one set of observations prevented those females from spawning after the next host molt, whereas females with males always released a new brood of embryos after ecdysis of the shrimp. These observations appear to be strong evidence in support of the hypothesis that males inseminate females prior to each spawning. However, Shuldt (in press) reports that, when deprived of males, females of *Probopyrus ringueleti* fail to complete vitellogenesis. Since none of the females from which males were removed in this study were incubating larvae (and thus would be likely to be near the next spawning), the lack of spawning by male-deprived females may also have been due to the effect on vitellogenesis reported by Schuldt (in press). Male removal experiments should be repeated in *Probopyrus* spp., using females incubating larvae and thus likely to have a fully mature ovary ready for spawning.

The reproductive morphology of bopyrid isopods is poorly known (Wilson, 1991), but Hiraiwa (1936) found that females of *Epipenaeon japonica* lacked the spermathecae for sperm storage that are common in other groups of isopods. Our observations, which should be extended and confirmed, support the hypothesis that bopyrid females do not store sperm and must be inseminated prior to each spawning. Male bopyrids lack gonopods characteristic of other isopod groups (Wilson, 1991). Hiraiwa (1936) hypothesized that the male released sperm into the female marsupium, without moving from its position on the female's abdomen, prior to or during spawning. Our observations shed no light on the actual process of sperm transfer, but do indicate that the male of *Probopyrus pandalicola* actively moves up near the female gonopores and/or inside the marsupium prior to spawning of a new brood of eggs. Our hypothesis is that the male inseminates the female just prior to each spawning, either by deposition of sperm into the female gonopores or by release of sperm inside the female marsupium.

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