

Reproductive Behaviour and Larval Development of *Tanais cavolinii* (Crustacea: Tanaidacea)

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Abstract

Mature female stages of *Tanais cavolinii* Milne Edwards are described according to degree of oostegite development. Sexual dimorphism of the chelipeds of copulatory males is explained functionally. An aberrant marsupium consisting of two closed ovisacs with a small slit for sperm transfer protects the brood from sudden unpredictable changes in salinity. Behavioural traits, courtship, copulation, and parental care are described and compared to those of *Heterotanais oerstedii* Kröyer. The different habitats selected by the two species are reflected by several adaptations in their morphology and behaviour as tube dwellers. A phenomenon unique among invertebrates is that the female *T. cavolinii* supplies additional yolk to the larvae just before their release. The production and timing of this food supply is described and its adaptive significance is discussed.

Introduction

With the exception of *Heterotanais oerstedii* (Bückle-Ramirez, 1965), only a few scattered details are known about the reproductive behaviour of tanaids. The Bückle-Ramirez study describes the behaviour of both sexes with regard to courtship, copulation, brood-care, etc.

Earlier works concerning the reproduction of the genus *Tanais* have primarily concentrated on the form (Moers-Messmer, 1936) and number (Iacobescu, 1970) of the ovisacs. Protogynic hermaphroditism has been reported within the genus *Tanais* by Lang (1958).

This investigation of the reproductive behaviour of *Tanais cavolinii* supplements a study on the functional morphology and life history of the species (Johnson and Attramadal, 1982). *T. cavolinii* is an almost cosmopolitan inhabitant of exposed tidal hard-bottom habitats and rock-pools. The body form and behaviour are adapted to tubiculous life. The tanaid is about five mm long and spends its entire life confined to the tube and its immediate

surroundings. The tube is spun with mucous threads from the spinning legs (pereopod II) and incorporates small fragments from the habitat and its own fecal products.

Material and Methods

Tanais cavolinii Milne Edwards builds tubes among the basal parts of the calcareous alga *Corallina officinalis* and in crevices on the calcified bottom crust. Individuals were collected from tidal pools on the islets Aspöya and Nord Oddane, near Bergen, on the west coast of Norway, twice a month throughout 1977. Morphological and histological analyses were performed on material fixed in 4% neutralized formaldehyde, alcoholic Bouin or 80% ethanol. In the laboratory, individuals were kept in circulating seawater of about the same salinity and temperature as their natural habitat (32–34‰ S, 7°–15°C). A few individuals were allowed to build natural tubes on pieces of *C. officinalis*, others were offered capillary glass tubes of varying diameters corresponding to different instars. These substitutes were readily accepted. For observation purposes, a Wild M5 dissection microscope movable in all directions above the aquaria, was used. The individuals were adapted to light of approximate day-light intensity which was kept constant during a 14- to 16-h daylength period, which ensured a continual growth of the microflora on the substrate. Under these circumstances, a large number of individuals could be kept alive for months to raise healthy broods. For more details see Johnson and Attramadal (1982).

Results

Development of Ovisacs and the Different Developmental Stages of the Female

The mature female stages are divided according to a terminology used by Gardiner (1975), into the preparatory,

copulatory and intermediate stages. The preparatory stage, which involves three moultings, is characterized by oostegites which are not yet developed into a marsupium (ovisac) (Fig. 1 A–C). After the first moult, a single pair of oostegites appear in the form of small lamellae borne on the coxa of pereopod V (Fig. 1 A). After the second moult, the oostegite increases in volume and has the form of a pear-shaped bulb (Fig. 1 B). The following moult increases the bulb to about three times the original volume (Fig. 1 C). The complete preparatory development takes about twenty days at a temperature of 15°–18 °C. Preparatory (immature) males were never found and are probably not distinguishable from juvenile or intermediate females without histological analysis.

The copulatory stage is characterized by fully developed ovisacs containing eggs or embryos. Copulation takes place after the ecdysis between Stages 3 and 4 (Fig. 1 C–D). The eggs are released via the gonopore which opens into the 'stalk' of the ovisac. The ovisacs are an aberrant form of marsupium, each ovisac is developed by only one oostegite. Occasionally, only one ovisac develops (Iacobescu, 1970). Just before copulation, the empty ovisac resembles a shrivelled plastic bag (Fig. 1 D). The eggs are fertilized by the male genital cone most likely entering through a small slit in the ovisac membrane. The copulatory female then carries the embryos during their 14- to 16-d development. The copulatory male is characterized by distinct sexual dimorphism of the chelipeds, genital cones and pigmentation, and by always being of the same size or larger than the copulatory female.

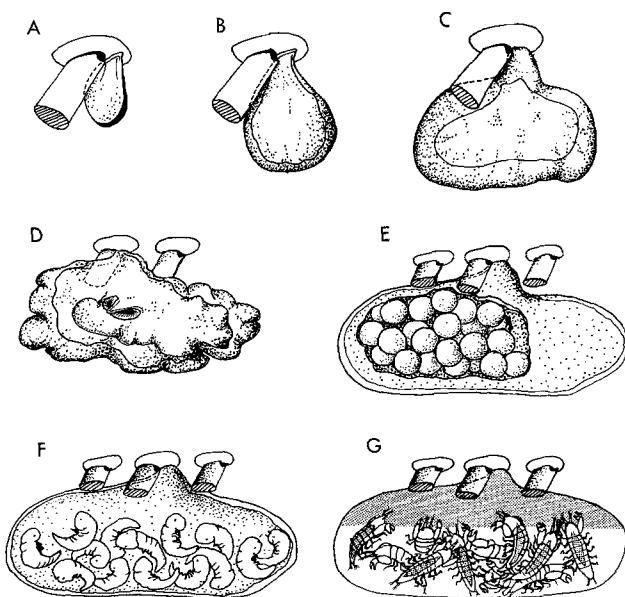


Fig. 1. The development of the oostegite into an ovisac. All views are lateral, except for D, which is a medial view. (A–D) Preparatory female Stages 1–4. Increase in size of oostegite by subsequent moultings. (D) Developed ovisac with genital slit through which sperm is transferred. No further moults occur during the marsupial development (D–G). (E) Ovisac filled with eggs or early embryos. (F) Embryos before hatching to manca I larvae. (G) Manca II larvae feeding on yolk

With the release of the young and the loss of the ovisacs, the female enters the intermediate stage. This is characterized by the absence of oostegites, and possibly by scars on the coxal plate of PV.

Precopulatory Behaviour of the Male

The only instar that left their tubes were copulatory males during the breeding season, when the copulatory females occurred (April to September). This was observed in laboratory and by *in-situ*-studies. The search for copulatory females exposes the males to predators such as the isopod *Idothea pelagica* Leach which was observed during day-time catching copulatory males.

When a male came in the proximity of a preparatory female (of Stage 3 or 4), he approached her directly, tore a hole in the tube wall close to the female with the chelipeds, and entered the tube. When the female inhabited a glass capillary tube, he entered through the tube opening.

Precopulatory Behaviour in the Tube

The behaviour in the tube did not seem to follow any strictly fixed pattern, but could be roughly divided into certain phases. At first the female reacted as she would to any intruder by reversing the pleopod beating. If the male touched her antennas, she pressed forwards trying to push him backwards out of the tube, or she used the more defensive method of blocking the tube with her carapace and chelipeds (Fig. 2 A). The male often pumped towards the female with the pleopods. The courtship continued and the turning inside the tube resulted in frequent antenna/antenna and antenna/uropod contact between the two sexes. After some time, the confrontations diminished in frequency and vigour and they seemed to tolerate each other. This usually ended with the male occupying one half of the tube, and the female the other half.

Copulation

Two complete copulations were observed and several copulations were observed in part. The behaviour of the male changed as the female moulted from Stage 3 to 4 (Fig. 1 D). The male began approaching the female. To reach her, he often had to tear away pieces of exuviae which blocked the tube. At first, the female resisted when the male tried to back up to her to place his posterior end ventrally under her, by bending down her carapace or pleon (Fig. 2 A) to block the tube. The male could withdraw and in the pause both cleaned themselves or ventilated the tube. After several trials, the male succeeded in sliding in under her ventral side, to a position shown in Fig. 2 B. Occasionally, the male was seen to grip the female chela with his own chela, whose aberrant structure seems to fit well around the female chela. In this copulatory position the

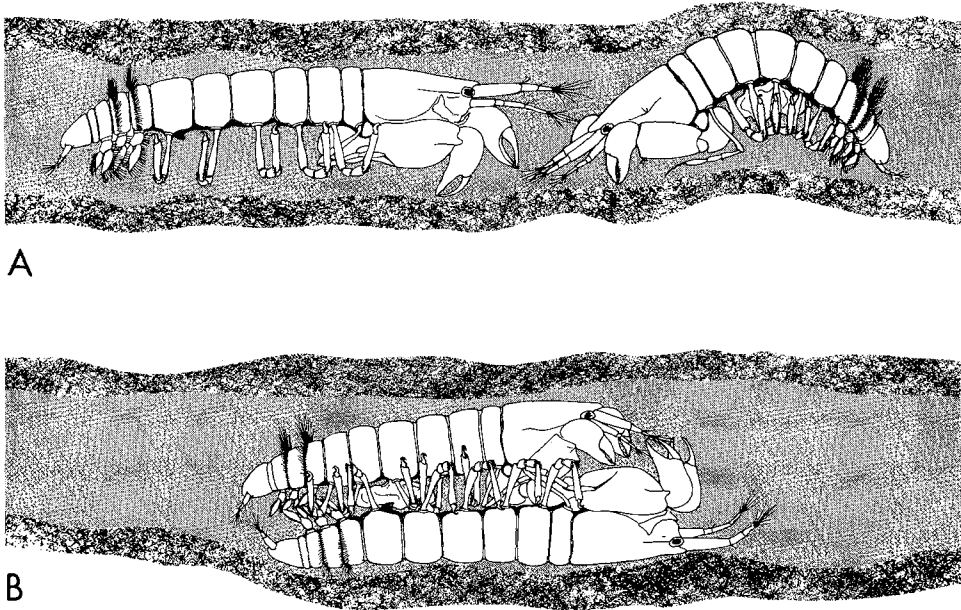


Fig. 2. Courtship and copulation. (A) Copulatory male entering female tube. Typical female pose when blocking the tube with the carapax. (B) Position during transfer of sperm with the male genital cones entering the female genital slits

male genital cones were close to the copulatory slits of the ovisacs for the insemination of the sperm (Figs. 1D and 2B). After a few minutes, the male distanced himself from the female who immediately started to release eggs into the ovisacs. Within about ten minutes, the ovisacs became voluminous and filled with eggs. During this procedure the female repeatedly kneaded the ovisacs with her pereopods, probably to distribute the eggs and sperm evenly. A few hours after the copulation, the male left the tube.

Brood Care

During the first postcopulatory period, the female carried out the normal activities described in Johnson and Attramadal (1982). The ovaries never completely displaced the intestine before copulation, and the general body-form was kept with only little ventral invagination after copulation. The membraneous ovisacs are thin and transparent, with the embryos visible inside. The female continued to knead the ovisacs frequently with the pereopods, which resulted in the embryos shifting position inside the ovisac. The ovisacs did not seem to hinder normal walking or turning inside the tube. Because of their elasticity, they could be squeezed out between the pereopods or wherever space was available.

After ten to fourteen days, the embryos developed into the larval stage manca II, and the female started spinning a brood-nursery of about three body lengths long (for spatial reasons, the length has been compressed in the drawing Fig. 3). The front section of the tube was partially closed by one or two nets with various openings. Although the rear end is normally closed, the brood-nursery was demarcated to the central part of the tube by an additional posterior net. After this, the female could not vacate the tube and she stopped eating.

In order to make the transverse nets, she first spun threads on one level, and proceeded by pulling cemented threads from the outside, through the net, and cementing them on the inside. This closed off the brood-nursery (Fig. 3). Sometimes, an additional net was made on the inside of the first net. The female regularly inspected the net with both pairs of antenna and reinforced it with more threads. Her activity of ventilating the tube resulted in particles getting caught in the nets, which had to be regularly cleaned. The uropods were moved dorsoventrally, brushing the nets with the bristles, and simultaneously the direction of the pleopod pumping was reversed so that particles brushed off were sucked away.

Behaviour of Larvae in the Ovisacs

Kneading by the pereopods continually shifted the position of the embryos. From 1–3 d before being released from the ovisacs, the larvae developed from the immobile larval stage manca I into manca II. At this stage they resembled the adults habitually, except that the anlagen of last pereopods and the pleopods were not visible. This manca stage is referred to by Bückle-Ramirez (1965) as the stage with embryogenesis sufficiently completed for the larvae to leave the mother's tube. He describes them as living off "deutoplasma" from the midgut, and thereafter feeding on bacteria, diatoms and detritus from the female's inner tube surface. In the manca II larvae of *Tanais cavolinii* the abdomen and most of the thorax were clearly translucent, showing the thin intestine and ventral cord. Two short midguts were developed, sometimes with a small remnant of "deutoplasma". The posterior body was not fully developed, lacking the last pereopod and pleopod anlagen.

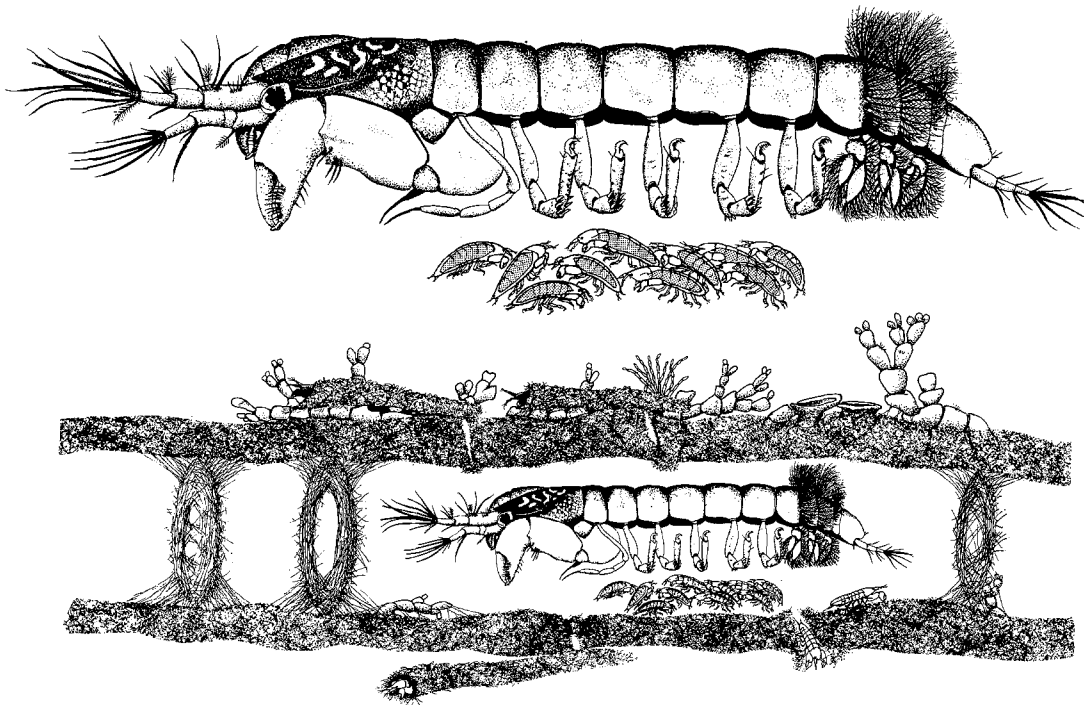


Fig. 3. Female and post-marsupial larvae in brood-nursery, showing transverse nets, a group of released larvae fastened to the tube wall digesting the yolk, and newly moulted manca III larvae leaving the brood-nursery, and making their own tubes

When the larvae reached the manca II stage they could walk, and incessantly pinched the ovisac membrane with the chelipeds. On the last day before the release of the young, the female injected a yellow substance through the gonopore, which we refer to as yolk. About one third of the dorsal part of each ovisac was filled up. The mancas immediately started to feed from this yolk, and after a few hours most of them had their dilated intestines completely filled up, and they had become yellow. Occasionally, a few mancas did not feed from the yolk and remained transparent. Whether they were delayed in embryogenesis is unknown. If yolk still remained after the release of the larvae, some of them could be seen hanging from it beneath the female, eating.

Release of Larvae

The young were released when the ovisac membrane disintegrated in a matter of seconds, without leaving any fragmental traces. This happened when most of the larvae were filled up with yolk. The two ovisacs disintegrated almost simultaneously. The larvae dropped down and attached themselves to the nearest site on the tube wall. Some were fastened to the wall by the female's spinning. This resulted in a cluster of larvae in the middle of the brood-nursery. The number of larvae varied between 15 and 20. Together with the manca II larvae approximately 3–7 unfertilized eggs or undeveloped embryos, and something which resembled remnants of shed membranes were released.

On one occasion, no yolk was supplied to one of the ovisacs. Only the yolk-filled ovisac disintegrated. The other broke loose at its coxal site after about one day, and was

thrown out of the tube by the female, while it still contained living larvae.

Intermediate Female and the Postmarsupial Larvae

The larvae remained immobile in their positions for about five days. Through the glass tubes, the manca II larvae could sometimes be seen scratching and tearing at the inner wall with their chelipeds. Occasionally, larvae loosened from their original places, and started to wander around sluggishly. The female seemed to sense these stray larvae, presumably through contact with her tactile setae, and immediately pushed or dragged them carefully back to the rest of the group with her chelipeds, attaching them to the wall with spinning threads.

With their first ecdysis, the confined situation of the larvae ended and they moved freely for the first time as manca III stages. By then, they had become larger and not so transparent. The average length of manca II was 1.0 mm, and manca III about 1.2 mm, but the volume increased approximately 100%. The pereopod and pleopod anlagen had by now developed. Due to feeding, the intestine became visible as a thin dark stripe. The first food consisted mainly of the diatoms, bacteria and the female faeces on the inner tube surface. After a short feeding period in the mother's pantry, the larvae left by tearing a hole in the tube wall of natural tubes. They made their own tubes using the outside of the mother's tube as substrate. The female soon sealed off the larvae by mending the inside of her tube. She then tore down the transverse nets which had closed off the brood-nursery, started feeding and resumed her normal behaviour.

Discussion

The reproductive behaviour of *Tanais cavolinii* shows a strong resemblance to that of *Heterotanaeis oerstedii* (Bückle-Ramirez, 1965). In both species, the precopulatory behaviour of the male and female is characterized by initial, strong resistance by the female which attempts to block the tube. This is followed by a period of frequent contact of the antenna, pumping by the pleopods towards each other, and finally by the acceptance of the male's presence.

The behaviour of the copulatory male of *Tanais cavolinii*, leaving its tube in search of preparatory females, resembles that of *Heterotanaeis oerstedii*. The image-forming capability of the eyes of *T. cavolinii* is poor (Andersson *et al.*, 1978) and certainly does not help locate the female in her well-camouflaged tube. It is possible that localization is assisted by release of a pheromone.

The use of the male chelipeds to grip the female's chela may be one explanation for the sexual dimorphism of this structure. The enlarged opening in the cheliped, caused by the slender shape of the propodus 'finger' and dactylus, fits around the female chela. It may protect the male from being injured if attacked by the female, without damaging her chelipeds. The cheliped may not, however, be essential for successful copulation. According to Bückle-Ramirez (1965), males of *Heterotanaeis oerstedii* with amputated chelipeds could still copulate successfully. In the case of *Tanais cavolinii* this would be most unlikely under natural conditions, as the male cheliped also has other functions. The copulatory male is usually the only free walking instar. When climbing among algae such as *Corallina officinalis*, the chelipeds are used to grip the rounded branches securely (Johnson and Attramadal, 1982). This is essential for living in a turbulent environment, and may favour the special structural development of the male cheliped.

The precopulatory behaviour of *Tanais cavolinii* resembles that of *Heterotanaeis oerstedii*, except for details of copulation which are probably a consequence of the morphological differences between the species. While the female *H. oerstedii* opens her marsupium widely to receive the sperm of the male, the two ovisacs of *T. cavolinii* only have small medio-ventrally positioned genital slits to meet the male's genital cones. Through close contact with the female's ovisacs and through his movements, the anteriorly directed genital cones probably open the slits and point directly into the ovisacs. To ensure fertilization of both ovisacs, it is essential that the genital cones are of the dikonophoran type. After the copulation, the female *H. oerstedii* moves her oostegite lamellae, probably to mix sperm and eggs, while the female *T. cavolinii* kneads the ovisacs to obtain the same result.

The eggs are laid into the ovisac via the gonopores which are the ovisacs' only connection with the body. In *Heterotanaeis oerstedii*, the egg-filled ovaries occupy the thorax almost completely, displacing other organs such as the intestine. After the eggs are released into the marsupium, the ventral side of the female invaginates, leaving additional room for embryos without considerably enlarging

the total diameter of the marsupium and the body. In *Tanais cavolinii*, the ovaries never completely displace the intestine and the normal body-form is kept by the female, with little ventral invagination. This allows the female to feed until she confines herself to the brood-nursery during the last days of the copulatory stage.

The function of the frequent kneading of the ovisacs is uncertain. Several other peracaridan crustaceans ventilate their brood more or less regularly by a pumping action of the marsupial lamellae, e.g. mysids (Tattersall and Tattersall, 1951), the isopods *Idothea neglecta* G. O. Sars (Kjennerud, 1950), and *Astacilla* sp. (Schiecke, 1973), the tanaid *H. oerstedii* (Bückle-Ramirez, 1965) and species of *Apeudes* (Schiecke, 1973). For *Tanais cavolinii*, this type of ventilation is impossible. The thin ovisac membrane probably permits some diffusion of oxygen into the ovisac. Diffusion of oxygen is probably enough for the metabolic needs of the embryos but the ovisac volume is too big for just diffusion so the kneading is necessary for mixing the diffused oxygen in the whole volume. The kneading also increases the diffusion out of smaller molecules.

Other crustaceans inhabiting osmotically variable environments, such as the mysid *Praunus flexuosus* (Müller) are known to osmoregulate the contents of the marsupium (McLusky and Heard, 1971). The closed off ovisacs of *Tanais cavolinii* may be necessary for protection of the embryos against the sudden extreme variations in salinity which frequently occur in tidal pools. We have not, however, tested the ability of *T. cavolinii* to osmoregulate the ovisac contents.

The brood-nursery behaviour resembles that of *Heterotanaeis oerstedii*, in that the larvae are kept in a restricted part of the tube, and the female retrieves stray larvae. The two species differ, however, in the timing of the construction of the brood-nursery. *H. oerstedii* seals the tube opening immediately after the male has left the tube, and it remains sealed until the larvae have left the tube. The female cannot feed during this period. The female *Tanais cavolinii* can feed during the copulatory stage until she constructs the brood-nursery a few days before the release of young. This additional feeding may be necessary for the large amount of yolk which is produced during this period.

According to Bückle-Ramirez (1965), the loss of embryos from the relatively open marsupium of *Heterotanaeis oerstedii* is a common event, and it is therefore necessary for *H. oerstedii* to seal off the tube directly after the copulation. The closed ovisacs of *Tanais cavolinii* completely eliminate this. The functions of the brood-nursery suggested by Bückle-Ramirez (1965) are: (1) 'Eggs' and larvae are protected from being washed out of the tube. (2) By the pleopod pumping of the female, diatoms are sucked into the tube, fasten onto the inner tube wall, and serve as food for the newly released larvae. (3) The brood-nursery serves as protection. The brood-nursery of *T. cavolinii* functions in much the same way. The female attempts to keep the larvae in a group, and transverse nets may serve as barricades, temporarily preventing stray larvae from leaving the tube until the female can locate and retrieve them.

Regular cleaning of the transverse nets of the brood-nursery prevents clogging, and may save energy by making the pleopod pumping more efficient. The cleaning procedure reveals an additional function of the uropods, besides that of being tactile sensory organs.

The yolk undoubtedly functions as additional nourishment for the larvae. In this case this is accomplished by transforming the succeeding generation of eggs in the ovaries into food for the larvae. This 'lactating' or second investment of yolk in the progeny is a previously unreported phenomenon, at least among crustaceans. A number of advantages may be put forward: the yolk may increase the growth rate and the size of the larvae, the female saves energy by not investing yolk in the eggs which do not develop into manca II larvae, and she has extra time to accumulate energy for the development of eggs and yolk.

The disappearance of the ovisac membrane does not seem to come about by mechanical means, because it happens abruptly and tracelessly, without any noticeable activity from the larvae or female. An enzymatic dissolving of the ovisac membrane is more probable. A similar dissolving of the oviductal sac is known among cirripeds (Barnes and Blackstock, 1977). The question is whether the female or the larvae produces the enzyme to set off the dissolving of the membrane, which does not occur until most of the larvae have their intestines filled with yolk. When the larvae did not receive any yolk, the ovisac membrane also did not dissolve. This points towards the larvae as releasers of the enzyme. The absence of yolk was, however, probably caused by a pathological disturbance which similarly may have prevented the female from releasing the enzyme.

Some undeveloped eggs or dead embryos and remains of shed membranes, were generally released with the manca II larvae. This is not reported for *Heterotanais oerstedii*. Bückle-Ramirez (1965) remarks that the female cleans out the marsupium with the pereopods. She also has a mechanism for returning lost embryos, except for those which deviate from normal size, which are eaten. The closed ovisacs of *Tanais cavolinii* prevent the cleaning out of unfertilized eggs, dead embryos and membrane remains, which are therefore released with the young.

Altogether, there is a considerable resemblance between the reproductive behaviour of *Heterotanais oerstedii* and that of *Tanais cavolinii*, although the environmental conditions in the habitats of the two species may differ considerably. *H. oerstedii* lives in soft mud, and *T. cavolinii* on the substrate of exposed rock-pools. This difference is reflected in several details of morphology and behaviour. Likewise the common features must have been acquired as a result of a tubicolous life strategy in a variable, shallow marine environment.

It is too early to determine whether this pattern applies to other tanaisids. As *Heterotanais oerstedii* and *Tanais cavolinii* are the only investigated species to date, more studies on biology, functional morphology, and behaviour are needed to establish the existence of a general tanaisid-pattern.

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