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A STUDY OF THE HATCHING PROCESS IN AQUATIC INVERTEBRATES, XXII. MULTIPLE MEMBRANE SHEDDING IN *MYSIDIUM COLUMBIAE* (ZIMMER) (CRUSTACEA: MYSIDACEA)[1]

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Abstract

Hatching of *Mysidium columbiae* was studied at Port Royal, Jamaica. Eggs in the brood pouch of the mother lose two egg membranes. Loss of the second egg membrane results in formation of a nauplius, with three pairs of free appendages. Continued development results in larvae that bear free thoracic legs, as in the adult, but are incapable of extensive movements, and in which the eyes point dorsally instead of laterally. Ecdysis is associated with liberation of the young mysids from the brood pouch. It is estimated that from 48 to 72 hours pass between oviposition and shedding of the second egg membrane, 48 hours between appearance of the free nauplius and shedding of the naupliar membrane, and another 48 hours between shedding of the naupliar membrane and emergence from the brood pouch. The stages in development are figured and described.

Introduction

Previous studies in the present series (listed in Davis, 1966) have indicated that aquatic invertebrates may hatch from their eggs either by 1) mechanical breaking of the membranes, 2) osmotic swelling of non-living egg membranes, 3) swelling of the enclosed embryo or larva through the uptake of water, 4) action of enzymes upon the membranes, 5) aid from water currents set up by the mother animal, or, more usually, 6) some combination of these methods.

The investigation was continued by study of the hatching mechanism of the common Jamaican mysid, *Mysidium columbiae* (Zimmer). The work was accomplished at the Marine Laboratory of the University of the West Indies at Port Royal, Jamaica from February to May, 1965. It is a pleasure to acknowledge the friendly cooperation of Dr. Ivan Goodbody, Director of the laboratory, in providing space and facilities for the investigation. Thanks are also extended to Sr. Alberto Namer of Montevideo, Uruguay, for aid with the Spanish summary.

Materials and Methods

Mysidium columbiae is a very common mysid in shallow lagoons of the mangrove areas adjacent to Port Royal, Jamaica. As a result of their

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schooling behavior (Steven, 1961), they occur in vast swarms and are easily collected in large numbers. The species has been shown by Goodbody (1965) to breed in the vicinity of Port Royal throughout the year. In this species, as in other mysids, the eggs and young are carried in a ventral brood pouch until a rather advanced stage of development, emerging essentially in the adult form (during the period of study from 1 to 11 young were encountered per brood pouch). Specimens for study were obtained frequently between February and May, 1965, in the mangrove lagoons near Port Royal. In the laboratory, the eggs, embryos, and young were extracted from the brood pouches and examined in Department of Agriculture type watch glasses using stereoscopic and compound microscopes under magnifications of 27 × and 100 ×. Undamaged specimens of early stages lived and developed well for 24 hours in small volumes of sea water, but later stages survived poorly, even with more water per individual. They fared better when ventilated by frequent movements of the water, accomplished by gentle currents from a medicine dropper.

Details of hatching could not be determined in embryos within the brood pouches because of the opacity of the pouch and the incessant movements of the females. In an attempt to ascertain the time necessary for the several steps of development in normal conditions, females were maintained by twos in ordinary finger bowls of sea water. They were removed periodically and examined intact in small quantities of water under a stereoscopic microscope at magnifications of 9 × and 27 ×. Only gross stages of development could be recognized under these conditions.

Observations

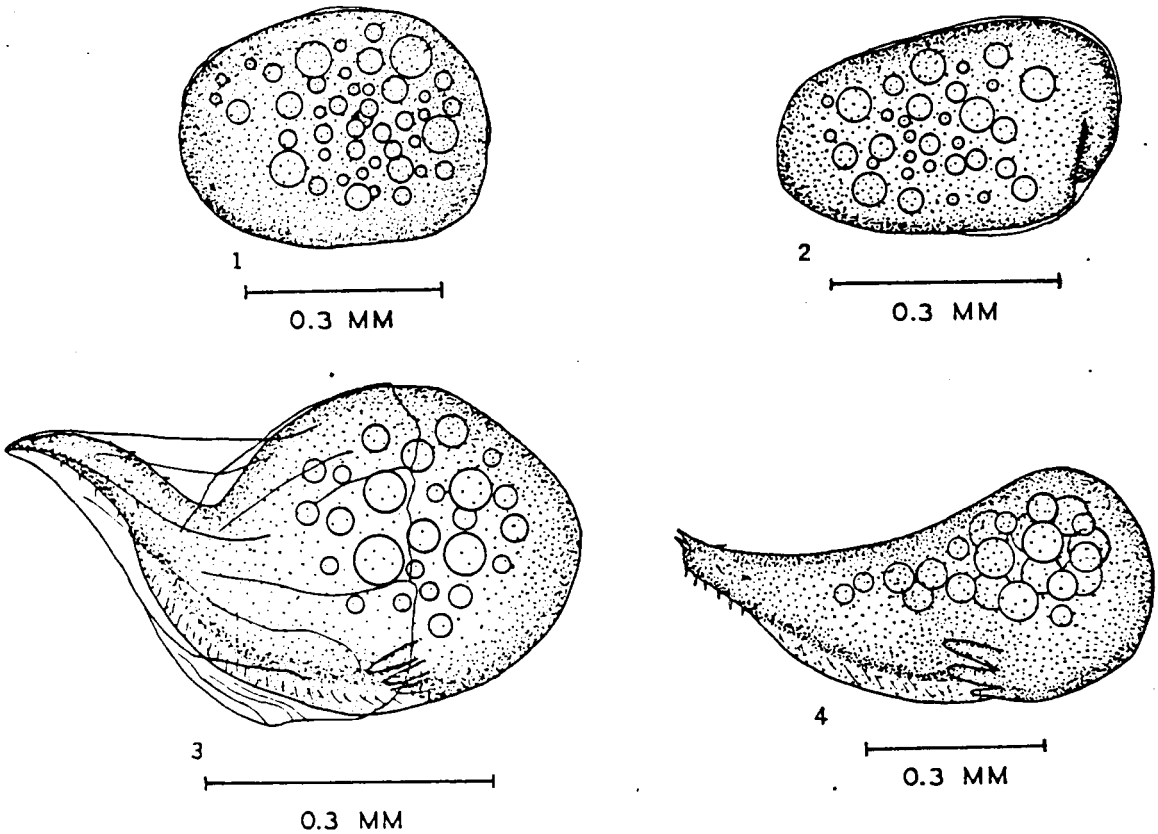
Oviposition occurred in the laboratory, but always at night, and was not observed. It was preceded, or accompanied, by ecdysis in the female. The actual egg-laying process could not be observed under field conditions, but in collections made from early morning to late afternoon, eggs, embryos, and young in the brood pouch, were encountered at all stages, from very early to near emergence. The earliest eggs were very delicate, and easily damaged or malformed during removal from the brood pouch. Within the intact pouch, however, and in most healthy specimens removed from the pouch, the eggs at this stage were somewhat oval; they contained a great number of large and small yolk spheres. A very thin outer membrane was apparent around the egg (Fig. 1), for the most part tight against the egg surface. In a few places, however, it was raised up in small blisters. The membrane was somewhat sticky, so that eggs tended slightly to adhere together, both within the brood pouch and in the observation dishes. The earliest eggs (an average of 10) measured 437μ × 372μ. They increased in size during development, evidently by absorption of water, becoming an average of 449μ × 375μ, at approximately which time the

outer membrane burst and sloughed off. The embryo, surrounded by a second, inner membrane, thereupon became rather more oval. At this time also the beginnings of the development of the abdomen could be clearly seen, folded rather tightly ventrally against the remainder of the body (Fig. 2).

Growth of the embryo then continued until an average size of 460μ × 378μ was attained. Previously, the embryo had remained completely without movement of its own, but at this time the small abdomen very gradually straightened until it bent dorsally. The entire embryo thus became "sway-backed." The combination of growth by absorption of water and the straightening of the abdomen resulted in breaking of the second membrane, always over the front of the head, and, gradually, the membrane was cast from the embryo (Fig. 3). Within the brood sac this process undoubtedly was aided by the ventilation currents produced in the sac by the mother, but it went to completion also in unventilated eggs in the laboratory.

It was not possible to determine exactly the time involved in the events described above, for eggs removed from the brood pouch lived poorly when kept unventilated for more than about 24 hours. The opacity of the brood pouch prevented *in situ* detection of the shedding of the first egg membrane, but emergence of the free larva could be ascertained due to the great difference of shape of the embryo occurring at that time. The eggs in most females isolated intact in finger bowls of sea water changed to the larval stage within 48 hours (few females were capable of living much longer than this in the artificial laboratory conditions). It is estimated that in nature, therefore, the egg stage persists from 48 to 72 hours. Of the several hundred eggs isolated from brood pouches, a number shed the first membrane during daylight hours, but only one shed the second membrane in the daytime. Large numbers, however, shed the second membrane during the night. It was not until observations were made after sundown that the actual process of hatching was observed, and then it was observed many times.

Immediately after emergence from the second egg membrane, three pairs of short, free, cephalic appendages were evident towards the anterior end. It was clear therefore that the larva corresponded to the nauplius of other crustaceans (the existence of such a naupliar stage had been recognized by previous investigators, particularly by Nusbaum, 1887; Manton, 1928; and Nair, 1939). Early rudiments of the thoracic appendages were indicated in the ventral region of the larva at this nauplius stage, and two small furcal spines were clear at the posterior end. There were several small hair-like setae ventrally on the abdomen (Fig. 4). No eyes were clearly developed, although the widest portion of the head lay at the position of the future development of the eyes. There were no further egg



FIGURES 1–4. Membrane shedding in *Mysidium*.—1, Early egg. Note that the outer egg membrane is raised very slightly above the egg surface as small blisters.—2, Egg some time after shedding of the outer egg membrane. The embryonic abdomen is folded ventrally down against the future cephalothoracic region. The second egg membrane exhibits small blister-like areas, as did the first.—3, Egg in the act of shedding the inner egg membrane. The ventrally flexed abdomen has bent dorsally, so that the dorsal surface of the emerging larva is concave. The membrane is being carried posteriorly off of the larva.—4, The nauplius stage after completion of hatching from the second egg membrane. The yolk mass is becoming concentrated towards the cephalic end of the larva, furcal rami are evident at the tip of the abdomen, and the three pairs of naupliar appendages lie ventrally towards the anterior end.

membranes, but the embryo was covered with a cuticle derived from the embryo itself—a first larval membrane. The newly-hatched nauplius averaged 589μ in length, and the head at its broadest region was 341μ wide.

After emergence, growth continued gradually until the length of body and greatest width of head became respectively 1,072μ and 369μ. By this time, the embryonic cuticle had become stretched; in parts of the body it was lifted well away from the surface of the embryo. The cephalic appendages had increased appreciably in length, and the yolk mass was much less diffused. The latter was lifted into a peculiar prominence over and just posterior to the head. The eyes were much better developed, and contained

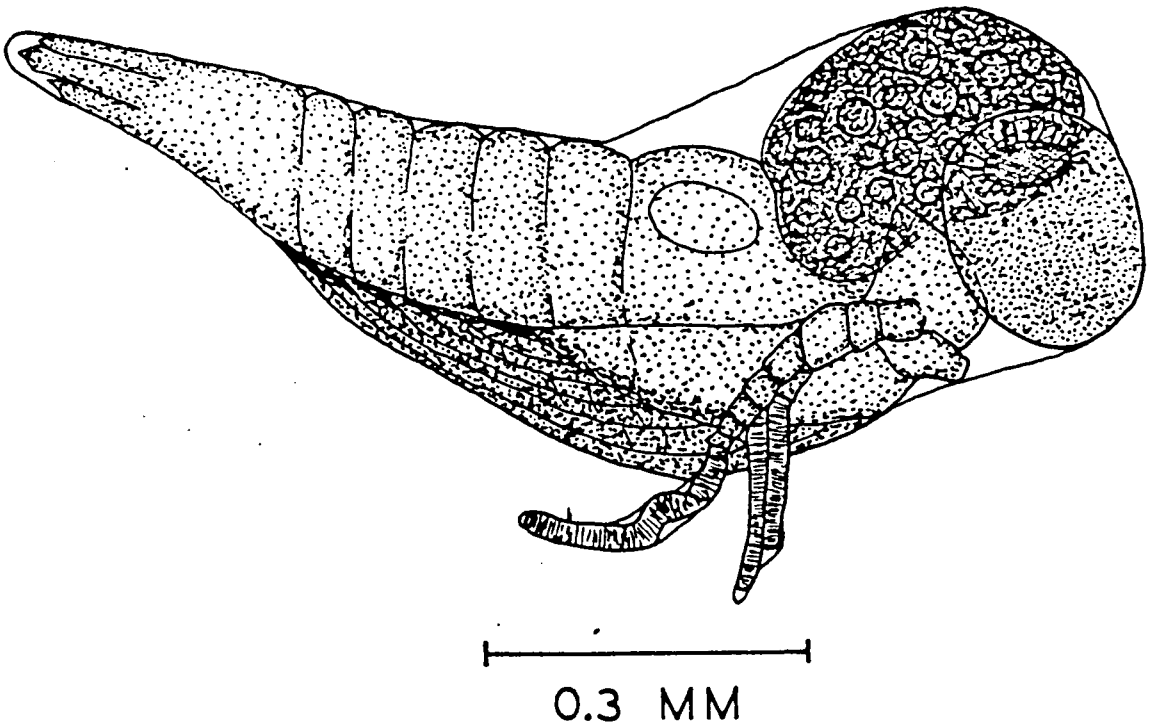


FIGURE 5. Late nauplius, shortly before ecdysis. Note that the yolk mass is highly concentrated in the head region, forming a high hump, over which the membrane will break during shedding. The naupliar appendages are much larger than earlier, and the thoracic appendages are well developed beneath the naupliar membrane. The large eyes are directed dorsally in the position they maintain during the remainder of the life of the larva within the brood pouch.

much more pigment than earlier, but they were pointed dorsally in the peculiar position that they maintained for some time later in development. The thoracic appendages were clearly developed beneath the cuticle, and exhibited occasional movements. The heart was beating regularly. Such a stage is shown in Figure 5. As ecdysis approached the embryo-larva increased its activity, mainly by moving the thoracic appendages and by stretching the tip of the abdomen and the front of the head dorsally to form even more of a sway-back condition.

Because of the considerable increase in length of the naupliar appendages, it was thought at first that there must be an ecdysis between the early and late naupliar stages, but an extensive search failed to uncover any definitive evidence for the existence of such a molt. A single specimen out of several hundred was seen to have short appendages on one side of the body and long appendages on the other. Therefore it is thought that no such ecdysis actually occurs. It is, however, not understood how the great elongation of the appendages and of the entire animal could come about without ecdysis, inasmuch as in the elongated animal and in the enlarged appendages, each is surrounded towards the end of naupliar existence by a rather loosely-fitting cuticle, as shown in Figure 5.

Naupliar ecdysis, which always occurred during the night or early morning, was associated with a movement whereby the tip of the abdomen and the head were moved repeatedly ventrally, thus greatly increasing the tension on the naupliar cuticle. Rupture of the cuticle was always observed to be over the anterior end of the dorsal yolk mass, towards the front of the head. Thereafter the cuticle was worked off posteriorly. Removal of the cuticle was slow in the isolated embryos in the laboratory. In nature, probably it was facilitated by the mother's ventilation movements when the embryos were in the brood pouch. This is suggested by the fact that it occurred much more rapidly upon gentle ventilation with a medicine dropper. After completion of ecdysis the thoracic legs were free. The larva now had much the appearance of the adult, except that the dorsal side remained distinctly concave, and the eyes remained pointing dorsally instead of laterally. Movements of the larva were limited, but much more extensive than in any previous stage.

From observations on isolated females in the laboratory, it is estimated that approximately 48 hours elapsed between first formation of the nauplius larva and the freeing of the thoracic legs by the ecdysis described in the paragraph above. Some uncertainty, however, was encountered in determining whether the ecdysis within the brood pouch had or had not taken place, so there is a degree of unreliability in the above time estimate. The young larvae remained in the brood pouch for an estimated 48 hours after freeing of the thoracic legs. During this period, movements of the animal were frequent, consisting mainly of a twitching of the legs, and of stretching movements in which the larva strained in the direction of straightening the still sway-backed dorsal region of the body. Final emergence, which always occurred at night, was associated with a larval ecdysis. This ecdysis resulted in freeing the eyes so that they took up their lateral adult position, and also resulted in a straightening of the back. In many instances, the mother animal also underwent ecdysis during the same night as the emergence of her young, but her ecdysis was not necessary for the liberation of the young from her brood pouch.

Discussion

The above observations suggest that removal of the first egg membrane in *Mysidium columbiae* was caused by intake of water and the consequent swelling of the embryo. The same process evidently was the main force in breaking the second membrane also, but removal was aided by a slow mechanical straightening of the embryonic abdomen. Subsequently, before emergence from the brood pouch, the embryo underwent two larval ecdyses, both of which were accomplished primarily by the struggles of the larva; in both instances, however, aid was derived from a continued increase in larval size. The entire period within the brood pouch appeared to be from 6 to 7 days, which is comparable to periods previously reported

for certain other species of mysids, as summarized by Zimmer (1933). Blegvad (1922), on the other hand, suggested somewhat longer incubation times of 14 to 25 days for mysids in Danish waters.

As far as can be ascertained, there are no reports that deal specifically with hatching in mysids. A few investigators, however, have mentioned in passing some of the phenomena of hatching, particularly in reports on mysid embryology. Among the earliest of these was the publication of Nusbaum (1887), who described and figured both external and internal structural features in the development of *Mysis chameleo* within the brood pouch. He mentioned both a membrane around the egg, and the straightening of the abdomen at about the time of shedding of this membrane; he then described and figured the embryonic or naupliar cuticle. In his figure, the most developed larva he studied was without this membrane, but there was no mention in the text of its loss. Nusbaum did not describe the increase of size of the embryo, nor anything about the methods of shedding the membranes.

Much later, Manton (1928) studied the embryology of *Hemimysis lamornae*. In this species she described properly the bursting of the (second) egg membrane primarily by pressure from within, caused by the absorption of water by the yolk, and secondarily by ". . . pressure exerted by the reflected caudal papilla." She also described the presence of the naupliar cuticle, and the fact that it "becomes widely separated from the body ventrally," but she said nothing about a first egg membrane, nor about the loss of the second membrane except that the embryos hatched from it. Furthermore, she did not mention the final molt at the time of emergence from the brood pouch, nor did she make a statement regarding the mechanism of shedding of the naupliar cuticle.

Finally, Nair (1939) studied the development of *Mesopodopsis orientalis*. As in Manton's description (*op. cit.*), he mentioned the bursting of an egg membrane by pressure from within caused by absorption of water into the yolk, and he figured a naupliar cuticle raised from the body; he did not, however, observe a first egg membrane, nor did he mention clearly the loss of the naupliar membrane, for he merely stated that there was a larval ecdysis after the young were dropped from the brood pouch of the female. It is not clear in his account whether he is referring to the loss of the first or of the second larval cuticle.

Sumario

Un Estudio del Proceso de Gestación en Invertebrados Acuáticos, XXII. Desprendimiento de Membrana Múltiple en *Mysidium columbiae* (Zimmer) (Crustacea: Mysidacea)

La eclosión del *Mysidium columbiae* fué investigada en Port Royal, Jamaica (W.I.). Los huevos en la bolsa de gestación de la madre pierden

dos membranas. De éstas, la primera se rompe y se muda enteramente a causa de una presión generada por una absorción de agua por el embrión. La segunda membrana del huevo se rompe y se pierde en primer lugar por el mismo mecanismo, pero también por un desarrollo del abdomen fuera de su posición embriónica. La pérdida de la segunda membrana, da como resultado la formación de un nauplius, con tres patas larvales libre. Más tarde la cutícula naupliar se levanta desde el cuerpo y forma una membrana larval. Ésta se estira a raiz de un crecimiento adicional de la larva, pero se rompe y pierde principalmente por movimientos mecánicos de la larva. El rompimiento ocurre sobre la masa de vitelo en la cabeza. La larva que aparece tiene patas torácicas como en el adulto, pero éstas no pueden moverle mucho, y los ojos larvales se extienden dorsalmente en lugar de lateralmente. La muda final ocurre asociada con la liberación de los jóvenes desde la bolsa de gestación. Se ha estimado que hay entre 48 y 72 horas entre oviposición y la muda de la segunda membrana del huevo, 48 horas entre esta muda y la muda de la membrana naupliar, y otras 48 horas entre la muda de la membrana naupliar y la salida desde la bolsa de gestación. Por lo tanto los jóvenes persisten durante seis o siete días en la bolsa de gestación.

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