自然教育園報告(Rept. Inst. Nat. Stu.)
第22号：55—62，1991

アゴトゲヨコエビの胚発生と交尾前行動

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Embryonic Development and Precopulatory Behavior of *Jesogammarus spinopalpus* (Amphipoda)

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ABSTRACT

Embryonic development, precopulatory behavior and precopula duration of *Jesogammarus spinopalpus* were observed under laboratory and field conditions. Five stages were recognized during the process of embryonic development until hatching ; Stage 1 : total and surface cleavages occur, Stage 2 : ectoderm appears, Stage 3 : a ventral cleft and appendage rudiments appear, Stage 4 : segmentation proceeds, and Stage 5 : eye pigmentation and the heart beating occur. The duration of each stage was measured at 6°C. The mean intermoult period and precopula duration were 28.1 days and 6.1 days at 6°C, and 43.4 days and 8.9 days at 10°C, respectively. Thus the relative precopula duration (RPD), the ratio of precopula duration to the intermoult period, was 0.22 and 0.21 at respective temperatures in the laboratory, where most males caught females carrying the embryos at Stage 5 to guard for subsequent copulation. In the field population, males preferred to females approaching to subsequent oviposition, with the RPD nearly equal to that in the laboratory.

INTRODUCTION

The life history of *Jesogammarus spinopalpus* inhabiting waters in the Institute for Nature Study has been described previously by Kusano et al. (1987). This species shows an annual life cycle. During the breeding season from winter to spring, the female deposits about three clutches of eggs. The body length is 10-12 mm and 9-11 mm for breeding males and females, respectively. During the breeding season, precopula pairs and ovigerous females appear. That is, the male guards the female before copulation for some days, and the female incubates her eggs until hatching.

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In the present study, the process of embryonic development, precopulatory behavior and precopula duration were observed under laboratory and field conditions.

METHODS

Laboratory Observation

All animals were collected from the western ditch (current speed <15 cm/s) under the aquatic plants garden in the Institute for Nature Study, Tokyo. Immature animals collected in November 1987 were reared at 10°C (11L : 13D). When they matured and formed precopula pairs, each pair was isolated in a small plastic vessel containing 50 ml of ground water. They were fed on decaying leaves and an artificial diet for Malacostraca, and the water was renewed once a week. For each pair, the intermoult period and precopula duration were recorded. Precopula pairs collected in February 1990 were reared at 6°C (8L : 16D) in the same manner as those at 10°C. For each pair, after the female had deposited eggs into her brood pouch, several eggs were raked out occasionally following anesthetization with bubbling CO_2 gas. The eggs were isolated in other Petri dishes for continuous observation of embryonic development. They were washed by pipetting in fresh ground water every day or every other day.

Field Survey

Breeding animals were collected randomly from the western ditch by scooping with a hand net on 12 January, 21 February and 20 April, 1990. They were fixed in 70% ethanol at the site, and the developmental stage of embryos carried by ovigerous females was examined in the laboratory. For non-ovigerous females, the presence of marginal setae on oostegites was examined, a feature which indicates previous oviposition.

RESULTS AND DISCUSSION

1. Embryonic Development

The process of embryonic development can be divided into five stages consisting of two or three substages (e : early, m :middle, l :late).

Stage 1e : Just after oviposition, a clutch of eggs is packed in two egg sacs within the brood pouch. The eggs in the sacs are fragile and have a variable shape. Before cell division, they become more stiff and emerge from the sacs (Fig. 1-1e).

Stage 1l : Total and surface cleavages occur, while all cells contain yolk (Fig. 1-1l).

Stage 2e : A few yolkless cells appear on the surface (Fig. 1-2e).

Stage 2l : The yolkless cells increase to form the dorsal organ rudiment and other parts of the ectoderm (Fig. 1-2l).

Stage 3e : A ventral cleft and appendage rudiments appear. The antenna rudiments are located

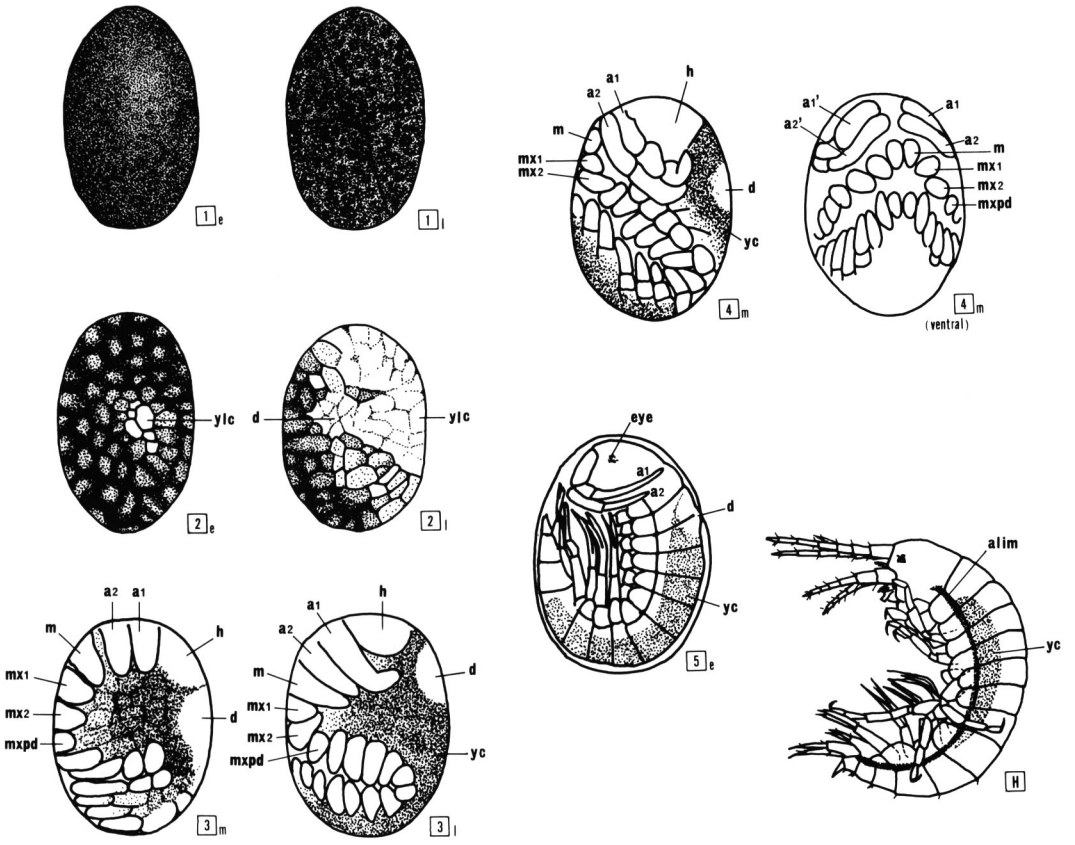

Figure 1. The process of embryonic development of *J. spinopalpus*. Stage 1 : total and surface cleavage occur. Stage 2 : yolkless cells appear and form a dorsal organ. Stage 3 : a ventral cleft and appendage rudiments appear. Stage 4 : appendages and a body become segmented. Stage 5 : eyes become pigmented and the heart begins to beat. Stage H : a juvenile hatched in a brood pouch. The abbreviations are as follows ; ylc : yolkless cells, d : dorsal organ, a1 and a2 : the first and second antennae, h : head, m : mandible, mx1 and mx2 : the first and second maxillae, mxpd : maxilliped, yc : yolkful cells, alim : alimentary canal.

on the dorsal side.

Stage 3m : The antenna rudiments move to the top of the embryo, and a head region becomes clear (Fig. 1-3m).

Stage 3l : All appendage rudiments move to the ventral side and the head is located at the top (Fig. 1-3l).

Stage 4e : The appendages become segmented.

Stage 4m : Coxal plates appear, and segmentation proceeds to cover the whole body (Fig. 1-4m).

Stage 4l : Non-pigmented eye rudiments appear.

Stage 5e : The eyes become pigmented (Fig. 1-5e).

Stage 5l: The heart begins to beat. The embryo sometimes moves within the egg membrane. Embryos isolated before Stage 2 showed hardly any further development by themselves, whereas after Stage 3, most embryos developed and hatched out in isolation. Hatched juveniles, with a body length of 1.2 mm, stayed for a while in the brood pouch (Fig. 1-H). They began to eat in the brood pouch, and were released from the pouch before their first moult.

In *J. spinopalpus*, no larval stage was recognized before hatching, and juveniles that hatched out were fully developed. The dorsal organ is considered to function as a respiratory organ in embryos of an isopod, *Asellus* (Weygoldt, 1960 after Anderson, 1982). *J. spinopalpus* also developed the dorsal organ in the early stage, and this was in contact with the egg membrane until just before hatching. These characteristics during embryonic development are similar to those of other amphipods (Weygoldt, 1958 after Anderson, 1982; Sheader & Chia, 1970).

The embryos isolated at early stages were prone to be attacked by bacteria and fungi, and required continuous washing with fresh water. This indicates the possibility that ovigerous females take care of their embryos. The water current produced by the pleopods of mother females would thus have a beneficial effect on embryonic development.

2. Duration of Each Stage, the Intermoult Period and Precopula Duration

For females, the intermoult period is equivalent to the clutch interval, since oviposition occurs just after moulting. The reproductive stages of females between ovipositions were determined according to the developmental stage of embryos in their brood pouches. The stages for females incubating juveniles and for non-ovigerous females were defined as Stages H and O, respectively.

The intermoult period was 28.1 days (n=22, SE 0.5) at 10°C. Precopula duration was 6.1 days (n=22, SE 0.6). Thus the relative precopula duration (RPD), the ratio of precopula duration to the intermoult period, was 0.22 on average. Most females were caught by males at Stage 5, forming precopula pairs for subsequent oviposition. The intermoult period at 6°C was 43.4 days, and the absolute and relative durations of each stage are shown in Table 1. The durations of Stages 1 and

Table 1. Mean duration(±SE) of each reproductive stage at 6°C. Relative duration is the ratio to the intermoult period.

Stage	n	duration(days)	relative duration
1+2	10	14.9±0.2	0.34
3	10	4.9±0.2	0.11
4	10	10.2±0.2	0.24
5	10	6.0±0.3	0.17
H+O		nd	0.17
The intermoult period			
	8	43.4±0.9	1.00

nd: Not determined.

2 were measured in total, since embryos at Stage 1 hardly developed by themselves. The durations of Stages H and O could not be determined for females whose eggs had been raked out. Precopula duration was 8.9 days (n=7, SE 2.0), and thus the RPD was 0.21 on average neary equal to the value at 10°C.

Sheader & Chia (1970) reported the duration of each stage during the embryonic development of an amphipod, *Marinogammarus obtusatus*. The relative duration of each stage for *J. spinopalpus* and *M. obtusatus* before hatching was nearly equal until Stage 3. However, Stage 4 for *J. spinopalpus* was notably longer than that for *M. obtusatus*, whereas the opposite was true for Stage 5. This indicates that the early process of development may be similar between the two species, whereas features of the late stage, for example, the timing of eye-pigmentation, may differ. Moreover, the period from hatching to subsequent oviposition in *M. obtusatus* was notably longer than that for *J. spinopalpus*. Thus the intermoult period might be regulated by factors independent of embryonic development time.

3. Mating Behavior

A male in precopula was observed to hook the fourth and fifth coxal plates of a female with the dactyli of his first gnathopods (Fig. 2). The coxal plates grasped by the male were on either the right or the left side of the female. In a plastic vessel, the male was easily able to catch the female. Precopulatory males were hardly able to pick up granules of artificial diet to eat, since the first gnathopods were thus occupied. The male copulated with the captured female within one day

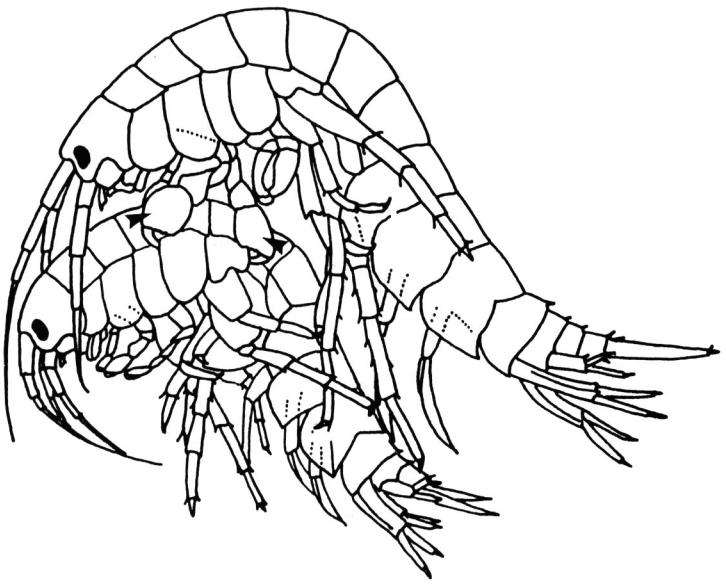

Figure 2. A precopula pair of *J. spinopalpus*. Arrows indicate the first gnathopods of the male.

after her moult. Since the position on the female's body where the male had hooked his dactyli rifted when the female moulted, the male was able to maintain his grip during the moulting. Copulation was completed within a few minutes, and then the male left the female. The female deposited a clutch of eggs in her brood pouch within 30 min after separation.

4. Relationship between Female Stages and Precopula Formation in the Field

The number of males decreased earlier than that of females during the breeding season. The sex ratio (male/female) was 0.884, 0.534, and 0.270 on 12 January, 21 February and 20 April, respectively, becoming significantly lower than one after February (χ^2 test, $P < 0.01$ and < 0.001 in February and April, respectively). Figure 3 illustrates the frequency of total and paired females at each stage in the field. All females at Stage O were virgin on 12 January, as shown by the lack of marginal setae on the oostegites. This indicated that reproduction began in late December. On the other hand, females at Stage O after February were all spent ones, having undergone oviposition. The ratio of paired to total females was 34.9%, 43.1% and 15.7% in January, February and April, respectively. Figure 3 indicates that males were likely to choose and guard females at stages later than Stage 4.

The tendency for males to choose females at later stages is in agreement with some reports for other amphipods (Hartnoll & Smith, 1978 ; Hunte et al., 1985). However, the stage at which females were chosen by the males appeared to be influenced by the frequency distribution of females. When the frequency of females at Stage O was low (i.e, in February), the males were likely to mate with females at earlier stages, whereas paired females were mostly restricted to within Stage O when the frequency at this stage was relatively high (i.e, in January and April). One further reason why the males mated with only Stage O females in April would have been the low sex ratio, paired females forming only a small part of the total population.

From Fig. 3, the RPD in the field was expected to be nearly equal to or slightly less than 0.2, which was observed in experimental pairs. If the precopulatory males can not forage thoroughly, as was observed in the laboratory, they must do so while they are free. This implies that availability of food resources influences the male's precopulatory behavior, i.e., the timing of precopula formation.

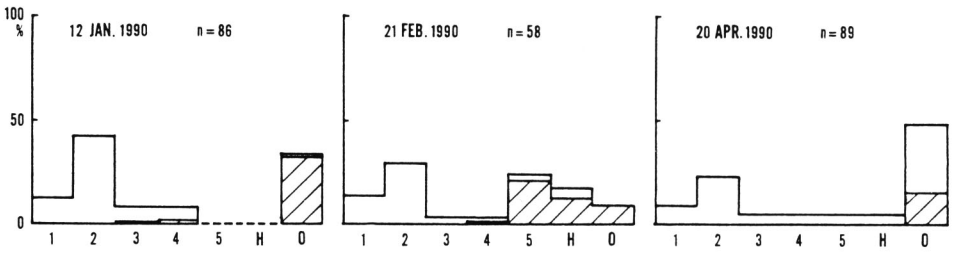

Figure 3. Frequency distribution of total and paired females in respective stages at each collection. Open and hatched areas show single and paired females, respectively.

ACKNOWLEDGEMENT

My thank is due to Mr. N. Hisai of the Institute for Nature Study, for his kind assistance in the field survey.

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摘　　要

1．アゴトゲヨコエビの胚発生：産卵直後の卵をペトリ皿に移して胚の発生過程を観察した。孵化までの発生過程は大きく５つのステージに区分できた。それぞれの発生段階は，次の様な現象で特徴づけられた。ステージ１：全割，表割が起こる，２：背器などの外胚葉を生ずる，３：付属肢の原基ができる，４：体節が生じる，５：眼に色素が沈着し，心臓の鼓動が始まる。また，水温６℃で各ステージに要する日数を測定した。

2．交尾前行動の観察：番いを隔離して飼育し，交尾前ペア形式から交尾，産卵までの行動を観察した。また，各番いについて産卵間隔と交尾前ペア期間を測定した。水温10℃での産卵間隔と交尾前期間は28.1日（n＝22，SE 0.5），6.1日（n＝22，SE 0.6）であり，相対交尾前期間（交尾前期間／産卵間隔）は0.22であった。水温６℃では，それぞれ43.4日（n＝８，SE0.9），8.9日（n＝７，SE 2.0）であり，相対交尾前期間は0.21であった。いずれの場合も，雌はステージ５の胚を抱いている時に次の産卵のための交尾前ペアになることが多かった。

3．自然教育園の水生植物教材園から流れる小川でアゴトゲヨコエビをランダムに採集し，雌が抱いている胚の発生段階を調べた。その結果，育卵嚢が空になっている雌が最も交尾前ペアになっている頻度が高く，ステージ3以前の胚を抱いている雌は全くペアになっていなかった。従って，野外の個体群でも雄は次の産卵が間近な雌を選択して抱接していると考えられた。野外での相対交尾前期間は，室内で得られた値とほぼ同じ程度であると推測された。