

NOTES ON THE BREEDING BEHAVIOR OF EUBRANCHIPUS HOLMANI (RYDER)¹

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INTRODUCTION

The breeding behavior of the branchiopod Anostraca has received little mention, especially that of species occurring in the New World. Except for the cosmopolitan *Artemia* (which is atypical in many respects) there is virtually no literature on the subject other than some notes by Gissler relating principally to *Eubbranchipus vernalis* (Verrill) (see Packard, 1883), the more detailed observations of Pearse (1912) on *Eubbranchipus serratus* Forbes, and a few notes by Johansen (1922) on the Arctic species, *Artemiopsis stefanssoni* Johansen. A useful review of the subject which deals with the European literature has been published by Mathias (1937) and includes a summary of his own important observations on *Branchipus stagnalis* (L.) and *Chirocephalus daphanus* Prévost.

In the course of studies by the junior author dealing with thermal thresholds in *Eubbranchipus holmani* (Ryder) certain stimuli were evidently produced which initiated a breeding response in the shrimp. The initial studies, based on 5 series of observations by Ogren in 1960, were supplemented by 10 additional series by the senior author in 1961. The following descriptions are a summary of the activities noted by both observers.

CONDITIONS OF STUDY

E. holmani is a stenothermal species occurring only during the winter months in certain heavily-shaded temporary ponds on the pine flatlands of southeastern St. Tammany Parish, Louisiana. Field collected specimens were brought to the laboratory in plastic buckets of habitat water and stored in a Precision Low Temperature Incubator at 12-13°C (approximately the habitat temperature at time of collection) until used, a period of a few hours to no more than 6 days. In 1961 some observations were made

on shrimp which had been segregated as to sex immediately upon return to the laboratory and stored in glass battery jars under similar conditions. Observations were carried out under conditions of normal room illumination on specimens in the stock buckets, in 2 liter battery jars, and (when photographs were being made) under the light of 2 No. 1 Photoflood bulbs in small, flat-sided museum jars. Temperatures were determined by a recently calibrated mercury thermometer; intervals were timed with a stopwatch.

OBSERVATIONS

When brought from the constant temperature box at 12-13°C shrimp of both sexes swam at random in the usual inverted position, with the horizontal axis of the body approximately parallel to the water surface except when they were moving from one depth to another. Under normal circumstances, occasional bodily contacts with one another were followed by a quick jerk of the abdomen and a rapid change of swimming direction by one or both shrimp.

A deviation in this normal swimming pattern was usually shown when the water temperature reached 13.5°C and it continued sporadically until the water had warmed to about 19°C. Above this temperature normal random swimming was again the rule. The first indication of sexual receptivity occurred in certain females which would assume a more or less stationary position at any depth, with the long axis of the body inclined at an angle of 35° to 45° with respect to the water surface. Such females would maintain this position for some time during which a male might approach from the rear and below, with his head at about her mid-body region. The pair would hold their positions, the male following any changes in the course of the female, for 1-5 seconds (see Figure 1).

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One instance of a pair holding their positions for 16 seconds was recorded, during which the male followed several turns by the female. This "station taking" by the male, as it will be referred to hereafter, was preliminary to one of several courses of action:

1. The male would abruptly break off and turn aside (see Figure 2).
2. The male would attempt to seize the female about her thorax just anterior to the egg sac with his sickle-shaped II antennae. On occasion this would be followed by copulation. Frequently the female would avoid the clasp attempt and both she and the pursuing male would swim in tight vertical circles for a short period (see Figure 3). Again, the male might succeed in clasp ing an unresponsive female and a short, violent struggle would ensue while she freed herself. The duration of these pre-

copulatory struggles rarely exceeded 1-3 seconds.

3. In cases where copulation took place the responsive female did not struggle after being clasped but continued to swim slowly about carrying the male. His II antennae were firmly clasped about her thorax just anterior to the ovisac; his long antennal appendages, normally carried coiled corkscrew-fashion, were laid out along the female's back.

Contact of the genitalia was effected by the male's efforts. He would twist his posterior thorax and abdomen over and around the side of the female's body in such a fashion as to bring their genitalia in contact. The precise alignment and introduction of the male's intromittant organ into the opening of the female's ovisac was not observed. His position in amplexus was similar to that shown in Figure 4, with his

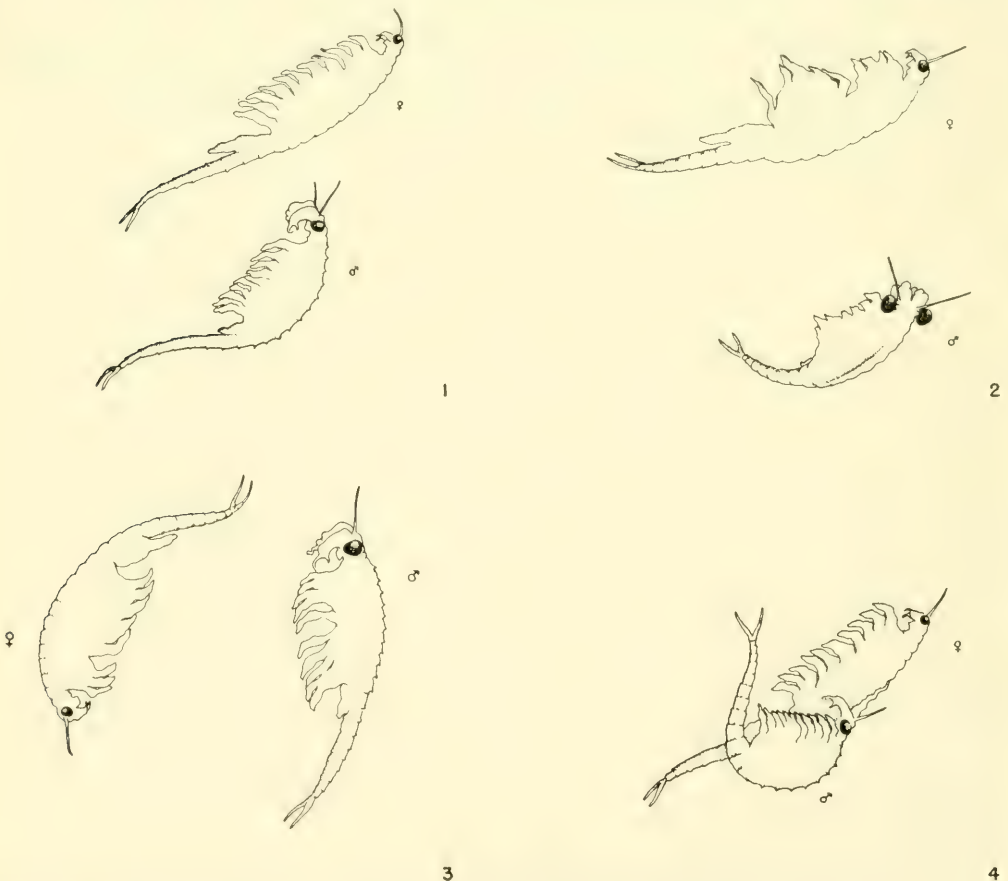


Figure 1-4. Breeding behavior of *Eubranchipus holmani*. For details see text.

anterior body nearly parallel, and his posterior thorax and abdomen carried vertically and at right angles, to the long axis of the female's body. In two cases in which copulation actually took place the duration of the act was timed at 1 minute, 10 seconds and 2 minutes, 10 seconds respectively, both preceded by a preliminary period of clasping lasting as long as 10 seconds. During copulation the female swam slowly about carrying the male, in the first case just above the bottom of the container, in the latter case near the surface. At the termination of the act a brief struggle of 2-3 seconds duration took place as the male disengaged his intromittant organ. It was not determined whether both, or only one, of the penes were involved in the contact. In a third instance, in which the male experienced difficulty in introducing his penes, the pair lay on the bottom in amplexus for 1 minute, 25 seconds before terminating an unsuccessful copulatory attempt.

Unfortunately no females involved in successful copulations were recovered for study. However four females which had been clasped for 3 to 5 seconds before they struggled free were captured and examined. Two of these had white, unshelled eggs in the lateral oviductal pouches, the third carried brown, shelled eggs in the median ovisac, and the fourth had an empty ovisac from which shelled eggs had recently been discharged. Presumably only the first two individuals were carrying eggs capable of being fertilized.

Certain instances of aberrant behavior were noted, one of the more interesting being the preliminary "station taking" of one male below a second (see Figure 1). As many as three males were seen taking station on each other simultaneously. The participants usually broke off without attempting to clasp (Figure 2), but occasionally one male would attempt to clasp a second. In such cases the passive participant struggled free immediately.

The influence of light intensity on behavior was also notable. In the shaded basins, which constitute the natural habitat, light intensity is low and all aspects of breeding behavior observed in the laboratory (except some preliminary "station taking") occurred under conditions of limited and diffuse illumination. When Photoflood lights were switched on to permit pho-

tography all the shrimp responded by sinking to the bottom of the container. Preliminary courting behavior started in dim light usually terminated abruptly when the bright lights were switched on.

DISCUSSION

The breeding behavior of *E. holmani* conforms in general pattern to that reported for other Anostraca. Pearse (1912) did not record for *E. serratus* any activity resembling the preliminary "station taking" noted in the present instance. Mathias (1937), however, reported that in *Chirocephalus grubii* (Dybrowski) the male swims parallel to and under the female for a few seconds before attempting to clasp; he noted similar behavior in the case of *Branchipus stagnalis*. These two species are also reported as copulating in bright light, unlike *Chirocephalus diaphanus* in which copulation was observed (as in the present instance) to take place under dim illumination.

The period of clasping preceding copulation is usually only a few seconds duration but *Artemia* may clasp and swim in tandem for several hours or days, and *Branchipus stagnalis* is said to maintain union for as long as 60 minutes (Mathias, 1937, p.47). We have observed that the long antennal appendages of the male *E. holmani* are laid out along the female's back; this does not conform strictly to Gissler's statement (in Packard, 1883) "Having observed them often in copulation I can state . . . the frontal tentacles do not come into play as auxiliary organs". Actual sexual contact appears to be rather brief in all anostracans; Mathias (1937) reported it to be from a few seconds to as long as one minute in various Old World species. In the present instance, it will be recalled, sexual union of over 2 minutes duration was noted.

The position of the male during sexual contact is apparently somewhat more variable, a condition attributed by Mathias (1937) to the different lengths of the ovisac in various species. *E. holmani* resembles *Chirocephalus diaphanus* in this regard, both having ovisacs of intermediate length. The male's position has been observed to be somewhat different in species such as *Branchipus stagnalis* which has a very short ovisac, and the planes of symmetry of the anterior regions of the two conjugants are presumed to be almost parallel in the genus

Streptocephalus (which has a very long ovisac) although there does not appear to be any published observations relating to the latter genus.

CONCLUSIONS

The courtship-breeding behavior of *E. holmani* appeared to be a series-succession of stereotyped events. Full knowledge of the cues involved and the environmental conditions necessary for courtship to be initiated is incomplete. However the most obvious condition, aside from sexual maturity of the participants, was a gradual warming of the surrounding water. Breeding activity usually occurred when the temperature ranged from 13.5° to 19°C; outside this range little or no such activity took place.

The female seemed to play a nearly passive role in the preliminaries although she may have initiated a response in the male by the angled, nearly stationary posturing that was so frequently observed. This response of the male took the form of "station taking" from below and to the rear of the female. However the fact that such females frequently resisted clasping attempts suggested that such posturing did not necessarily indicate sexual receptivity.

It has been repeatedly observed (Packard, 1883; Mathias, 1937; Pearse, 1912) that only females with eggs in the lateral oviductal pouches accepted copulation, although the last-named noted several instances in *E. serratus* in which males attempted to copulate with females carrying shelled eggs in the median ovisac. Since the females which actually accepted males were not recovered in the present study no evidence on

this point can be provided. However clasping attempts on mature females in all stages of egg formation were observed indicating that highly stimulated males will attempt to clasp any female or even other males. The role of the female is positive to the extent that she accepts or rejects a male depending upon the condition of her eggs.

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ABSTRACT

Breeding activities of *Eubbranchipus holmani* are described and the environmental factors, especially water temperature, known to be associated with such activity, are reviewed. Various aspects of breeding behavior including preliminary positioning, clasping attempts, and successful and unsuccessful copulation are described and compared with published accounts of other species of Anostraca.