

Technical Note

RAPID IDENTIFICATION OF REPRODUCTIVE STATE AND THE RECEPTIVE PERIOD OF FEMALES IN POND POPULATIONS OF *MACROBRACHIUM ROSENBERGII* — A NEW TECHNIQUE

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ABSTRACT

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A technique for rapid identification of the reproductive state and the receptive period of *Macrobrachium rosenbergii* females was developed. Such a technique allows selection of those females which would go through a pre-mating molt within a period of 7 days. This technique is based on external examination of gonadal development of the female and on typical reproductive behavior, as displayed by the dominant males, which is directed towards receptive females only. Identification of the reproductive state and the receptive period of a female is useful for improving broodstock management, directed breeding, artificial insemination and for various other experimental purposes.

INTRODUCTION

Prediction of pre-mating molt in females

Fully developed ovaries of *M. rosenbergii* females lie dorsally to the stomach and the hepatopancreas, just behind the eyes and beneath the rostral crest, reaching the abdominal segment (Ling, 1969; Sandifer and Smith, 1979). During gonadal development, the ovaries grow from the posterior part of the cephalothorax, and the fully developed ovaries become easily identifiable through the carapace as large orange-colored masses, occupying a large portion of the cephalothorax, approximately from the heart to the base of the rostrum. Usually, once the gonads become fully mature, the female goes through a 'pre-mating molt' which is followed by egg laying and fertilization (Ling, 1969; personal observation).

In general, prediction of ecdysis in *M. rosenbergii* can be achieved by using Peebles' molt-staging technique (1977). However, prediction of the

female pre-mating molt can be done much faster and without physical stress to the prawns, by determining the state of gonadal development. By this method, a female population which is synchronized with respect to its molt cycle may be established.

Determination of female receptivity using a 'male detector'

A *M. rosenbergii* female stays in its receptive state, ready for mating, for a period of 3–6 h following the pre-mating ecdysis (Ling, 1969). Receptivity for artificial insemination (by the attachment of spermatophores to the female's abdomen) may last for 10–15 h after molting takes place (Chow, 1982). Due to this fairly restricted period of the receptive state, it is necessary to develop a means for identifying a single female which is nearing this state. To achieve this, we have adapted the 'male detector' method, commonly used in cattle husbandry, to the prawn population. In this method, a set of behavioral cues displayed by the male towards the receptive female can be easily detected.

A male population of *M. rosenbergii* may be divided into three main types, differing in certain morphological characteristics (Cohen et al., 1981) as well as in their behavior and functions within the population (Ra'anan, 1982; Sagi, 1984). The blue claw males (BC) are the largest dominant males, and are characterized by long, thick, dark blue claws. BC males are attracted to females which are nearing their pre-mating molt. The male tries to capture the female between its claws and protect her from other individuals, while cleaning the ventral portion of her thoracic shell with its other legs (Ling, 1969; Ra'anan, 1982). BC males become attracted to such females from a few hours before the pre-mating molt until mating takes place.

The 'male detector' system involves a BC male whose gonopores are blocked so that no spermatophores are released. This ensures that no fertilization by the 'male detector' occurs. The male's typical reproductive behavior remains unaffected, so that it can identify only those females which are in their receptive state.

METHODS

Females' mature ovaries

Fifty-three females with fully developed gonads (as illustrated in Fig. 1) were selected from a random sample of a prawn population which had been reared in a large earthen pond. The selection was performed by a rapid external examination of gonads through the carapace. Females were then kept individually in separate aquaria. All aquaria were held at a temperature of $26 \pm 1^\circ\text{C}$ and were equipped with built-in biofilters to ensure good water quality. Prawns were fed with fish pellets (20% protein), supplemented with chopped fresh fish fillets and live *Daphnia*. Each aquarium was checked

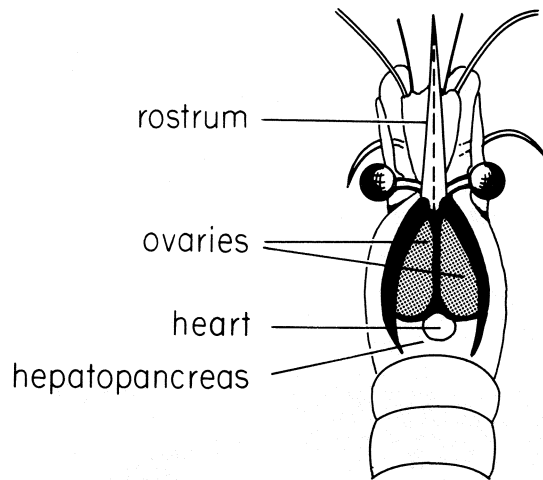


Fig. 1. Dorsal view of the head of a female: the location of the fully developed ovaries.

twice daily for exuviae, and the time between determination of the fully matured gonads and the actual ecdysis was recorded.

Male detector

The 'male detectors' were BC males selected from a pond-reared population. These males were observed in aquaria to ensure normal courting behavior prior to their selection as detectors.

Once a BC male was picked, its gonopores were sealed by the application of a drop of quick-set adhesive cyanoacrylate (commercial name: Super Glue), in order to prevent it from releasing its own spermatophore while courting. In order to ensure absolute sealing, each male was stimulated by an electric shock applied to the base of the gonopore bridge to examine spermatophore release. (Electric stimulation for male ejaculation is a common procedure in artificial insemination of prawns; Sandifer and Smith, 1979.)

Blocked BC males were then marked by a colored plastic tag (1 cm × 1 cm) attached to the carapace, using the same glue. The main reasons for tagging these males were to make them conspicuous when placed together with other prawns, and to make their molting easily detectable so that the gonopore may be resealed. Neither sealing nor tagging affected the courting behavior of the treated BC males. Generally, a male treated in this way serves as a detector for from 2 weeks to over 2 months. Once a receptive female is detected and removed, the male is capable of immediate response towards another receptive female.

Three such male detectors were then placed in three separate 100-l glass aquaria. Females with fully developed gonads were added to each aquarium

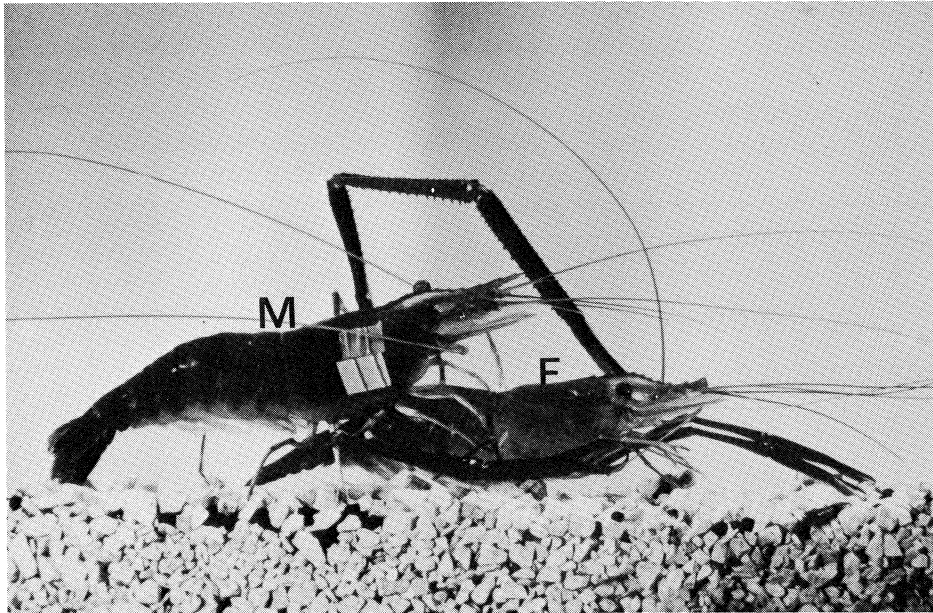


Fig. 2. Typical courting behavior: a blue claw male detector (M) holding a receptive female (F).

at a ratio of 7–10 females per male at all times. All aquaria were kept under environmental conditions similar to those described above. The aquaria were checked twice daily for exuviae, and when found, the female with which the male detector was interacting (Fig. 2) was removed and replaced by a new female.

Altogether 37 such females were selected, all newly molted. At this stage, these females were artificially inseminated in order to examine receptivity to fertilization, and were then kept in separate aquaria. Embryonic development was verified by a microscopic examination of the eggs 15 days after insemination, and was the ultimate criterion for female receptivity at the time of identification by the male detector.

RESULTS AND DISCUSSION

Prediction of molting of females by gonadal development

All females with fully developed gonads were also examined according to Peebles' molt-staging technique (1977). They were all included within stages D_3' to D_3''' , thus predicting 5–8 days to ecdysis. Table 1 shows the observed molting event for each female. Forty-six of the 53 females (86.7%) had molted within 7 days, with an average duration of 5.3 days after selection by gonadal development. These results show that molt prediction by

TABLE 1

Prediction of pre-mating molt by gonadal development

Time (days)	1	2	3	4	5	6	7	8	9	10	11	12	25	Total
Females (number)	1	6	9	8	12	6	4	2	2	0	0	2	1	53
Frequency (%)	1.9	11.3	17.0	15.1	22.6	11.3	7.5	3.8	3.8			3.8	1.9	100

←————— 86.7% —————→

gonadal development in *M. rosenbergii* females can be as accurate as that achieved by the molt-staging technique. While Peebles' method involves the removal of a pleopod and a microscopical examination for each individual, our system requires only a few seconds of external examination, and is therefore particularly suitable for working under field conditions and for the rapid selection of large numbers of such females.

The advantages of applying this method lie mainly in the ability to synchronize a female population for purposes such as broodstock management, where the degree of synchronization in egg laying and hatching has significant economic merit (Ra'anan and Cohen, 1982).

Selection of sexually receptive females using a male detector

The benefit of using a male detector depends on its ability (a) to indicate a newly molted female in a group, thus making it unnecessary to examine all females individually, or alternatively keeping them in separate compartments, and (b) to indicate only those newly molted females which are still within the period of receptivity to fertilization.

In our study we have found that all of the 37 females which were indicated by the male detectors were indeed those which had just molted. Whenever an exuvia was found in an aquarium (which contained up to ten females) selection of the newly molted female was immediate, with no need for handling the other females in the same tank. In some cases we observed an interaction of the male detector with a female before any molt was noticed. In these cases the females would go through their pre-mating molt within a period of 24 h. These preliminary observations indicate that the male detector can identify a female which is close to being receptive, and is starting to display its typical courting behavior (Ra'anan and Cohen, 1985).

Individual follow-up of each of these females after artificial insemination revealed that in 34 of the 37 cases (91.9%) normal embryonic development occurred, proving that the females indicated by the male detector were indeed in their receptive period following their pre-mating molt. The failure

of fertilization in three cases may be attributed either to the fact that artificial insemination is not an absolutely reliable method, or to a possible 10% error in identification by the male detector. Studies involving artificial insemination in *M. rosenbergii* (Sandifer and Smith, 1979; Chow, 1982) report less than 100% success in the application of this technique, possibly due to a misidentification of the receptive females or faults in technique.

The common system applied for the selection of receptive females (Malecha, personal communication, 1981) involves their maintenance in separate compartments and the twice or thrice daily examination of each cell to identify molts. Then, once a newly molted female is found, it is still necessary to verify whether it is in the pre-mating molt, which is essential for successful fertilization.

It should be noted that not all molting events are followed by a receptive period. Some molts are probably associated with somatic growth and not necessarily with reproduction, even after a female has become sexually mature (personal observations). Therefore an individual follow-up of females for identification of receptivity is time and space consuming and might become a serious limiting factor in designing large-scale directed breeding experiments for genetic studies (Malecha et al., 1984), and for the understanding of mating behavior (Sagi, 1984).

The combination of both techniques examined in this study, i.e. selection of females according to the state of gonad development and the introduction of a male detector into the system, enables investigators to upscale their experimental systems while saving much time, space and effort and at the same time improving the accuracy of female selection.

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