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An Analysis of Sufficient Stimuli for the Oviposition in the Medaka *Oryzias latipes*

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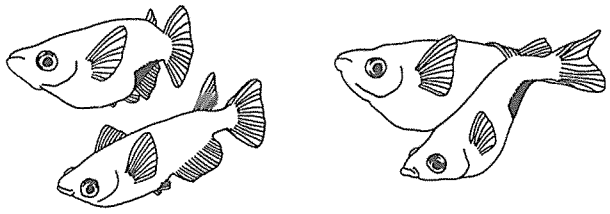
Abstract Specific stimuli required for initiating oviposition of the spawning medaka and sufficient duration of the stimuli were examined by means of measuring time durations of selected stages of the sequential spawning behaviour of the medaka. The female medaka oviposits the eggs after receiving tactile stimuli applied by the male as a vigorous body vibration, quivering, for a certain period (8.7±3.2 sec, mean±SD). By a forced separation of spawning pairs, it was determined that the sufficient duration of the stimulation for initiation of oviposition is longer than 4 sec. After the separation it was often observed that a fully stimulated female made oviposition whether willing or not. The automatism of the ovarian contraction indicates that oviposition of the medaka is a reflex mediated by the autonomic nervous system. The excitatory ovarian nerve is speculated to be emerged from the spinal cord at the level near the 10th vertebrae.

INTRODUCTION

The ovary of the medaka, *Oryzias latipes*, is an unpaired, hollow organ covered with a muscular ovarian wall which is connected with a short, muscular oviduct (ROBINSON and RUGH, 1943; YAMAMOTO, 1963; TAKANO, 1968; HOSOKAWA and NAMBU, 1971). It has been suggested for cyprinodontids, *Oryzias* (ROBINSON and RUGH, 1943) and *Fundulus* (MATTHEWS, 1938; BRUMMETT *et al.*, 1982) that these muscular structures might serve for ovulation and oviposition through facilitation both of follicular rupture and egg transport.

The medaka spawns in almost every morning during the spawning season. According to its mating ethogram described by ONO and UEMATSU (1957), the mating behaviour progresses as in the following order; courting orientation (male), head-up I (female), courting round dance (male), head-up II (female), floating-up (male), crossing (male), copulation (male), ejaculation (male), spawning (female), fertilization and separating. However, accurate measurement of time allocation through the behaviour has not yet been carried out. In connection, I think "embracement" is a more suitable term than "crossing" to express the posture of a spawning pair of the medaka, because a male tightly embraces a female with his dorsal and anal fins (Fig. 1). On this reason, I will use a term "embracement" or "embracing" instead of "crossing" below. A male who is embracing a female begins a spasmodic body vibration after a while, leading to oviposition. I will also use a term "quivering" for this behaviour.



a b

Fig. 1. Spawning posture of the medaka. a)After a courtship behaviour, the male (lower) approaches the female ventrolaterally with pectoral fin fanning and expansion of the anal and dorsal fins. b)If the female does not represent a head-up display, the male embraces the caudal portion of her body with his anal and dorsal fins. After a while, he starts quivering his body. This embracement lasts until about 10 sec after completion of oviposition.

EGAMI and NAMBU (1961) persuited factors initiating oviposition of the medaka by means of removal of skin or fins and spinal transection, and have concluded that the body vibration of a quivering male worked as the mechanical stimuli to elicit oviposition in the female and that command impulses stimulated a secretion of neurohypophyseal hormones. However, they carried out no actual observation of the spawning behaviour of the operated fish and instead they only compared the egg numbers laid by the fish several hours after completion of the behaviour. In addition, they operated fish on the previous day of the experiment. Thus, this problem might exert possible mal effects on the reproductive rhythm of the female fish, leading to failure in ovulation. Furthermore they did not check after experiments whether or not females had been ovulated.

In the present study, I conducted a careful observation of the spawning behaviour of the medaka and measured the time required for some contexts of the behaviour. Then, the effects of spinal transection on oviposition were investigated to verify the involvement of the spinal cord in ovarian contraction.

MATERIALS AND METHODS

Fish

The red variety of the medaka *Oryzias latipes* were perchased from a local pet shop and reared in pair in 1 litre glass beakers. They were being subjected to the experiments in July which corresponds to the middle of the natural spawning season in Japan, under natural day length and temperature. In every morning it was checked for each pair whether or not they spawned. Fish were fed *Tubifex* sp. after the check.

Females, who had been proved to spawn every days for 10 or more days, were used as subjects of the experiments. The female, isolated from a male on the previous day, would be ovulated at the morning of experiments.

Experiment 1.

Time Mesurement of Some Selected Behavioural Sequences of the Spawning Medaka.

A female was introduced into a glass observation aquarium (measuring 15 cm in length and 10 cm in width, water depth is about 10 cm), which had been occupied by a male beforehand. Time durations of selected behaviour of the spawning pairs were measured with a stop-watch. They were embracement, quivering and oviposition. Twenty-two pairs were used in total.

Experiment 2.

Measurement of a Male-Female Contacting Time Sufficient for the Initiation of Oviposition.

The female and male were mated as in the experiment 1. An embracing male was forced to separate from a mate female by being startled by tapping a wall of the observation aquarium. Both durations from onset of the embracement and the quivering to the separation were measured. Twenty-nine females were used in total and 41 times of measurements were done.

Experiment 3.

Effects of the Spinal Transection on Oviposition.

A female, whose spinal cord was transected the day before the experiments, was introduced into an aquarium which had been occupied by a male beforehand. The spinal transection was done as follows. After anaesthesia in 50-100 ppm tricaine methansulfonate, the spinal cord of the female was transected through the lateral musculature with a 25G injection needle at a level between the 4th and 21st vertebrae. Succcess of the spinal transection was determined by immobilization and darkening of the body lower than the spinal level transected. The levels of transection were verified after the experiments with the aid a soft X-ray photography. Failure in oviposition in the female being embraced by the male might be possibly due to absence of ovulated eggs in the female. Thereupon, it was ascertained by an intraperitoneal injection of 2 mM acetylcholine chloride in a saline. The injection into an ovulated female can induce oviposition artificially. Fifteen females were used in this experiment.

RESULTS

Experiment 1.

Most of the male began to court a female immediately after introduction of the female into the aquarium. On the other hand, reactions of the female to male's atraction were different from each other individual. Some accepted the male within a few minuites and others did until nearly one hour after. However, after a female once received the males's embracement, each behavioural sequences of spawning pairs progressed in almost similar timings (Fig. 2). The male began quivering within 8 sec after he embraced the female with his dorsal and anal fins and it continued until the female had finished oviposition. Five to 12 sec after onset of the quivering, ovipositon occurred and lasted for 6.5 to 11 sec. The male released the female 5 to 21 sec after completion of oviposition and consequently contacting time of the male and the female fish was about 40 sec in total.

Experiment 2.

Every pair in any stages of spawning behaviour could be separated compulsorily by tapping to aquarium wall (Fig. 3). This figure shows that (1) oviposition takes place in a female

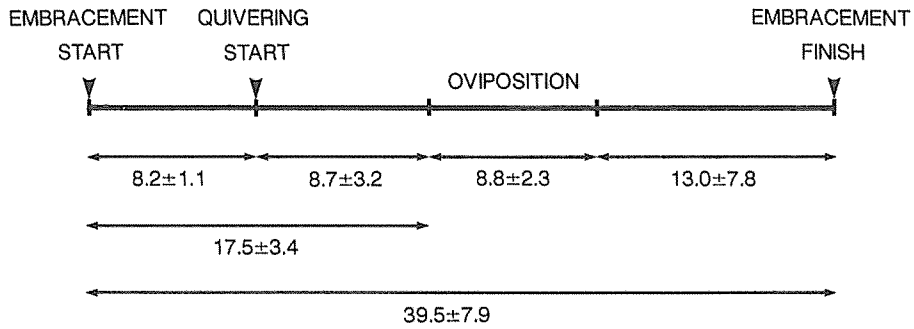


Fig. 2. A diagram showing time durations required for some selected stages of the sequential spawning behaviour of the medaka. Numbers under bars with arrowheads represent time (mean±and SD in seconds) required for each stage indicated with the bar.

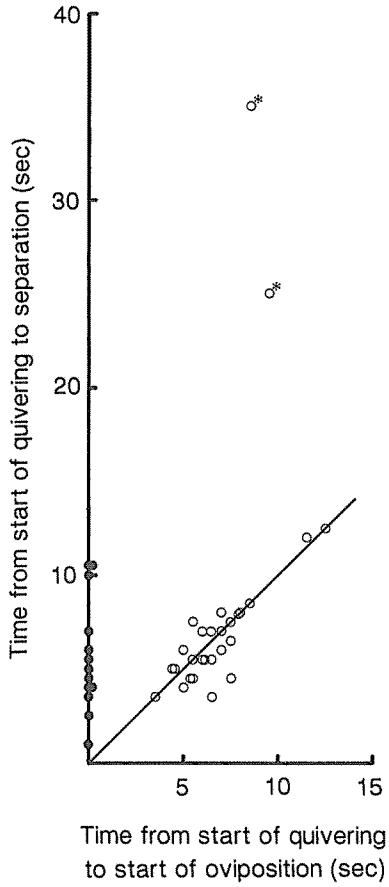


Fig. 3. Plot showing chronological relationships of separation and oviposition of the spawning medaka pairs. A filled circle means a spawning act that was cancelled before initiation of oviposition. Open circles are the acts that resulted in oviposition. Open circles on the bisector of the angle between the X- and Y-axis means that separation and oviposition occurred at the same time. Spawning acts in which oviposition was initiated after forced separation are plotted below the line. Unusual pairs would not break soon after completion of oviposition (asterisks).

Table I. Effects of the spinal cord transection on oviposition of the medaka.

Fish No.	Site of the Spinal Cord Transection (No. of Vertebrae)	Ovulation	Oviposition
1	4	○	×
2	4	○	×
3	6.5	○	×
4	6.5	○	×
5	8	○	×
6	9	○	○
7	9	○	×
8	10	○	×
9	10	○	○
10	10	○	○
11	14	○	○
12	14	○	○
13	15	○	×
14	21	○	○
15	21	○	○

○ : occurred; × : did not occur

that has been stimulated by quivering of a male at least for 3.5 sec, (2) oviposition can take place even after separation of a vibrating pair, (3) oviposition once commenced can not be held in any situations and (4) exceptional males (asterisks) would not release mate females for severeal 10 seconds after completion of oviposition.

Experiment 3.

None of 5 females whose spinal cords were cut at the level between the 4th and 8th vertebrae, oviposited eggs even after receiving stimulatory vibration from the male for several 10 sec. (Table 1). In addition, 1 fish out of 2 that were cut at the 9th vertebral level and 2 fish out of 3 that were cut at the 10th level did not oviposit (Table 1). When the spinal cord was transected between the 14th and 21st vertebral level, most females (4 out of 5) did oviposition. Every female used was checked immediately after experiments and they were ovulated.

DISCUSSION

Spontaneous contractions of the muscular hollow ovary of the medaka had been observed (Hosokawa and Nambu, 1966). It is also known that acetylcholine injection can produce strong ovarian contractions leading to oviposition in the ovulated medaka (Nambu and Hosokawa, 1971). However knowledge about neural control of the movement is still scanty.

Egami and Nambu (1961) demonstrated that numbers of the female medaka which laid eggs (oviposition rate) was decreased by removal of the anal and dorsal fins in the male, and the skins overlying the lateral body surface in the female. Based on these results, they speculated that tactile stimuli that were applied by the male as a spasmodic body vibration (quivering) and were received with sense organs on the body surface of the female,

mediated through the central nervous system and elicited oviposition. Although I feel their conclusions are probable, differences in the oviposition rate between the control and the operated groups appeared too small in their study. I speculate that some of the operated female might oviposited spontaneously and that they overlooked the egg release, because they did not observe actual spawning behaviour itself and only counted the results, the egg numbers. Spontaneous egg releases in the female fish was reported in the blue gourami (POLLAK *et al.*, 1978). I conducted the present study as a reflection upon their study.

I measured time durations required for some stages of the sequential spawning behaviour of the medaka as accurate as possible. As a result, it was confirmed that the female medaka oviposits eggs after receiving tactile stimuli from a male for a certain period (8.7±3.2 sec, mean±SD) as indicated by EGAMI and NAMBU (1961). The large variance in the period appeared to be caused not only by a technical error in measuring time with a stop-watch but also due to short-tempered males which embraced a female before she accepted the male completely. In this situation time duration for the period took longer than that of the usual spawning. An extreme case of this situation was observed in the experiment 3. Some males kept holding a spinal female which was wriggling to escape from the male and no oviposition occurred. For this reason, I suppose that the period might be estimated longer than an actual time. Viewing this from another aspect, although it digresses from the subject, the male medaka will not release female until she completes oviposition.

From the results of the experiment 2, it was indicated that tactile stimuli lasted for at least 4 sec is sufficient for initiation of ovposition, since some females, that were separated from a male 4 to 5 sec after onset of the quivering, began oviposition 1 to 2 sec after the separation. During the rest of the spasmodic period, the male might be solely ejaculating sperm, on the other hand the female having finished her job, oviposition.

Additionally, in the present study we made some interesting observations, firstly a female received stimuli strong and long enough to elicit oviposition could oviposit even when the stimulation was interupted by separation at that time. Second, ovarian contraction once commenced could not be stopped, indicating that oviposition in the medaka is a reflex mediated by the central nervous system, like micturition and ejaculation in mammals. Moreover, these observations also indicated that the medaka oviposition involves the autonomic nervous system, since a fully aroused female made oviposition inevitably whether she was willing or not. Autonomic innervation of the teleost ovary has been demonstrated in some fishes (UEMATSU, 1985 and 1986; UEMATSU *et al.*, 1989), but not in the medaka. Lastly, the medaka oviposition was severely inhibited by the spinal transection made at the levels rostral to the 10th vertebrae. Although which pathways, afferents or efferents, were blocked by the transection is still unclear, it is unlikely that every afferent that is essential for initiation of the oviposition was blocked by the transection, since the male quivering shakes vigorously the mate female and every sense organ on the whole body surface must be stimulated by the vibration, leading to cause enough sensations for initiating oviposition. Similarly, EGAMI and NAMBU (1961) demonstrated that nearly 10% of the female, in which the skin of the caudal part of the body was removed, could oviposit. Upon them, I consider that the motor pathway was possibly cut by the operations and the path are emerg-

ed from the spinal cord around the 9th vertebra.

In conclusion, the oviposition of the medaka is an autonomic reflex involving the spinal cord and the ovary is innervated by at least an excitatory cholinergic nerves related to the spinal cord near the 9th vertebrae.

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メダカ *Oryzias latipes* の放卵機序

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メダカの放卵を誘起するサイン刺激と放卵の神経支配系を明らかにすることを目的として，一連の産卵行動のうち放卵に関わる過程の時間的推移を測定した。産卵行動の末期にメダカの雄は背鰭と尻鰭で雌を抱擁しながら，魚体を細かく振動させる。この間に放卵放精が行われる。22組のペアの産卵行動を観察した結果，メダカの雌は抱擁している雄の与える機械的刺激をある時間感受し続けると放卵することが明らかとなった（8.7±3.2秒，平均±S.D.）。抱擁の開始から放卵が起こるまでの間の適当な時点で人為的に雌雄を分離させることにより，放卵が起こるために必要な雌雄の接触時間を測定した。その結果，雌は雄からの刺激を少なくとも4秒間感受すると放卵でき，一度開始された放卵，つまり卵巣の収縮は決して中断できないことが分かった。これにより，メダカの放卵は自律神経系の関与する反射であることが示唆される。また，第10椎骨より吻側で脊髄を切断すると雌は雄からいくら刺激を受けても放卵できない。以上より、メダカの放卵は脊髄以上の中枢神経系が関与する反射であり，卵巣の収縮には第9椎骨付近の脊髄から出る神経が関与することが明らかとなった。この神経は交感神経幹を経て卵巣にいたるアセチルコリン作動性の神経であると推察される。