Effect of early experience and age on initial responsiveness of *Tilapia mossambica* fry to a maternal model

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Fry of the maternal mouth brooding fish *Tilapia mossambica* were tested for the effects of (1) rearing and (2) increasing age on initial responsiveness to a gray disk covered with small black pits that served as a maternal model. In the first experiment, the eggs were removed from their mother's mouth shortly after spawning, hatched, and the fry raised in groups, singly in complete visual isolation, or by the mother (control) until testing. When free swimming, the fry were tested individually for responsiveness to the model. High levels of responsiveness were exhibited by all fry regardless of rearing experience, indicating that responsiveness to the mother's mouth is fully developed at birth and needs no previous social experience for its expression. In the second experiment, naive group-reared fry were tested individually for initial responsiveness to the model at 13, 16, 20, or 26 days postspawning. It was found that naive fry were strongly attracted to the model at 13 and 16 days of age but were neutral or actively avoiding it at 26 days of age. This decline is almost identical to the decline in responsiveness of normally reared fry toward their actual mother.

Under natural conditions, the eggs of the Cichlid fish *Tilapia mossambica* are taken into their mother's mouth at spawning, hatch there (4-6 days postspawning), and remain there until 1 or 2 days after the fry are physically capable of swimming (10-12 days postspawning). They are then released from her mouth and aggregate as a swarm near the mother for another 12-16 days. During these 12-16 days, the fry reenter their mother's mouth at night and whenever danger threatens. At such times, the mother performs a jerky "calling movement" and backs slowly away from the young. This movement elicits an approach response and the fry congregate in the dark areas below the mother's mouth, around her eyes, and at the corners at the base of her pelvis and pectorals. From there they work their way into her mouth. As the parental care period progresses, the fry respond less and less frequently to the "call." By the time the maternal care period ends (24-28 days postspawning), the fry are actively avoiding their mother's mouth region. A review of these behavior patterns can be found in Baerends and Baerends-VanRoon (1950) and Neil (1964).

Baerends and Baerends-VanRoon (1950) have determined that *Tilapia mossambica* fry will approach any large object that makes jerky motions, is not bright in color, and which contains dark areas. The fry tend to congregate on the underside or on the dark areas of the models. The strongest response from the fry is elicited by flat silver disks 3.5-10.0 cm in diam and containing black pits. Therefore, it must be concluded that *T. mossambica* fry under natural conditions possess at birth, or acquire soon afterward, an approach response to certain stimuli which under natural conditions would lead them to the safety of their mother's mouth.

The purpose of the present study was to determine whether the identification of maternal stimuli is learned soon after birth (and, therefore, is subject to the

paradigms of what has been traditionally thought of as "imprinting") or whether the responsiveness to appropriate stimuli is ready to be exhibited at hatching without previous experience (Experiment I). A second purpose was to determine if the fry's responsiveness to such stimuli extinguishes if the fry are kept naive until after the age they are naturally exposed to the mother (Experiment II). In other words, we wished to determine if there is a relationship between the age at which the behavior is first allowed to be expressed and the rate of extinction of responsiveness.

EXPERIMENT I

Method

Subjects and Rearing. The Ss were 90 fry of the species *Tilapia mossambica* obtained directly from four brooding females. They varied in age at testing from 10 to 12 days postspawning. The fry were reared in one of three manners—(1) Fry raised by the brooding mother: The brooding mother was moved to a 5-gal aquarium soon after spawning. The development of her eggs was checked several times a day to determine as closely as possible the time of hatching. The fry were removed from her mouth within 1 h prior to testing. (2) Fry raised in groups: The eggs were removed from the mother's mouth 24-48 h after spawning and placed as a group in an opaque white jar. The jar contained a solution of commercial "fungus stop" in aged water, and the water was continuously aerated by an air stone. The fry hatched in these jars and remained in them until immediately before testing. (3) Fry raised in visual isolation: The eggs were treated exactly the same as the group-reared eggs until 24-48 h before hatching. When an egg showed visible signs that the hatching process was beginning, it was placed in an isolation chamber. The isolation chambers were made of sections of biologically inert black polyethylene tubing approximately 7.0 cm high and 1.5 cm in diam. A piece of fine plastic screening attached to one end with a rubber band provided a floor for the chamber. Seven to ten of these chambers were placed in jars containing "fungus stop" solution as described above; an air stone below all the chambers served as a common oxygen source. The eggs hatched in the isolation

Table 1
Effect of Rearing Conditions on the Initial Response of
T. mossambica Fry to a Surrogate Mother Stimulus
(10-12 Days Postspawning)

Rearing Conditions	N	Number of Fry Responding in a Given Manner			
		++	+	0	-
With Mother	26	26 (7.6 Sec)*	0	0	0
In Groups Without Mother	32	22 (23.2 Sec)*	4	5	1
Complete Visual Isolation	32	26 (13.4 Sec)*	2	4	0

*Average response latency

chambers and the fry remained in them until immediately before testing.

Procedure. The fry were tested individually in a white opaque plastic container (28.5 x 19.5 x 13.0 cm) filled with aged water. A piece of black slate (16 x 16 cm), located 6 cm from one end of the test chamber, formed a physical and visual barrier.

At the beginning of the test, the fry was placed behind the barrier and given 1 min to accommodate to the chamber. At the end of 1 min, the visual stimulus was placed in the water on the other side of the barrier. The barrier was then removed, revealing the stimulus to the fry.

The visual stimulus consisted of a gray wooden circular disk 4 cm in diam and 1 cm thick. On each side of the disk were four circular black depressions 0.6 cm in diam. A 26-cm metal rod protruding from the disk served as a handle.

After the barrier was removed, the disk was gently waved and bobbed in position for 90 sec or until the fry approached. The disk was turned so that the dots were constantly facing the fry regardless of the fry's location, but the disk itself stayed in the same place. If approach was not elicited within 90 sec, the visual stimulus then approached the fry to within 1-3 cm. If the fry ignored or avoided the disk after five such approaches, the test was terminated.

Once the fry approached the stimulus the disk was moved slowly away to determine whether the fry would follow. The fry that responded to the stimulus were scored as "++" if they responded during the initial 90-sec period or "+" if they responded only after the stimulus approached them. The fry that never exhibited a positive response were rated as "0" if they ignored the disk or "-" if they actively avoided it.

Results

There was an extremely high level of responsiveness to the stimulus regardless of the rearing conditions of the fry (Table 1). Of the 90 fry in the study, only 10 failed to respond to the stimulus and of these only 1 actively avoided it. All fry that eventually approached the stimulus also followed it and made contact with the black pits. The percentage of the fry eventually responding ranged from 81% in the group-reared fry to 100% in the control fry raised by their mothers.

Because of small numbers in some cells of Table 1, the first two columns (++ and +) and the last two columns (0 and -) were combined for purposes of analysis. A 3 by 2 contingency chi-square test reveals no significant differences between the three groups. Despite large differences in the means, a one-way analysis of variance

reveals no significant differences between the approach latencies of the ++ fry of the three groups, probably because of the high variability within groups.

Discussion

The most direct conclusion that can be drawn from these results is that *T. mossambica* fry are born with a behavioral predisposition to respond to stimuli at least generally resembling the head region of the brooding female. This can be deduced from the high level of responsiveness of both groups of experimental fry, neither of which had any maternal experience after the first 24 h postspawning.

It is highly unlikely that any experience could have been acquired prenatally during these first 24 h. Gideiri (1969) has determined that the sensory systems of *T. nilotica* do not start differentiating until after 44½ h of embryonic life. Shaw and Aronson (1954) state that the *T. macrocephala* embryo cannot see until at least 3-5 days postspawning.

We are in agreement with Baerends and Baerends-VanRoon (1950), who also found that isolate fry respond to maternal stimuli. However, the group they termed "isolates" had not been taken from their mother's mouth until several days after hatching and, therefore, had not been completely isolated from maternal care. Peters and Bretowsky (1961) gave strong evidence for a genetic basis for the contact behavior of the fry while studying hybrids of a *T. mossambica* by *T. tholloni* cross. However, they also did not eliminate the possible effects of maternal care before testing.

The presence of initial behavioral predispositions regarding the parents is not unique to the fry of *T. mossambica*. The fry of other Cichlid species such as *Haplochromis multicolor* (Peters, 1937), *Cichlasoma bimaculatum*, *Hemichromis bimaculatus* (Noble & Curtis, 1939), and *Cichlasoma biocellatum* (Kuhme, 1962) have been found to have an initial preference for the color of their parents; a parental care period is a characteristic of all of these species. Such predispositions may be of some importance when studying the attachment behavior of these fish, since it may well be that the fry can only form a permanent bond to objects containing the elements of their predispositions.

EXPERIMENT II

Method

The Ss were 80 fry of the species *T. mossambica* obtained as eggs from a brooding female 24 h after spawning. Immediately after removal from the female, they were placed in an opaque white jar and raised in a group, as described in Experiment I.

The Ss were tested individually at 13, 16, 20, or 26 days of postspawning; each S was tested only once. Twenty fry were tested at each age using the maternal model, the test of responsiveness, and the behavior classifications described in Experiment I.

Results

There was a marked change in responsiveness to the model by fry that were tested at 20 or 26 days

postspawning as opposed to those that were tested 13 or 16 days postspawning (Table 2). Because of small numbers in some cells of Table 2, the first two columns (++) and (+) and the last two columns (0 and -) were combined for purposes of statistical analysis. A 4 by 2 contingency chi-square test reveals significant differences among the four groups ($p < .001$). These differences are accounted for largely by the changes occurring between Day 16 and Day 20 and between Day 20 and Day 26 (2 by 2 contingency chi-square tests, $p < .005$ in each case).

Discussion

The data clearly indicate that responsiveness of the fry to the maternal model changes from approach to avoidance with age. However, the most interesting aspect of the change is that it takes place at about the same rate as it would if the fry had not been kept naive of maternal stimuli but had been with their mother the entire time. Right after being released from the mother's mouth for the first time, normally reared fry are highly responsive to their mother's mouth area; at that age (13 and 16 days postspawning) our isolate fry were highly responsive to the maternal model. At 20 days postspawning normal fry are responding much less strongly to their mother; at that age our maternally isolated fry were either neutral or mildly positive to the model. Finally, at 26 days postspawning experienced fry either ignore or actively avoid mouth-size holes, since an attraction to such holes could be fatal once they have left their mother; maternally naive fry of this age exhibited neutral or negative reactions to the maternal model.

GENERAL DISCUSSION

The rate of extinction of responsiveness observed in Experiment II combined with the evidence that initial responsiveness to maternal stimuli is basically unlearned (Experiment I) throws doubt on the applicability of using the concept of a sensitive period (as a time when exposure to certain stimuli can have maximal effects) to help explain the early behaviors of these fish. Our data suggest that responsiveness to maternal stimuli is maintained in these fry during the normal maternal care period and then declines when the care period is ending, regardless of whether or not the fry have actually had any experience with maternal stimuli. In other words, our data suggest that there is no sensitive period within which the fry must be exposed to the mother in order for them to respond to her later in the maternal care period.

The absence of effects of exposure during the sensitive period is, perhaps, not as surprising as it might appear at first. The concept of the sensitive period was formed mainly on the basis of information obtained from precocial birds which remain with their mother for several months after hatching. However, *T. mossambica*

Table 2
Effect of Age on the Response of Naive *T. mossambica*
Fry to a Surrogate Mother Stimulus

Age (In Days Postspawning)	N	Number of Fry Responding in a Given Manner			
		++	+	0	-
13 Days	20	18 (27.8 Sec)*	1	1	0
16 Days	20	18 (25.3 Sec)*	2	0	0
20 Days	20	5 (53.0 Sec)*	8	3	4
26 Days	20	1 (48 Sec)*	3	5	11

Note—Fry normally reared with a parent would be highly responsive (attracted) at Day 13 and avoiding or neutral by Day 26.

*Average response latency

fry stay near their brooding mother only about 2-3 weeks after being released from her mouth for the first time. It is possible, then, to picture an innate mechanism which "run its course" in 2-3 weeks regardless of experience and accounts for responsiveness to maternal stimuli in these fry. This is, perhaps, a more primitive mechanism than "imprinting" for the establishment of parent-young interactions.

Of course, our data alone do not prove the existence of this primitive mechanism. To do so it must first be determined if exposure to maternal stimuli early in development prevents, or slows down, the decline we have observed. Goude et al (1971, 1972a, b) exposed fry in groups to a model similar to ours starting on Day 9, 11, 13, or 15 postspawning for 20 min/day until 19 days postspawning. They concluded (1972b) that all fry of the same age reacted to the model in the same manner regardless of the amount of exposure they had received or their age at onset of stimulation. Therefore, their data support our hypothesis of an innate mechanism controlling responsiveness.

Second, it must be determined if exposure to either nonmaternal stimuli or a different "maternal" stimulus very early in development affects later responsiveness to our original maternal model. This experiment is in progress in our laboratory.

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