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Maturation and Spawning of the Dragonet, *Callionymus enneactis*, in an Aquarium

Toru Takita, Teruyuki Iwamoto, Shuya Kai, and Ichiro Sogabe

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Abstract *Callionymus enneactis* was reared in a tank to observe its spawning behavior. Changes in ovarian tissue were also examined. Females spawned every night for at least seven days. By day, males fought with each other and displayed to females. Males came out of the sandy substrate at about 8: 45 p.m. after a stay of approximately one hour and a half in the sand. The males then enticed females to move out of the sand. Then, the pair rose up in the water to spawn in darkness. Spawning in the tank by two males and 14 females continued for about 45 minutes. The ovary contained eggs in the yolk globule stage and earlier stages just after spawning. These measured less than 0.32 mm in diameter. A part of the eggs reached the migratory nucleus or prematuration stage at 18 h after the previous spawning, and was ovulated at 23 h. A comparison of the natural condition of ovaries from fish in the field with the developmental cycle of females in the tank suggested that spawning in nature may have occurred at the same time as observed in the laboratory.

Some observations on the characteristic spawning behavior of callionymid fishes have been reported by Holt (1898), Kato (1936), Abel (1955), and Takita and Okamoto (1979), and others.

Callionymus enneactis Jordan et Fowler (Japanese name, hanabi-numeri) occurs from mid-Japan through Okinawa to Australia (Fricke, 1981, Nakabo, 1983). It is rather rare and little is known about its biology beyond the taxonomic description. In this study, we collected living specimens of this species and observed their spawning in a tank during 1979. Here we report several characteristics of the spawning behavior which are different from those described in earlier studies of spawning in callionymids. We also provide information on changes in ovarian tissue between successive spawnings.

Material and methods

Collection of specimens. Mature individuals of *C. enneactis* were collected on July 8 and 25, 1979 in the shallow innermost area of Shijiki Bay (33°12′N, 129°23′E) of Hirado Island, Nagasaki. The habitat was a beach of fine sand with patches of *Zostera nana* in the intertidal zone and those of *Z. marina* off the zone of *Z. nana*. Each patch of vegetation was separated by a canal-like sandy bottom.

A 2.8 m wide trawl net with 3 mm mesh was used to collect the fish. The net with ropes attached to each wing was pulled by hand in areas with *Zostera*, sandy canal, and open sandy areas, at depths of 50～100 cm. *C. enneactis* was netted in areas of *Z. nana* and over the neighboring sandy bottom. One female was observed on the sand of a canal-like region. No specimens were collected in the open area or the area of *Z. marina* in spite of many trials. In the area of *Z. marina*, the bottom of the net was lifted up by thick vegetation, making it difficult to collect fish on the bottom, even though they may have been present.

Specimens were placed in a bag containing sea water and oxygen, and transported to the Fisheries Experimental Station of the Faculty of Fisheries, Nagasaki University in Nomo, Nagasaki. The vivid coloration of males and well-developed ovaries of females observed through the abdominal muscle indicated that all specimens were sexually mature. Male specimens measured 53～65 mm in standard length and weighed 2.6～4.6 g; females ranged from 35～49 mm and 1.0～2.2 g.

Rearing. Six males and 14 females were kept in a round plastic tank, 135 cm in bottom diameter and 76 cm deep. The transparent side wall of the holding tank was covered with a dark curtain to prevent the disturbance of the spawning behavior of the study fish. The bottom of the tank was covered with fine sand. Sea water

flowed into and out of the tank continuously at night (about 70 liters an hour). Sea water flow was interrupted in the daytime for convenience while feeding plankton to the study fish. Fish were fed small polychaetous annelids and microplankton mainly composed of copepods and decapod larvae.

C. enneactis shows distinct sexual dimorphism. Males are consistently larger than females, moreover, there are differences in size and coloration of fins. The first dorsal fin of males is elongated and bright yellow, whereas that of females is small and inconspicuously colored. The second dorsal and caudal fins are much larger in males than females. Although all specimens were judged to be mature when caught, they initially failed to spawn in the laboratory. On July 30 both males and females were injected with a sexual hormone (Gonatropin, Teikoku-zoki Co. Ltd. Japan). Half of the individuals received 25 units each and the other half, 12.5 units. They began to spawn on July 31, but the effectiveness of the hormone could not be confirmed because of the absence of a control population.

Since *C. enneactis* spawned at night, a faint light was introduced to the holding tank, so that the behavior of individuals on the bottom could be observed.

Examination of ovary. Following observations of spawning behavior, all fish were sacrificed and used in an examination of ovarian maturation. After the last observed spawning, one individual was killed every three hours until the next spawning time, and each ovary was fixed in a 20% formalin solution. After confirming lack of difference in development among parts of ovaries of one individual, a section of the central portion of an ovary was used for measurement of egg diameter and histological examination. Specimens, which were injured during the initial field collection, were killed and fixed in formalin solution just after being caught, and their ovaries were examined to determine their natural status. In examination of egg diameter, 500 ova, larger than 0.1 mm in diameter, were taken at random from each individual. Cross sections of ovary were made using the celloidin method in 12 μm thickness and stained with Meyer's haematoxylin-eosin solution. Developmental stages were classified according to Yamamoto (1956).

Results

Behavior in daytime. From the time the fish were put in the holding tank, we stayed beside the tank throughout the daytime to study the behavior of the fish, until we found eggs and larvae in the water on August 2. These eggs had apparently been spawned during the previous nights. *C. enneactis* almost always remained in contact with the sand substrate in the holding tank. While feeding, *C. enneactis* moved forward intermittently as if crawling across the bottom on their pelvic fins. Both sexes frequently buried themselves in the sand and stayed motionless. Two or occasionally three males displayed to females and often fought with each other throughout the daytime; other males remained buried. Interactions were not observed between females.

The pattern of display by males was simple. When approaching a female, the male pushed the side of his body toward the female, then momentarily spread the colorful long dorsal fin in front of or beside the female. Males of *C. enneactis* neither kept spreading each vertical fin, nor lifted the body up by the fins, as other callionymid species do (Takita and Okamoto, 1979). The male with intermittent forward motion achieved by sporadic movement of the pelvic fins pushed the female. The male and female sometimes positioned themselves side by side as if they were beginning the upward spawning trip which is known in callionymid fishes (Holt, 1898; Takita and Okamoto, 1979). However, they did not maintain this position for more than a few seconds.

Fights between males were intense. When a male recognized another male on the sand, it darted and rushed forward, often biting at the rival's mouth. Frequently, males would continue biting and twisting their bodies for more than a minute.

Both males and females ceased movement around sunset each evening, and buried in the sand.

Spawning behavior. The eggs and larvae which were found in the tank on August 2 were judged to have been spawned at night on August 1 and July 31. Thereafter, each female spawned daily until August 6, when observations were ceased. Two males came out of the sand around

8: 45 p.m., about 1½ h after sunset and began to move. They moved about on the bottom alternating short advances with short rests and appeared to be rubbing the bottom with their abdomens. Each male occupied about half of the tank bottom and would never enter the other half which was occupied by the other male. Even if they encountered each other at the border of their areas, they were not seen fighting at night. Each male sometimes maintained a fixed position for a brief time, then, a female suddenly emerged from the sandy substrate. The male and female would assume a position parallel to each other. A male sometimes moved around a point above the bottom, as if signalling to the female to come out. Sometimes males repeatedly visited a particular location on the bottom and moved around it several times. Although we were unable to confirm their presence, it is possible that a female was buried in the sand and that it did not react to the signalling male.

After a female came out of the substrate, the male and female usually started an upward rise immediately. Sometimes they remained on the bottom for several seconds and the male pushed the female with the side of his body as if urging the female to start the spawning sequence.

When making the upward rise, the pair first ascended in a straight line at an angle of about 60° to the bottom, inclining their body axis at about 45°. Several seconds later, the angle of their body axis became somewhat less, and they swam upward in a slight curve. Then the male and female flexed their tail acutely outwardly as in other callionymids when releasing gametes (Takita and Okamoto, 1979). Although gametes were not seen, the fish were regarded to spawn at that moment. *C. enneactis* made a very prompt and short spawning rise, requiring only about five seconds to complete. The spawning pairs were 30 to 40 cm above the bottom when spawning. After spawning, the male and the female parted and descended quickly. As the female buried itself in the sand, the male began searching for a second female.

All males in the tank came out of the sand at the spawning time. However, spawning activities were observed in only two males which visited particular locations on the bottom. Other males did not appear to seek females, only repeatedly dashing forward, sitting quietly on the sand momentarily or burying themselves in the sand. During observations over four days, the males which did not participate in the spawning activity were observed several times to dash up and strike the ascending pair.

After being attacked by another male or for unknown reasons, a pair sometimes interrupted the spawning rise prior to the release of gametes. The male and female parted, and the male began seeking another female. The female moved around on the sand, and would approach the same male which was moving on the sand as females of other callionymids do in daytime (Takita and Okamoto, 1979). However, the male did not immediately respond to the female. It appears to be easier for males to court a female in the sand than to do so to an exposed female. Although it was difficult in darkness to count exactly the number of females which had spawned, judging from frequent spawning rises of pairs observed, almost all females were regarded to spawn everyday. It took about 45 minutes to complete all spawning activities in the tank.

If *C. enneactis* spawns at the same time in nature as observed in the laboratory, they must go through the process in darkness without recognizing individuals of the other sex by sight. In order to confirm this hypothesis, the tank was maintained in total darkness on August 6, the last day of this study. The holding tank in an unlit room was covered by a light proof sheet at sunset. The sheet was removed at 10: 00 p.m. Spawning in complete darkness was confirmed by a number of fertilized eggs floating in the tank.

Ovarian aspects. After completion of behavioral observations, ovaries of each female were taken and examined. A frequency distribution of egg diameter for each individual (moving average of three) is shown in Fig. 1. Aspects of the ovaries taken 0 h (A), 15 h (B), 18 h (C), and 21 h (D) after spawning are shown in Fig. 2.

Ovaries just after spawning contained eggs in the yolk globule stage and earlier stages, measuring smaller than 0.32 mm in diameter. The ovaries also contained new postovulatory follicles and the frequency distribution of egg diameter was monomodal. The frequency distribution was not clearly bimodally divided until 9 h after spawning (Fig. 1). Based on the increase in yolk globule size in a group of larger

eggs (eggs of a larger mode), the globules appeared to begin to fuse around 15 h after spawning. However, the increase in egg size was rather slow until 18 h. At 18 h after spawning, ovaries had eggs in the migratory nucleus and prematuration stages. A notable increase in egg size was recognized in the group of larger eggs at 21 h after the previous spawning. The larger eggs were completely separated from the younger eggs in the frequency. The group of larger eggs reached the mature stage in the ovary 21 h after the previous spawning, and was ovulated at 23 h.

A bimodal distribution was recognizable in the younger eggs at 15, 21 and 23 h after spawning, although it was not obvious (Fig. 1). There might be two or more groups of eggs in the ovaries just after and 3 and 6 h after spawning, whereas the frequency distributions did not clearly show this. In other words the younger egg group possessing a small amount of yolk is found just after spawning. This suggests that the accumulation of yolk begins at least two days before ovulation.

Postovulatory follicles were clearly seen only in the ovaries just after and 3 h after spawning, thus indicating prompt disappearance as new eggs began to develop. They were not seen in ovaries 15 h after spawning and subsequently fixed ovaries.

Fish were caught from the sea in the afternoon before sunset. Those fixed immediately after being caught were in essentially the same stage of ovarian development as observed in individuals maintained in the laboratory and sacrificed at the same time of day. Our observations on the condition of ovaries, therefore, indicate that spawning in nature and in the laboratory may be synchronous.

Discussion

Callionymus lyra has been regarded as a territorial species (Wilson, 1978), whereas a territory could not be recognized in *C. flagris* and *C. richardsoni* (Takita and Okamoto, 1979). Sexual dominance is suggested among competing males of *C. enneactis* as determined from observations of fights in daytime. The two males which elicited spawning at night were considered to establish their dominance during daytime, although they could not be specifically identified at night as those which were displaying on the substrate in the daytime. The fact that the males did not fight at night suggests that antagonistic behavior is elicited only when they recognize rivals by sight.

In the case of *C. flagris* and *C. richardsoni*, severe competition among males was regarded to be attributable to unnatural overcrowding in an artificial and limited area (Takita and Okamoto, 1979). A similar violent fight by *C. enneactis* as observed in our study was documented by Mr. H. Usuki (photograph appearing in the magazine, Anima, 7 (7), 1979, p. 9). Thus, the violent fights which we observed in the laboratory also occurs in this fish in nature.

In *C. flagris* and *C. richardsoni*, the vertical fins, especially the first dorsal fin, has been recognized as important in attracting females and displaying to other males (Takita and Okamoto, 1979). Males of *C. lyra* (Holt, 1898) and *C. festivus* (Abel, 1955) have been reported to erect the vertical fins when attracting females. In *C. enneactis*, males erected the first dorsal fin only briefly when either attracting females or fighting other males, even though the fin is conspicuously colored.

In *C. flagris* and *C. richardsoni*, less-dominant males also had opportunities to mate with females (Takita and Okamoto, 1979). However, in *C. enneactis*, only two males out of six elicited spawning activity. The other males did not actively court females, although on several occasions they interrupted the ascending pair. We infer that only dominant males elicited spawning activity. The relation between a male and females, established during the day, may be rigid.

Callionymid fishes have been observed to spawn in daytime except for *Diplogrammus xenicus* which spawns at dusk (Fricke and Zaiser, 1982). Our observation of *C. enneactis* is the only report of spawning activity at night, as far as we know. Spawning behavior of callionymids takes some time to be completed, exposing spawners to the danger of predation. Thus, spawning at night in *C. enneactis* might be an effective predator defense.

The spawning rise of *C. enneactis* was of a short duration compared to spawning rises of other species (Takita and Okamoto, 1979). However, *C. enneactis* might make spawning

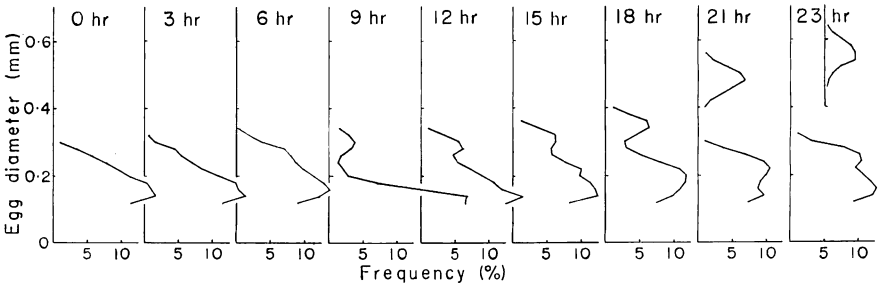

Fig. 1. Frequency distribution of egg-diameter of the individuals fixed every three hours after spawning. The ovulated eggs at 23 h are shown separately.

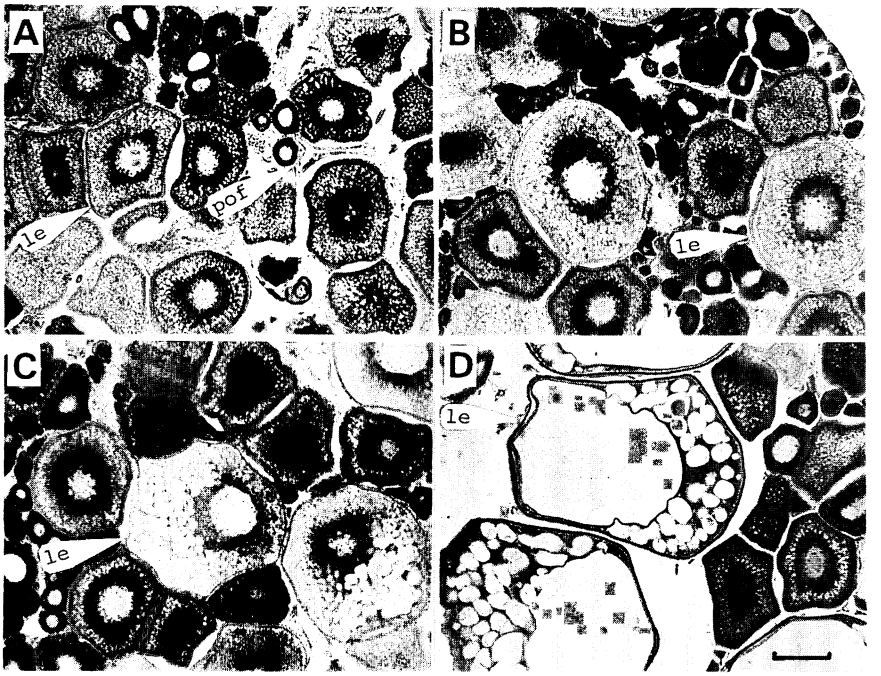

Fig. 2. Photomicrograph of the ovaries. A, just after spawning; B, 15 h after spawning; C, 18 h; D, 21 h. le, egg to be spawned next; pof, postovulatory follicle. Scale indicates 0.1 mm.

rises of much longer duration in nature. In entire darkness, without observational disturbances, the spawning trip produced a delicate belt-like egg mass (Takita, 1983). Such an egg mass must have taken some time to be produced.

The daily maturation process of ovaries can be divided into three intervals. The first interval is the stage where the group of eggs which is to be released next is not easily distinguished in the egg diameter frequency. This stage occurs when females are buried in the sand at night after they have spawned. In the second stage, the egg-group to be spawned is easily recognized, but growth in egg size increment is slow. The eggs grow rapidly in size in the third stage.

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(Faculty of Fisheries, Nagasaki University, 1-14, Bunkyo-machi, Nagasaki 852, Japan)

ハナビヌメリの成熟と水槽内産卵

田北　徹・岩本輝幸・甲斐修也・曾我部一郎

ハナビヌメリを水槽で飼育し，産卵行動を観察した．成熟過程についても知見を得た．日中，雄は雌に求愛誇示をし，他の雄と争う．夕刻，全ての個体は一旦砂にもぐって静止する．午後9時前後に，暗夜の中で，6尾中2尾の特定の雄が砂中にひそむ雌を探し，次々に産卵した．他の雄は産卵に加わらなかった．これらの行動から，雄は日中，行動域と雌の確保のために争い，それを確保した雄だけが自分の行動域内の雌と産卵すると考えた．同一の雌がある期間にわたり毎日産卵する．次回に産み出される予定の卵は，産卵時刻の約6時間前に胚胞が移動し，1時間前までに排卵される．海から採集した直後の個体の卵巣成熟度から，本種は自然でも夜間に産卵すると考えられた．

(852 長崎市文教町 1-14 長崎大学水産学部)