

Nocturnal hatching timing of mouthbrooding male cardinalfish *Apogon niger*

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Abstract We observed hatching behavior by mouthbrooding males of the cardinalfish, *Apogon niger*. Mouthbrooding males showed no feeding activities at night, in spite of their nocturnal feeding habit. On the day of hatching, they released newly hatched larvae from their mouths on average 81 min after sunset. Semilunar hatching periodicity was significant, but its diel pattern was independent of the tidal rhythm. Sunset hatching may be advantageous not only to offspring because of their low predation risk but also to parental males because they can resume feeding sooner, thereby reducing the energetic loss from fasting while mouthbrooding.

Key words Apogonidae · Adult biology hypothesis · Larval biology hypothesis · Lunar periodicity · Reproductive synchronization

Reef fishes have evolved a variety of behavioral traits to increase their offspring survival (Thresher, 1984; Sale, 1991). One such adaptation is associated with preference for a particular timing and location of offspring dispersal when and where the mortality risk to offspring will be minimized. In general, reef fishes live an early life offshore as planktonic propagules (Leis, 1991). Because predation pressure on offspring is extremely high in the reef environment, effective dispersal of planktonic propagules would be advantageous to their parents (Johannes, 1978).

Reproduction in reef fishes often shows periodicity or synchronicity on various temporal scales. For instance, the timing of propagule dispersal coincides with physical environmental fluctuations such as the lunar cycle, tidal rhythm, and photoperiod (reviewed by Robertson, 1991). It is believed that the temporal pattern of reproduction has been selected to transport eggs or larvae offshore in the timing most appropriate for their dispersal, thus resulting in mitigation of predation on offspring (Johannes, 1978). Alternatively, such a reproductive pattern might have been controlled on the basis of parental interests, for example, through reduction in predation risk on parents or enhanced efficiency of their feeding, mating, or parental activity (Conover and Kynard, 1984; Foster, 1987; Kohda, 1988). In terms of larval and adult interests, such alternative explanations are called the larval biology hypothesis and adult biology hypothesis, respectively

(*sensu* Robertson, 1991). Many studies have hitherto discussed the relative importance of these two hypotheses to explain the evolution of reproductive pattern in reef fishes.

The subject species, *Apogon niger* (Apogonidae), is a paternal mouthbrooder. Mouthbrooding is one of the care styles most effective in protecting offspring under high predation pressure (Oppenheimer, 1970). For some apogonid species, spawning and mouthbrooding behaviors have been described in detail (Kuwamura, 1983, 1985). However, it has not been well known in nature how and when parental males hatch eggs in their mouths and release larvae into the water column. In this article, we report hatching and larvae-releasing behavior by mouthbrooding males of *A. niger* and discuss which of the alternative hypotheses can explain its temporal pattern.

Materials and Methods

Study species.—*Apogon niger* inhabits sandy reefs in the shallow waters. In the study site, Murote Beach (33°00' N, 132°30' E), Shikoku Island, Japan, this fish is found mainly in the sandy area where poritid coral colonies are sporadically distributed (Okuda, 1999). The coral colonies are used as courtship and spawning sites as well as shelter against sand-dwelling predators. The fish

leave their shelter at dusk to feed on small crustaceans and fishes, and return there at dawn, showing site fidelity (Kuwamura, 1985; N. Okuda, personal observation).

The breeding season is from May to October in this study site (Okuda, 1999). Before spawning, courtship displays are exhibited by a pair, a male and a female that live in the adjacent shelters. Courting males and females touch their lateral side to each other, with their bodies quivering (Fig. 1a). Body coloration is markedly different between the sexes during courtship displays. Spawning usually takes place at the same site where the courtship is observed. The prespawning behavior and coloration are very similar to those during the courtship (Kuwamura, 1985), but some morphological changes are found in females, which have a swollen belly and a prolapsed cloaca on the day of spawning (N. Okuda, personal observation). The male and female release gametes almost simultaneously in a parallel position, with vents close together (Fig. 1b). As soon as an egg mass bound with adhesive threads is fertilized, the male takes it into his mouth (Fig. 1c). While the male repeatedly tries to settle his egg mass into the right position in his mouth, the female swims briskly around him, pressing her lateral side to him (Fig. 1c). Such postspawning behavior weakens as the egg mass is packed into his mouth. A few hours after spawning, the pair separates and returns to their own shelters.

Both males and females repeat breeding cycles in a season, but mate fidelity is low (i.e., the mating pattern is promiscuous; Kuwamura, 1985; Okuda, 1999).

Field observations.—We conducted field research in 1992 at Murote Beach. At the study site, Okuda (1999) previously observed reproductive behavior of *A. niger* in the daytime for a total of 257 days, but hatching behavior was never seen. Thus, we made nocturnal observations of mouthbrooding males on the expected day of hatching of their broods, from dusk (within 1 h before sunset) to midnight (up to 2240), with the aid of SCUBA. We used a submerged flashlight with a red filter so as not to disturb a focal fish (see Kuwamura, 1987). We recorded a behavioral sequence of the focal fish and its occurrence time. In some cases, we could not see the hatching event directly but judged that it occurred if a brood disappeared from the male's mouth between two consecutive visits in one night.

To examine a diel pattern of habitat usage by *A. niger*, we made nocturnal censuses of marked fish within a 20 m × 60 m quadrat set in their diurnal habitat for a total of 20 days between 30 July and 1 October (for details of methods, see Okuda, 1999).

We performed the autocorrelation analysis and the Rayleigh test to see if spawning and hatching show periodicity and if they coincide with any particular lunar phases, respectively, using data of Okuda (1999), in which

almost all spawnings and hatchings by 60 marked individuals were identified throughout the breeding season of 1993. For the former analysis, the time lag was set at 1 day. For the latter analysis, dates on the solar calendar were transformed into the age of the moon, and the number of spawnings or hatchings observed in each day were collapsed onto a single cycle (Zar, 1999). In addition, to view a relationship between a diel pattern of hatching and a tidal rhythm, we referred a tide table issued by the Uwajima Observatory of Japan Meteorological Agency (33°13' N, 132°33' E) nearest (about 26 km apart) to the study site.

Results and Discussion

Egg hatch and larval release. Females and non-brooding males began to move around actively at dusk and left their shelters by sunset to forage on the sandy bottom. In nocturnal censuses, they were never found within a quadrat, whereas most mouthbrooding males stayed still near their shelters (in 68 of 86 cases). The mouthbrooding males showed no feeding actions during the nocturnal observations. It is therefore unlikely that males feed during the mouthbrooding period, as in other apogonids (Okuda and Yanagisawa, 1996a; Okuda, 2001).

At the early parental stage, mouthbrooding males remained with their mouths shut, rolling the egg mass in their mouth at intervals of a few minutes. At the moment of rolling action, the egg mass and its development were observable. As the egg hatch approached, their mouths became half-opened, with an increasing frequency of egg rolling (Fig. 1d).

On the day of hatching, males started chewing actions about an hour after sunset. This behavior continued on average for 13 min (± 2 min SD, $n = 9$). Just after stopping the chewing actions, they slowly spiraled upward, with their mouths closed (Fig. 1e). As soon as they reached the water surface, they spat their brood out (Fig. 1f,g). At this time, the eggs had already hatched and the newly hatched larvae had separated individually. The time of larval release was on average 81 min after sunset (± 25 min SD, $n = 11$; Table 1). For some males, the chewing actions and larval release did not occur during the nocturnal observations, but their broods had disappeared by the next forenoon (Table 1). It is unclear whether the delay of larval release was attributed to disturbance by observers or to prematurity of embryonic development.

After newly hatched larvae were scattered just under the water surface, males quickly descended headfirst toward the bottom (Fig. 1h). Their descent was so fast that we sometimes missed them. Returning to the bottom,

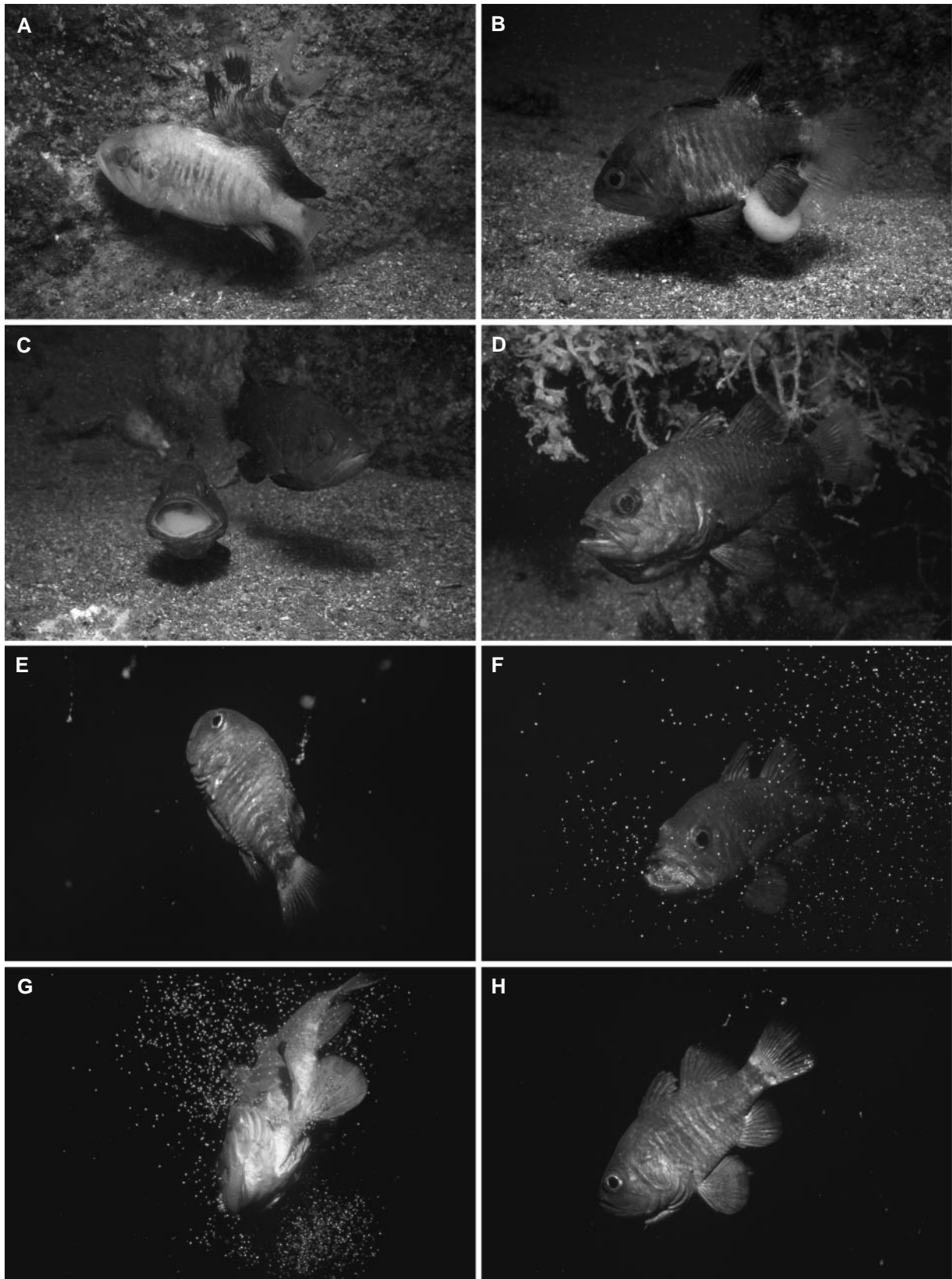


Fig. 1. Reproductive behaviors by *Apogon niger*. **A** A courtship display shown by a pair, male (*behind*) and female (*front*). **B** The female (*front*) releases an egg mass, which was simultaneously fertilized by the male (*behind*) in a parallel position. **C** The male has just taken the egg mass into his mouth (*left*), and the female is frequently pressing her lateral side against him (*right*). **D** The male mouthbrooding an egg mass, which was ready to hatch. **E** The male ascending toward the water surface. **F**, **G** The male spitting eggs out into the water column. **H** The male darting back to the bottom

Table 1. Time of day at larval release by mouthbrooding male *Apogon niger*

Date	Male	SL (mm)	Sunset time	Time at larval release	Tide at larval release ^a		
July	30	M1	85.5	1911	2039	Ebb (2012HT–0200LT)	
August	5	Unmarked	—	1906	2009	Flood (1749LT–2347HT)	
	7	M2	84.5	1904	2009	Ebb (1558HT–2056LT)	
	11	M3	73.5	1900	1930–1950 ^b	Ebb (1904HT–0038LT)	
	11	M4	84.5	1900	1930–2032 ^b	Ebb (1904HT–0038LT)	
	14	M5	79.0	1857	1950	Flood (1343LT–2013HT)	
	16	M6	85.5	1855	2003	Flood (1443LT–2103HT)	
	23	M3	73.5	1847	2010	Ebb (1606HT–2134LT)	
	25	M7	78.5	1844	2100 before ^c	—	
	September	2	M6	85.5	1833	2030 after ^d	—
		5	M8	70.0	1830	2018	Ebb (1539HT–2056LT)
		6	M4	84.5	1829	2007	Ebb (1654HT–2233LT)
7		M9	79.0	1827	1916	Ebb (1735HT–2317LT)	
11		M10	76.5	1821	1955	Ebb (1910HT–0107LT)	
11		M7	78.5	1821	2033	Ebb (1910HT–0107LT)	
14		M5	79.0	1817	2040 before ^c	—	
21		M6	85.5	1808	2100 after ^d	—	
23		M11	82.5	1805	2045 after ^d	—	
23		M7	78.5	1805	2045 after ^d	—	
October		1	M4	84.5	1754	2240 before ^c	—

^aTime of high tide (HT) and low tide (LT) are shown in parentheses

^bLarval release occurred during this period though we could not observe it directly

^cLarval release had already occurred until this time when we visited a focal male

^dLarval release had not yet occurred at this time when nocturnal observation ended, but it was completed by the next forenoon

they started feeding actions on average 23 min (± 8 min SD, $n = 7$) after the larval release.

In some demersal egg spawners, the egg hatch can be induced by changing the light condition, without the help of the parent (Doherty, 1983; Kohda, 1988). In *Apogon niger*, in contrast, the egg hatch occurred only when parental males initiated the chewing actions, irrespective of photoperiod. In this fish, the eggs of which development is advanced can be hatched artificially by physical stimuli, such as churning them in a polyvinyl pouch, just after removing them from the male's mouth (N. Okuda, personal observation). These results imply that chewing actions by male *A. niger* have a function of inducing the egg hatch and that the parent can adjust its timing.

Ascent behavior performed by male *A. niger* before larval release is similar to the spawning ascent common to pelagic egg spawners, which is assumed to be an adaptation for the effective propagule dispersal by the tide (Johannes, 1978). In *A. niger*, such a behavior will expose a parent to danger of predation by piscivores, whereas it may have a great advantage of avoiding predation on offspring by nocturnal plankton feeders, such as apogonids, which forage actively in the bottom or midwater.

Reproductive synchronicity and periodicity. Spawning did not occur frequently early in the breeding season of 1993 when water temperature was relatively low (Fig. 2). After 28 July, when the water temperature

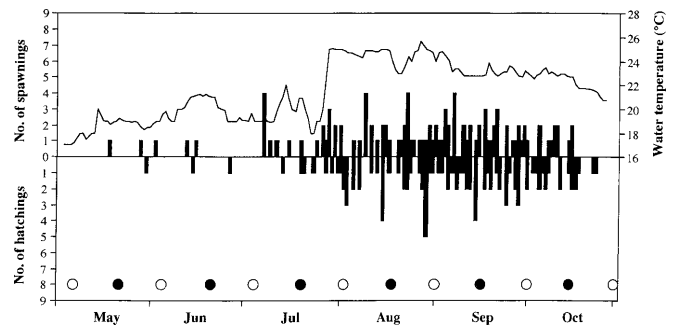


Fig. 2. Daily occurrences of spawning and hatching by marked fish in 1993. *Line* indicates water temperature; *open* and *closed circles* are full and new moon, respectively

rose drastically, there were several small peaks of spawning and hatching around the full and new moon, but such a reproductive synchronization was not significant (Rayleigh test, spawning: $z = 0.30$, $n = 106$, $P = 0.74$; hatching: $z = 0.39$, $n = 99$, $P = 0.68$).

Autocorrelation analysis showed no remarkable spawning or hatching periodicity either during the period when water temperature was low (18 May–27 July) or when it was high (28 July–24 October) (Fig. 3). However, the analysis revealed that spawning peaks had a statistically significant tendency to fluctuate at periods of 25 days ($r = 0.34$, $P = 0.022$) early in the season and 14

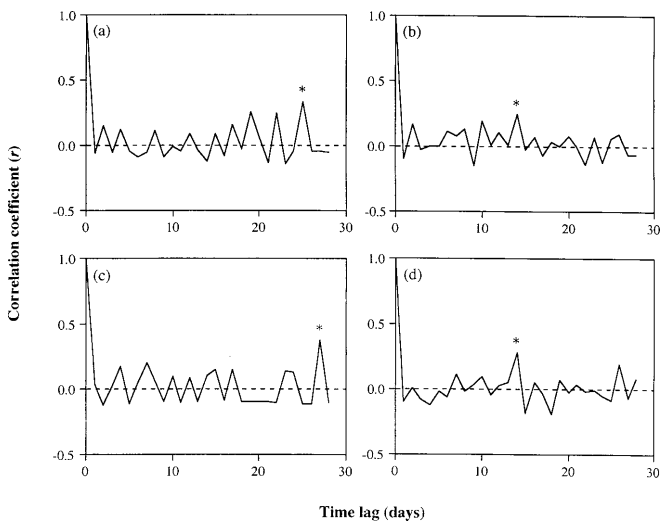


Fig. 3. A correlogram of reproductive periodicity for spawning peaks in the early (a) and late (b) breeding season, and for hatching peaks in the early (c) and late (d) season. Asterisks indicate a significant correlation coefficient ($P < 0.05$)

days ($r = 0.25$, $P = 0.035$) late in the season, and hatching peaks occurred at periods of 27 days ($r = 0.38$, $P = 0.012$) early in the season and 14 days ($r = 0.28$, $P = 0.015$) late in the season. Fortnightly periodicity in the late season is a consequence of sporadic reproductive synchronization by some fish rather than a consequence of regular reproductive cycle for each individual, because their interspawning intervals greatly deviated from 14 days (Okuda, 1999).

In general, the highest tide occurs near sunset at the full or new moon, with the subsequent ebb tide. Thus, newly hatched larvae of *A. niger* will be swept away by tidal currents most effectively when parental males release them shortly after sunset at such lunar phases. The lunar or semilunar reproductive cycle has been also reported for the tropical apogonid *Sphaeramia orbicularis* (Allen, 1975). By contrast, in a population of *A. doederleini* in the same site as the present study, spawning peaks did not coincide with any particular lunar phases (Okuda and Yanagisawa, 1996a). In *A. doederleini*, in which females compete intensely for sexually receptive males in short supply (Okuda and Yanagisawa, 1996b), females can benefit more by giving their clutch to a vacant male as soon as they encounter than by adjusting the timing of spawning to hatch eggs during the lunar phase most appropriate for larval dispersal. This is not necessarily the case for *A. niger*, in which female mating competition is less intense because of low population density and high mortality risk of mate searching (Okuda, 1999). Nevertheless, lunar periodicity and synchronicity by *A. niger* were weak or not signifi-

cant. For temperate demersal egg spawners such as *A. niger*, which experience drastical seasonal changes in water temperature, it may be difficult to coincide hatching precisely with a particular lunar phase because the embryonic developmental time greatly varies depending on the water temperature (Ochi, 1986).

In *A. niger*, even though the hatching date deviated from the full or new moon, males usually released larvae just after sunset (Table 1). Sunset hatching has been reported for many reef fishes with demersal eggs (Johannes, 1978; Ross, 1978; Doherty, 1983; Ochi, 1986; Foster, 1987; Kohda, 1988) and viviparous fishes (Fujita and Kohda, 1998). In such species, predation on hatchlings is expected to be low because diurnal plankton feeders are inactive during the nocturnal dispersal. Moreover, if a parent releases larvae sooner after sunset, then the larvae can have more time to pass through reefs under low predation pressure (Fujita and Kohda, 1998). There is another reason why the sunset hatching is favored in *A. niger*. In this fish, the sunset hatching may be advantageous to parental males because they can resume feeding sooner, thereby reducing the energetic loss entailed by fasting during the mouthbrooding period. In many reef fishes, there exist conflicts over the timing of offspring dispersal between parent and offspring (Robertson, 1991). In *A. niger*, in contrast, the occurrence pattern of hatching could be beneficial both to parental male and to their larvae. In conclusion, the larval and adult biology hypotheses mutually explain the timing of hatching in *A. niger*.

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