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Reproductive Behavior, Eggs and Larvae of a Caesionine Fish, *Caesio caerulaurea*, Observed in an Aquarium

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Abstract The reproductive behavior and early life history of *Caesio caerulaurea* are described from aquarium observations. Spawning occurred mainly between a pair plus sneakers from June 11 to July 16, 1994. Six easily distinguishable behavior phases were recognized in the spawning sequence: 1) Nuzzling; 2) Several males joining in courtship; 3) Spiraling towards the surface; 4) Pair spawning; 5) Sperm release by sneakers; and 6) Post spawning. Fertilized eggs were spherical, transparent, buoyant and unpigmented. They averaged 0.82 mm in diameter and contained a single oil globule averaging 0.17 mm in diameter. Hatching began about 23 hours after fertilization at 24.0 ± 0.5°C. Immediately after hatching, larvae were 2.02–2.46 mm in total length and had a large, round, ellipsoidal yolk. A single oil globule with about 10 melanophores protruded extensively from the anterior end of the yolk. In 36 and 48-hour old larvae, one or two dendritic melanophores were observed just posterior to the auditory vesicle. Larval development of *C. caerulaurea* during the first 48 hours after hatching is described.

The reproductive behavior and early life histories of species in the family Lutjanidae (*sensu* Johnson, 1993) are poorly known (Thresher, 1984; Grimes, 1987; Leis, 1987), the former having been reported only seven times (Wicklund, 1969; Starck, 1971; Arnold et al., 1978; Suzuki and Hioki, 1979; Bell and Colin, 1986; Hamamoto et al., 1992; Yokoyama et al., 1994), including two reports for the subfamily Caesioninae. Bell and Colin (1986) and Yokoyama et al. (1994) described the reproductive behavior of *Caesio teres* in the field and *Pterocaesio digramma* in an aquarium, respectively.

Early life histories of the Lutjanidae have been reported for many species in recent decades; nevertheless, difficulty in identifying material at the species level because of the lack of information on egg and larval characters. This is especially so in the subfamily Caesioninae, the subject of only four reports, *viz.* preflexion (except in the yolk sac stage) to postflexion larvae of *C. cuning* (Leis and Rennis, 1983), a juvenile of *Caesio* sp. (Kojima, 1988), embryonic development and preflexion larvae of *P. digramma* (Yokoyama et al., 1994) and preflexion (except in the yolk sac stage) to postflexion larvae in 7 species (Reader and Leis, in press). Data of embryonic and yolk sac larval development of the Caesioninae is nothing, other than those of Yokoyama et al. (1994).

Therefore the purpose of this study was mainly to clarify early ontogeny of *C. caerulaurea.* This paper describes reproductive behavior and embryonic development from newly-hatched to 48-hour old larvae of *C. caerulaurea* observed in an aquarium. In addition, comparisons are made between reproductive behavior, embryonic development and larvae of *C. caerulaurea* and other lutjanid species where known.

Materials and Methods

Observations of reproductive behavior

The adult brood stock of 67 *Caesio caerulaurea* were captured in a drive-in net (oikomi-ami) and a stationary trap net (teichi-ami) off the north coast of Okinawa Island, Okinawa Prefecture from 1988 to 1993. The fish were reared in a rectangular concrete tank (27.0 m × 12.0 m × 3.5 m deep; water capacity 1100 t) at the Okinawa Expo Aquarium (*sensu* Yokoyama et al., 1994), Okinawa Prefecture. The surface of the tank allowed indirect natural light to enter from the northeast side, after being reflected from the glass ceiling (10 m×8 m) of an adjacent tank.

Thirty-seven *C. caerulaurea* remaining alive at the end of May, 1994, measuring approximately 30 cm in total length (TL), were employed for observations. Five other lutjanid species of approximately 1200 individuals and 38 other species of marine fishes, totalling approximately 600 individuals were reared in the same tank. The fishes were fed every day, except Thursday, at approximately 15:00. Water temperature was measured every day at 09:00 throughout 1994.

Observations of reproductive behavior of *C. caerulaurea* were made everyday between about 16:00 and 20:00 from June 9 to July 16, 1994, and behavior of this species was sometimes observed for the year. The whole of the school was observed through an acrylate panel (27.0 m×3.5 m high) of the tank, and photographic records were also used for the analyses of behavior. No lighting equipment was used during observations, because the surface of the tank was lit up with the light of the tank's keeper deck throughout the day. Additionally, there was no obvious sexual dimorphism in *C. caerulaurea*, thus a parental fish having swollen abdomen, was regard as a female. However, sex ratio of this species in the tank could not be clarified.

Rearing of eggs and larvae

Eggs of *Caesio caerulaurea* spawned naturally on June 27 and 30, 1994, and collected immediately by dip net, were used for observations of embryonic and prelarval development. Because spawnings of *Pterocaesio digramma* were also observed in the tank at the similar period, the collection of these eggs was made as far away as possible from the spawning site of *P. digramma* in order to prevent mixing of the eggs of the two species. Mixing of these eggs occurred or not, was distinguished by the color of their oil globule: the oil globule of *C. caerulaurea* was orangish; that of *P. digramma*, slightly yellowish. These eggs collected were maintained in a 1 *l* glass beaker, the temperature during the rearing trials being kept at 24.0±0.5°C (mean±standard deviation [SD]). At observations of embryonic development, over ten eggs were observed at each observation time.

Illustrations of live specimens were made with the aid of a drawing attachment on a microscope, and many color photographs were also taken. All measurements were from live specimens. Myotome counts followed Leis and Rennis (1983), postanal myotomes including the terminal myomere from which the urostyle will form.

Serial samples of egg and larval specimens observed during the study were deposited in the Department of Animal Science, Fisheries Science Course, Miyazaki University (MUFS): eggs, MUFS 11051–11064 and larvae, MUFS 11065–11075.

Results

Reproductive behavior

Reproductive behavior of *Caesio caerulaurea* was observed almost everyday from June 11 to July 16, 1994 (Fig. 1), but the spawnings of this species were not observed during other months. Reproductive behavior took place for 2–3 hours each evening, with between 1–7 spawnings by different females being recorded each time. Location of spawnings within the tank appeared to be random. The time of first spawning on each day changed from 16:45 to 20:05 (i.e. changing from 156 minutes before sunset to 41 minutes after sunset), being not associated with the lunar cycle (Fig. 1). Six easily distinguishable behavior phases were recognized in the spawning sequence (Fig. 2A–D).

Nuzzling.—For most of the day the fish swam slowly, forming a few schools in the lower part of the tank. However, about 1–1.5 hours before spawning occurred, one or two males in the school initiated courtship behavior toward a selected female by pecking and pushing her swollen abdomen with their snouts (Fig. 2A). In this early stage, such courtship behavior was always interrupted with the spawners returning to the school.

Several males joining in courtship.—As spawning time drew near, 2–6 males within the school participated in courtship behavior, each male attempting to position its abdomen close to that of the female (Fig. 2B).

Spiraling towards the surface.—One of the males and the female assumed the position which their abdomens were orientated towards each other, but the male and female forming a pair, were random each spawning. At this point the pair began to ascend quickly in a spiral, keeping their abdomens side-by-side. In addition, the remaining males

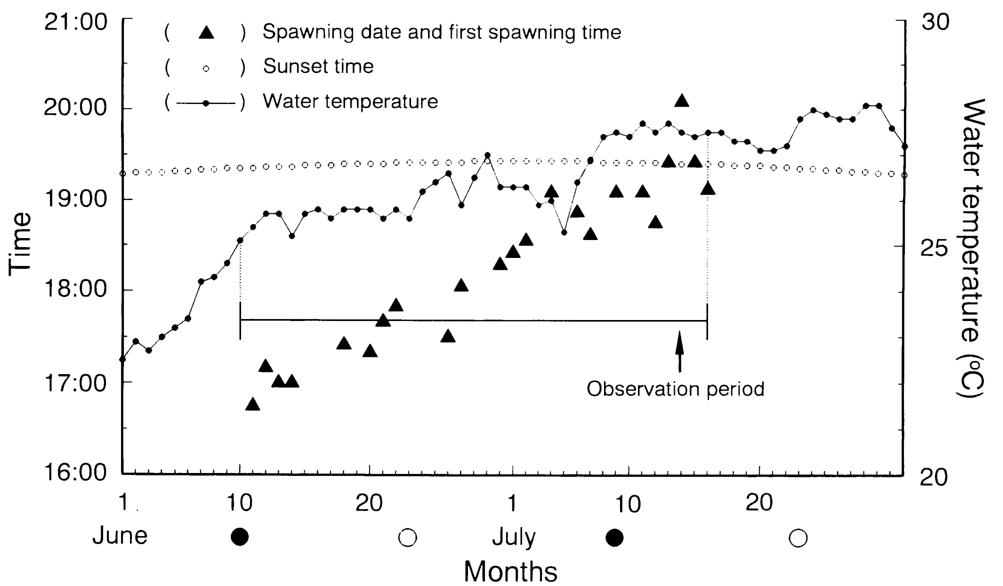

Fig. 1. Reproductive behavior of *Caesio caerulaurea* in an aquarium; spawning dates and daily changes in time of first spawning. Dotted and open circles indicate a new moon and a full moon, respectively.

(sneakers) pursued the couple (Fig. 2C). However, there was no clear difference in appearance between the one and remaining males, no aggressive or territorial behavior among the males being evident.

Pair spawning.—The initial pair released eggs and sperm in the middle-upper part of the tank.

Sperm release by sneakers.—Just after the initial spawning, the sneakers released sperm together at the pair spawning spot (Fig. 2D).

Post spawning.—After the release of gametes, the pair and sneakers scattered and returned to the school.

Occasionally, spawnings unaccompanied by sneakers were observed. In such cases, the behavior differed only in that the spawning pair ascended directly rather than in a spiral. No predation of eggs by any of the spawning adults of *C. caerulaurea* were seen, although schools of *Pterocaesio digramma* were often seen eating the spawned eggs just after the release of gametes.

Eggs and embryos

The newly-fertilized eggs were spherical, transparent, buoyant and unpigmented. Eggs ranged from 0.80 to 0.84 mm in diameter (0.82 ± 0.02 mm, $n = 20$) (mean$\pm$SD) and had a single, orangish oil globule measuring 0.16–0.18 mm in diameter (0.172 ± 0.006 mm, $n = 20$), a clear and unsculptured chorion and homogeneous, unsegmented yolk.

Embryonic development is summarized in Table 1 and Figure 3. Fifty-two minutes after fertilization, the eggs reached the eight-cell stage (Fig. 3A). At 10 hr 22 min, the blastoderm covered three–fourths of the yolk and the embryonic body was visible (Fig. 3B). At 13 hr 57 min, 12 myotomes and several punctate melanophores on the oil globule were visible (Fig. 3C). At 22 hr 57 min, immediately before hatching, 28 myotomes were apparent, the posterior half of the body being separated from the yolk (Fig. 3D). In addition, punctate or dendritic melanophores were observed along the dorsal side and on the posteroventral part of the embryonic body, and 0 or 1 melanophore on the yolk and about 10 melanophores on the oil globule were visible. Hatching began about 23 hours after fertilization, 60% of the eggs having hatched within the following 2 hours at 24.0 ± 0.5°C.

Larval morphology

Total lengths of larval *Caesio caerulaurea* at various times of development are given in Table 2. Just-hatched larvae (Fig. 4A) were transparent with some pigment, ranging from 1.90 to 2.28 mm (2.11 ± 0.15 mm, $n = 5$) in notochord length. The larvae

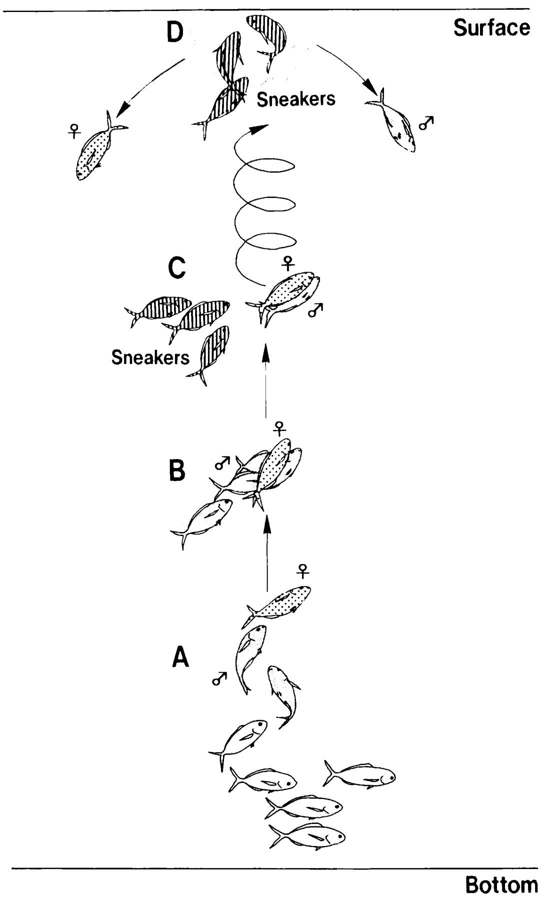

Fig. 2. Stages in the spawning sequence of *Caesio caerulaurea* in an aquarium as observed from the side of the tank. See text for explanation.

had a large yolk, no mouth and limited swimming abilities. The anus was not contiguous with the posterior end of the yolk. Small granules on the surface of the finfold and yolk, and a tortoiseshell-like pattern of short lines on the surface of the finfold were observed, but are not illustrated. The granules became thickened in 12-hour old larvae. The total number of myomeres ranged from 28 in just-hatched larvae (Fig. 4A) to 26 in 48-hour old larvae (Fig. 4D). Preanal myomeres numbered 10; postanal, 18 to 16.

Just-hatched larvae (Fig. 4A) had a large, round, ellipsoidal yolk that extended slightly beyond the anterior portion of the snout. A single oil globule was situated at the anterior tip of the yolk with its anterior two-thirds protruding out of the latter. The diameter from the anterior tip to the posterior tip of the yolk decreased from mean 1.08 mm in just-hatched larvae (Fig. 4A) to mean 0.25 mm in 48-hour old larvae (Fig. 4D). The diameter of the oil globule decreased from mean 0.17 mm to mean 0.11 mm. The gut began to coil in 18-hour old larvae. In 24-hour old larvae (Fig. 4C), the urinary bladder was visible, and the pectoral fin rudiments had become clear. In 48-hour old larvae (Fig. 4D), the mouth and anus opened.

Larval pigmentation

Just-hatched larvae (Fig. 4A) had many dendritic melanophores along the body axis from the head to the caudal section. In 6-hour old larvae (Fig. 4B), the melanophores on the body, except 1–2 melano-

Table 1. Embryonic development of *Caesio caerulaurea* at water temperatures of 24.0±0.5°C

Time after spawning	Developmental stages observed
52 min	8-cell stage (Fig. 3A)
3 hr 42 min	Early blastula stage
6 hr 37 min	Gastrula stage
10 hr 22 min	Beginning of embryo formation (Fig. 3B)
10 hr 57 min	Appearance of 4 myotomes
11 hr 37 min	5-myotome stage: formation of optic and Kupffer's vesicles
13 hr 23 min	10-myotome stage: appearance of melanophores on embryo and yolk
13 hr 57 min	12-myotome stage: appearance of melanophores on oil globule (Fig. 3C)
15 hr 37 min	16-myotome stage: formation of auditory vesicles
16 hr 27 min	18-myotome stage: formation of optic lenses
17 hr 12 min	19-myotome stage: appearance of heart
18 hr 32 min	22-myotome stage: disappearance of Kupffer's vesicle
19 hr 12 min	23-myotome stage: beginning of heart pulse
20 hr 17 min	27-myotome stage: appearance of pectoral fin rudiments and beginning of embryonic movement
22 hr 57 min	28-myotome stage: immediately before hatching (Fig. 3D)

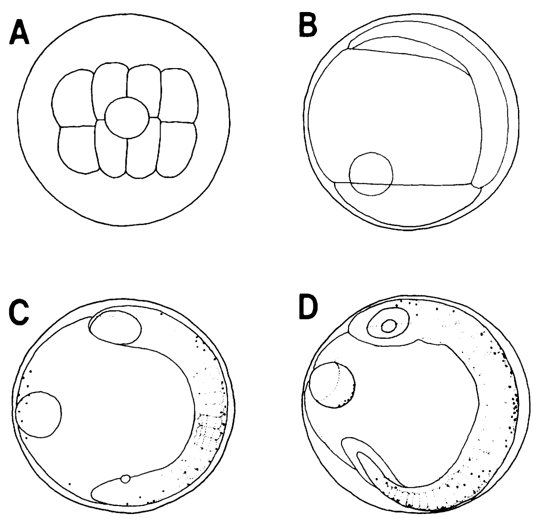

Fig. 3. Embryonic development of eggs of *Caesio caerulaurea*. A) 8-cell stage, 52 min after fertilization, MUFS 11051; B) beginning of embryo formation, 10 hr 22 min, MUFS 11054; C) 12-myotome stage, 13 hr 57 min, MUFS 11057; D) 28-myotome stage, 22 hr 57 min, MUFS 11064.

phores on the posterodorsal surface of the notochord, began to move towards the lateral and ventral surfaces of the body. One dendritic melanophore appeared on the posterior tip of the notochord in 12-hour old larvae. In 24-hour old larvae (Fig. 4C), the melanophores on the body were confined to the ventral midline of the trunk and tail posterior to about the 7th myotome on the trunk with the melanophores on the posterodorsal surface of the notochord having disappeared. The number of melanophores on the body decreased from 52–66 in just-hatched larvae (Fig. 4A) to 23–29 in 48-hour old larvae (Fig. 4D).

Just-hatched larvae (Fig. 4A) had 8–10 melanophores on the surface of the oil globule, but the yolk was unpigmented. The melanophores on the oil globule had disappeared in 18-hour old larvae, however. In 6-hour old larvae (Fig. 4B), 10–25 melanophores were visible on the yolk, with 9–12 melanophores observed on the yolk sac in 48-hour old larvae (Fig. 4D).

Just-hatched larvae (Fig. 4A) had several dendritic melanophores on the posterodorsal surface of the head, but the eye was unpigmented. Eye pigmentation started in 6-hour old larvae (Fig. 4B). In 36-hour old larvae, one or two dendritic melanophores were present just posterior to the auditory vesicle, becoming strongly dendritic in 48-hour old larvae (Fig. 4D).

Table 2. Total length (including the anterior portion of the yolk sac membrane protruding beyond the larval snout) of larval *Caesio caerulaurea*

Time after hatching	Range, mean and SD of TL (mm)
Just-hatched	2.02–2.46 (2.27±0.16, *n* = 5, Fig. 4A)
6 hr	2.32–2.62 (2.43±0.10, *n* = 5, Fig. 4B)
12 hr	2.32–3.06 (2.61±0.28, *n* = 5)
18 hr	2.42–2.78 (2.61±0.11, *n* = 5)
24 hr	2.64–3.00 (2.76±0.13, *n* = 5, Fig. 4C)
36 hr	2.90–3.20 (3.04±0.10, *n* = 5)
48 hr	2.78–3.09 (2.95±0.10, *n* = 5, Fig. 4D)

Discussion

Reproductive behavior

The reproductive behavior of *Caesio caerulaurea* was similar to that of *Pterocaesio digramma* (Yokoyama et al., 1994), spawnings of both the species occurring between a pair plus sneakers. On the other hands, the genus *Lutjanus*, *viz. L. synagris* (Wicklund, 1969), *L. kasmira* (Suzuki and Hioki, 1979), *L. stellatus* (Hamamoto et al., 1992) were group spawners, spawnings of all taking place among plural fish simultaneously. Thus, reproductive behavior of the family Lutjanidae may be different between the Caesioninae and Lutjaninae. However, Bell and Colin (1986) reported spawning of *C. teres* as follows, "Finally these subgroups began to swirl more rapidly, releasing gametes within the main mass." Therefore, it could not be determined if spawnings of *C. teres* occurred between a pair plus sneakers.

In addition, spawnings of the above three caesionines, *C. caerulaurea*, *C. teres* and *P. digramma*, were similar in that they took place essentially before sunset. In contrast, spawnings of lutjanine fishes occurred after sunset (Wicklund, 1969; Suzuki and Hioki, 1979; Hamamoto et al., 1992). Such spawning differences may be explained by differences in life-style in the same way (Yokoyama et al, 1994), namely, the Caesioninae are diurnal fishes, whereas the Lutjaninae are nocturnal in many cases (our observation and Randall et al., 1990).

One different spawning feature was confirmed between *C. caerulaurea*, and *C. teres* and *P. digramma*

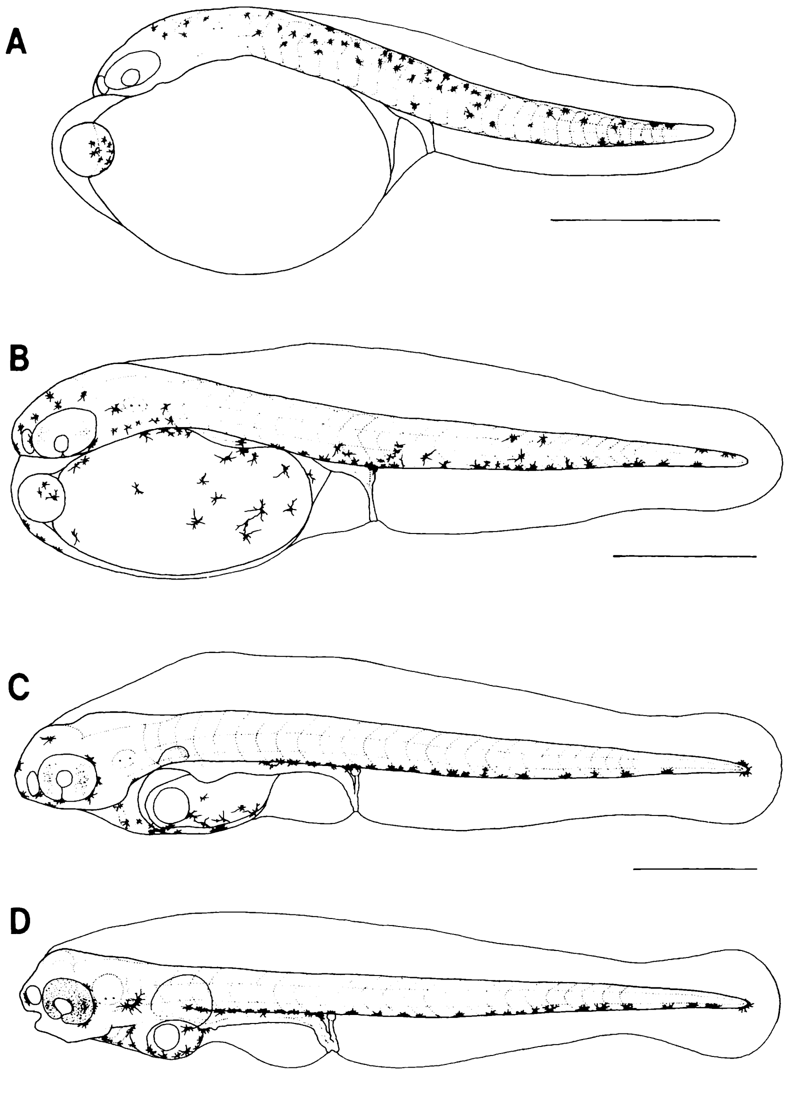

Fig. 4. Yolk-sac larval stages of *Caesio caerulaurea.* A) Just-hatched larva, 2.02 mm TL, MUFS 11065; B) 6 hrs after hatching, 2.62 mm TL, MUFS 11066; C) 24 hrs after hatching, 3.00 mm TL, MUFS 11069, 11070; D) 48 hrs after hatching, 3.09 mm TL, MUFS 11073–11075. Scale bar indicates 0.5 mm.

(Bell and Colin, 1986; Yokoyama et al., 1994). During spawning of the latter species, a large school of spawners (ca. 1000–1500 individuals) performed up and down swimming movements, whereby they periodically rose to the surface, ascending almost vertically as a column and descended in a similar manner. Such movements by schooling *C. caerulaurea* were not observed. The primary reason for this may be the small population size of *C. caerulaurea* ($n=37$) in the aquarium. Because, the movements of the large school may be useful for the spawning fish to avoid their peril by predators, conversely, in the small population size, such movements are considered to be danger of predation.

Eggs and larvae

General morphology of the eggs and early larvae of *Caesio caerulaurea* was similar to that of other lutjanid fishes for which information is available, *viz.*

Lutjanus kasmira (Suzuki and Hioki, 1979), *L. campechanus* (Rabalais et al., 1980; Minton et al., 1983), *L. russelli* (Liu and Hu, 1980), *L. vitta* (Lu, 1981; *L. vitta*? in Iwatsuki et al. [1993]), *L. erythropterus* (Zhang et al., 1985), *L. lutjanus* (Zhang et al., 1985), *L. stellatus* (Hamamoto et. al., 1992) and *Pterocaesio digramma* (Yokoyama et al., 1994).

However, the following two differences in *C. caerulaurea* were confirmed. In this species, the oil globule of just-hatched larvae protruded extensively from the anterior end of the yolk, whereas in the other lutjanids the oil globule of just-hatched larvae was situated either close to but inside the anterior margin of the yolk or protruded only slightly beyond the anterior tip of the yolk (Suzuki and Hioki, 1979; Rabalais et al., 1980; Liu and Hu, 1980; Lu, 1981; Minton et al., 1983; Zhang et al., 1985; Hamamoto et. al., 1992; Yokoyama et al., 1994). Furthermore, in *C. caerulaurea*, a few, well-defined dendritic melanophores just posterior to the auditory vesicle were present at the late yolk-sac stage (Fig. 4D), whereas in the other lutjanids, such well-developed melanophores were not present at any time during the yolk-sac stage (Suzuki and Hioki, 1979; Rabalais et al., 1980; Lu, 1981; Minton et al., 1983; Zhang et al., 1985; Hamamoto et. al., 1992; Yokoyama et al., 1994). Thus, the larvae of *C. caerulaurea* might be distinguishable from other lutjanid fishes by these two differences at the yolk-sac stage.

Yokoyama et al. (1994) compared embryonic and larval characteristics between *P. digramma* and the subfamily Lutjaninae, noting as unique in the former the presence of melanophores on the oil globule of the eggs and just-hatched larvae. In the eggs, such melanophores were first seen 12 hr 31 min after fertilization. Eggs and just-hatched larvae of *C. caerulaurea* also had melanophores on the oil globule, those in the eggs first seen 13 hr 57 min after fertilization. On the other hand, this characteristic has not been reported from similar stages in Lutjaninae eggs and larvae (Suzuki and Hioki, 1979; Rabalais et al., 1980; Liu and Hu, 1980; Lu, 1981; Minton et al., 1983; Zhang et al., 1985; Hamamoto et. al., 1992). Accordingly, melanophores on the oil globule in both the egg and just-hatched larval stages, as seen in these two Caesioninae species, may possibly be a character distinguishing between the Caesioninae and Lutjaninae.

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タカサゴ亜科魚類ササムロ *Caesio caerulaurea* の飼育下における産卵行動とその卵内発生および仔魚

横山季代子・亀井良昭・戸田　実・
平野克己・岩槻幸雄

タカサゴ亜科魚類ササムロ *Caesio caerulaurea* の飼育下における産卵行動とその卵内発生および孵化後48時間目までの仔魚を記載した．本種の産卵は，1994年6月11日から7月16日までほぼ毎晩観察された．産卵行動は1日2–3時間観察され，1晩につき1–7回の産卵が確認された．産卵開始時刻は16時45分から20時05分で，これは日没156分前から41分後に相当した．産卵行動は6つの段階に分けられた．1) 1–2尾の雄が1尾の雌に対し求愛行動を始め，2) 産卵が近づくと，2–6尾の雄が求愛行動に参加した．3) これらの雄の中の1尾が雌と共に急上昇し，残りの雄，すなわちスニーカーがペアを追尾した．4) ペアによる産卵後，5) スニーカーによる放精が行われた．6) 放卵放精後，親魚は群れに戻った．

本種の受精卵は，油球1個を有する卵径0.80–0.84 mmの無色透明な球形分離浮遊卵で，水温24.0±0.5°Cで受精後約23時間で孵化を開始した．孵化直後の仔魚は平均全長2.27 mm，卵黄は楕円形で大きく，その先端は吻端よりわずかに前方へ突出していた．油球は卵黄の前端より大きく前方に突出し，その表面には約10個の樹枝状黒色素胞が認められた．本種の孵化後48時間目（平均全長2.95 mm）の仔魚は，耳胞後方の良く発達した樹枝状黒色素胞によって，既知のフエダイ科魚類と識別できた．

（横山・亀井・戸田：〒905–03 沖縄県本部町石川424番地　国営沖縄記念公園水族館；平野・岩槻：〒889–21 宮崎市学園木花台西1–1　宮崎大学農学部動物生産学科）