Natural Spawning of Four *Epinephelus* Species Reared in the Laboratory

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Abstract

Natural spawnings of four *Epinephelus* species reared in the laboratory were observed from 1987 to 1992. These species are: *E. summana, E. caeruleopunctatus, E. macrospilus* and *E. fuscoguttatus.* Spawning was serial, usually occurring at night, on or 1-6 days after the new moon. Egg characteristics of these four species were compared. Fertilized egg and early larval development of *E. summana* and *E. fuscoguttatus* are discussed.

Introduction

Groupers of the genus *Epinephelus* (Perciformes: Serranidae) are known for their tasty flesh and rank among the highly priced food fishes of tropical seas. They are also the basis of important sport and commercial fisheries around the world, and thus, are confronted with increasing fishery pressure in many areas (Colin 1989).

The mariculture importance of groupers is now recognized in many countries in Southeast Asia. Groupers are found to be suitable for cage and pond culture (Chua and Teng 1978, Chen 1979, Elizalde and Marcial 1983, Kohno et al. 1988, 1989) but these activities have not reached commercial scale. Fry or juveniles for culture still depends on wild catches which is dwindling, probably due to habitat destruction and overfishing. A major constraint to the large-scale production is the shortage and uncertain supply of fry in the wild (Kohno et al. 1988). Serranid larval recruitment around the central Philippines also proved to be low (Alcala and Cabanban 1986). In addition, broodstock management of groupers is difficult since most groupers show no sexual dimorphism and are reported to be protogynous hermaphrodites (Smith 1965, Tan and Tan 1974, Leis 1987).

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Successful induced breeding have been documented for *E. akaara* (Ukawa et al. 1966), *E. tauvina* (Chen et al. 1977), *E. amblycephalus* (Tang et al. 1979), *E. malabaricus* (Sutemechaikul et al. 1985), *E. salmoides* (Kungvankij et al. 1986), *E. fuscoguttatus* (Kohno et al. 1988) and *E. septemfasciatus* (Kitajima et al. 1991). Only a few species, however, were reported to spawn spontaneously in the laboratory. To date, only *E. suillus* has spawned spontaneously in captivity in the Philippines (Quinitio and Toledo 1990).

This paper describes the natural spawning of four *Epinephelus* species at Silliman University Marine Laboratory (SUML). The egg characters and early development of *E. summana* and *E. fuscoguttatus* are described.

Materials and Methods

Broodstock Collection and Maintenance. *E. suillus, E. summana, E. macrospilus, E. caeruleopunctatus* and *E. fuscoguttatus* were collected from 1984 to 1986 through an IDRC-funded Fish Seed Resources Survey Project, an SUML-BFAR collaborative project. These fishes were caught as fry and reared to adult stage. Additional juveniles were also bought from fishermen from nearby localities. They were segregated by species in one 20-ton, two 4-ton and two 2-ton cement/wooden tanks provided with sand-filtered, aerated, free-flowing seawater. The lay-out of water and aeration systems in rearing tanks is shown in Fig. 1. Broodstock were fed fish-by-catch at a rate of at least 5% body weight daily, except during spawning when feed was withdrawn from two to three days. Salinity (S) was 33-35 ppt, dissolved oxygen (DO), 4-5 ppm, and temperature, 26.5-29.5 °C.

Egg and Larval Rearing. After each spawning, eggs were siphoned into a 35-µm mesh net, washed and placed in a 10-l container. Total number of eggs was approximated by counting aliquot samples. At least half of the fertilized eggs from one spawn (first or second day spawn) were collected and transferred to 3-ton circular hatching/larval rearing tanks filled with preconditioned seawater. Seawater preconditioning included aeration, filtration, and addition of unicellular algae (i.e. *Tetraselmis* sp., *Isochrysis* sp., and *Chlorella* sp.) at a density of 500-1000 x 10^3 cells/ml 2-3 days prior to stocking with eggs. During the larval stages, different food sources were introduced to the tanks (Figs. 2 and 3). Sediments in the bottom of the rearing tanks were siphoned off daily and the volume of water siphoned was replaced with filtered seawater from the spawning tank.

Sampling of eggs was done hourly within the first 24 h and larvae were sampled twice daily during the next four days after hatching. Sampling frequency was reduced thereafter to minimize disturbance of the larvae. Egg and larval characters were noted from live specimens observed with compound microscope or stereoscope provided with an eyepiece micrometer.

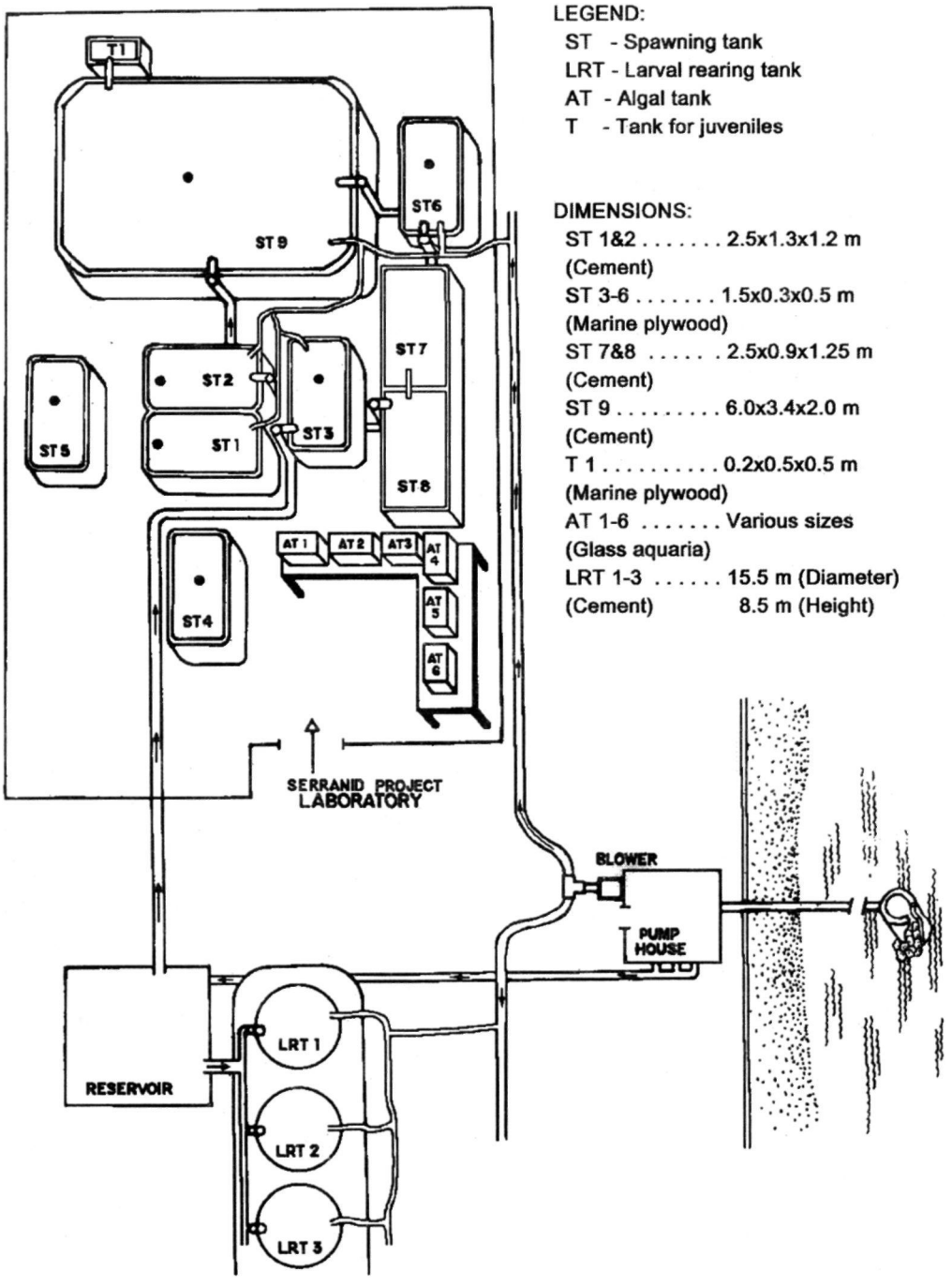

Fig. 1. Lay-out of water and aeration systems in serranid culture and rearing tanks.

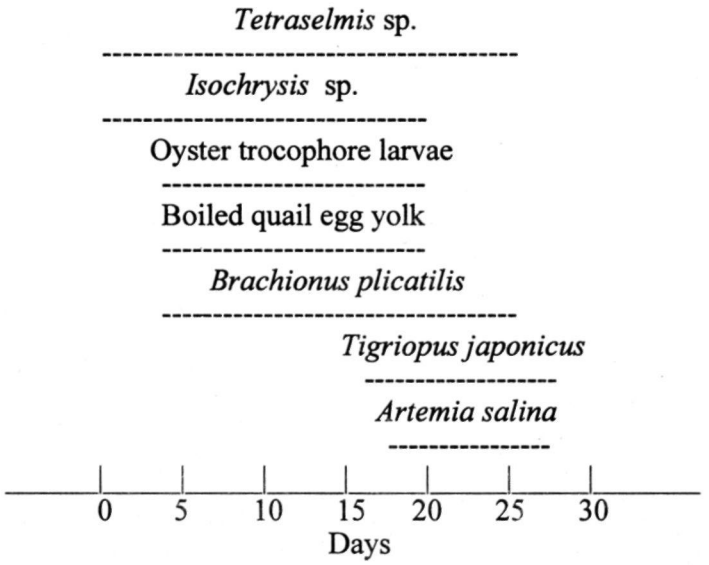

Fig. 2. Food used for rearing larvae of *Epinephelus summana* in the laboratory.

Results

Spawning. Except for *E. suillus,* all species of captive groupers spawned spontaneously in the laboratory (Table 1). *E. summana* was first observed to spawn on 12 October 1987. *E. macrospilus, E. caeruleopunctatus* and *E. fuscoguttatus* spawned in July and October 1989. Fertilized spawns were obtained from *E. fuscoguttatus* (100%) and *E. summana* (71%) while all spawns of *E. macrospilus* and *E. caeruleopunctatus* were unfertilized. Sizes of grouper broodstock at first maturity are given in Table 1.

Spawning normally occurred at night between 2100 h and 0200 h, 1-6 days after the new moon phase. Spawning for all species were most often simultaneous (i.e., occurring at the same night) and serial, continuing for 1-4 nights in a row except for *E. macrospilus* where 1- to 2-day gaps sometimes occur between spawnings. Although direct observations of spawning behavior was not done, spawners seem to leap out of the water during spawning. This behavior may have contributed to at least three spawner mortalities as they leap out and land outside the tanks until covers were provided.

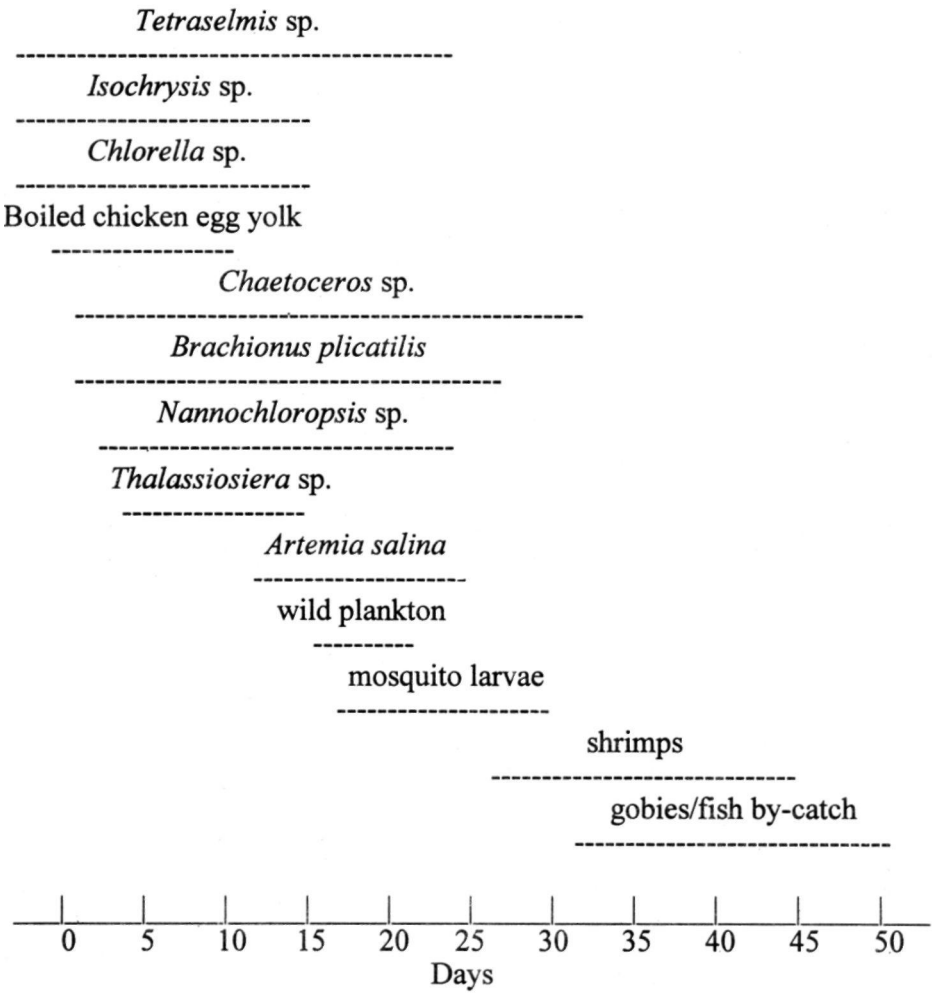

Fig. **3.** Food used for rearing larvae of *Epinephelus fuscoguttatus* in the laboratory.

Spawning occurred 1-8 months in a year. Each species exhibit peak spawning months (i.e., highest number of spawning days in a month): July-August and October for *E. fuscoguttatus,* August-October for *E. summana,* March-April for *E. caeruleopunctatus,* March-April and August-September for *E. macrospilus* (Table 2). About 45,000-1,872,000 eggs per spawning were released by these species, with *E. fuscoguttatus* producing the highest number of eggs per spawn.

Table 1. Size of broodstock, number of spawnings, fertilization success per spawning of grouper (*Epinephelus*) species reared in the laboratory.

Species	Number	Total length at 1st spawning (cm)	Dates of 1st and last recorded spawning	Number of spawnings	%Batch fertilization success	%Egg viability
E. suillus	4	50-60	-	-	-	-
E. fuscoguttatus	2	41-47	10/12/90-08/01/92	25	100	>50
E. summana	3	25-28	10/27/87 - 09/27/91	66	71	<50
E. caeruleopunctatus	2	25-28	10/03/89 - 06/06/92	25	0	0
E. macrospilus	2	28-30	07/06/89-04/27/91	48	0	0

Table 2. Periodicity and seasonality of reproduction of four grouper (*Epinephelus*) species in the laboratory.

Species	Number of spawnings Days/Mo.	Mos./Year	Months	Peak months	Approx. number of eggs released per day (x 10^3)
E. fuscoguttatus	1-4	1-6	Jun-Dec	Jul-Aug, Oct	350 - 1,872
E. summana	1-4	2-7	Feb-Dec	Aug-Oct	65 - 750
E. caeruleopunctatus	1-3	1-6	Mar-Jan	Mar-Apr	45 - 1,200
E. macrospilus	1-2	1-8	Mar-Jan	Mar-Apr, Aug-Sep	75 - 1,100

Eggs. Eggs of the four species were pelagic, spherical and non-adhesive with a transparent and smooth chorion and vitelline membrane. Yolk was colorless, translucent, unpigmented, and homogeneous. A single oil globule was present in eggs of *E. summana, E. caeruleopunctatus* and *E. macrospilus. E. fuscoguttatus* had 1-10 oil globules which coalesced in time. Perivitelline space of unfertilized eggs in all species was narrow and indiscernible from the chorion. Unfertilized eggs became white, opaque, and sink after some time,

Fertilized eggs of *E. summana* had the smallest egg diameter (0.78±0.04 mm) while *E. fuscoguttatus* had the largest (0.89±0.02 mm) (Table 3).

Table 3. Egg and oil globule diameter of four grouper species spawned in the laboratory.

Species	Egg diameter, mm		Number	Oil Globule diameter, mm	
	Average	(Range)		Average	(Range)
E. caeruleopunctatus	0.84±0.02	(0.82-0.86)	1	0.20±0.01	(0.18-0.22)
E. macrospilus	0.84±0.01	(0.82-0.86)	1	0.17±0.02	(0.13-0.20)
E. summana	0.78±0.02	(0.75-0.77)	1	0.20±0.02	(0.15-0.24)
E. fuscoguttatus	0.89±0.02	(0.85-0.91)	1-10	0.17±0.06	(0.02-0.26)

Embryonic and Larval Development. Fertilized eggs of *E. summana* and *E. fuscoguttatus* exhibited meroblastic cleavage. Embryonic development was similar in the two species, however, *E. summana* developed faster than *E. fuscoguttatus.* Incubation period at 26-30 °C varied from 16-19 h for *E. summana* eggs , and 18-22 h for *E. fuscoguttatus* at 26.8-28.5 °C.

Newly-hatched larvae of *E. fuscoguttatus* (TL, 1.64±0.19 mm) were larger than those of *E. summana* (TL, 1.46±0.67mm) (Table 4). In both species, larvae were transparent, had limited motility, unpigmented eyes and body, differentiated brain parts, and simple alimentary canal, closed at both ends. Sequence of development were similar in both species. Yolk and oil globule resorption occurred within 2-3 days after hatching with a concomitant increase in mouth size and larval lengths. Characters associated with feeding and swimming developed first: mouth (day 2-3), pigmented eyes with transparent lenses (day 2-3), looping of the alimentary canal and formation of the anal vent (day 2-3), pectoral fin and caudal fin ray differentiation (day 1-2). Differences occur in development time and in sizes at each stage of larval development.

Table 4. Characteristics of *Epinephelus summana* and *E. fuscoguttatus* newly-hatched larvae reared in the laboratory.

Species	Total length (cm)	Yolk sac length (cm)	Oil globule diameter (cm)
E. summana	1.46 ±0.67	0.58 ±0.67	0.19 ±0.10
E. fuscoguttatus	1.64 ±0.19	1.11 ±0.74	0.23 ± 0.03

Transformation of transient larval characters was not observed in *E. summana* since the larvae died at day 27. Mortality was caused by total power failure caused by typhoon "Ruping" in 1990. In *E. fuscoguttatus,* some individuals started to settle on day 36-47 at the average size of 26.57±9.48 mm TL. Sizes of larvae at this age, however, ranged from 7-65 mm TL. Heavy cannibalism was observed during transformation and early juvenile stages.

Discussion

Spawning. The relationship between spawning periods of groupers and water temperature and other factors have been examined by several workers (Leis 1987). However, no factor or combination of factors was found to clearly explain variability in time of year at which groupers spawn. Groupers seem to spawn during a restricted period: from 1-2 months to 6-8 months, more often 1-5 months in a year. Spawning season for *E. akaara* was from July to September (Ukawa et al. 1966), July to August (Maruyama et al. 1992), or April to June with a reproductive peak in May (Tseng and Ho 1988). For *E. tauvina,* spawning season was from April to May at 25 °C (Hussain and Higuchi 1980). *E. suillus* spawned from July to September (Quinitio and Toledo 1990).

Epinephelus tauvina was reported to spawn only during days 27-13 of the lunar month, *E. striatus* during lunar days 14-18 (Johannes 1978), and *E. merra* for 3-4 days out of each reproductive month (Randall and Brock 1960). *E. suillus* was observed to start spawning 2-3 days before the last quarter of the lunar phase between 1700-1900 h (Quinitio and Toledo 1990). Factors to explain variability in the time or day at which groupers spawn need to be examined.

Eggs. There seems to be little morphological variation exhibited by eggs of *Epinephelus* species which have diameters ranging from 0.70-1.20 mm. Similarities in egg characters typify that of the family Serranidae (Leis 1987). Differences in average diameter of the eggs, however, occur among species. In terms of egg sizes, it

could be inferred that *E. fuscoguttatus* has a higher aquaculture potential than *E. caeruleopunctatus, E. macrospilus,* or *E. summana.* Egg size has direct correlation to the subsequent survival and development of the individual or species since it influences rate of development, larval size, and survival potential (Bagarinao and Chua 1986, Blaxter 1988).

Embryonic and Larval Development. Except for development time, no remarkable differences in embryonic development was observed between *E. summana* and *E. fuscoguttatus,* and among other *Epinephelus* species studied (Ukawa et al. 1966, Tseng and Ho 1979, Huang et al. 1986, Hussain et al. 1975, Chen et al. 1977, Kohno et al. 1990). *E. summana* eggs (smaller type) had a faster rate of development than *E. fuscoguttatus* eggs (larger type) due to the relatively small size of their yolk. Development time of *E. fuscoguttatus* in this study and in Kohno et al. (1990) is similar.

Temperature influences incubation time. Incubation of *Epinephelus* eggs takes about 19-43 h after fertilization under various water temperatures (Leis 1987). *E. akaara* eggs hatch within 23-25 h after fertilization at 22-27 °C (Ukawa et al. 1966, Tseng and Ho 1979, Xu et al. 1985, Maruyama et al. 1992), *E. gigas* took 38-43 h (Barnabe 1974), while *E. striatus* eggs which are large (1.02mm) after 40 h at 25 °C (Guitart Manday and Juarez Fernandez 1966). Eggs of *E. summana* and *E. fuscoguttatus* similarly hatched within the reported incubation period for groupers.

Newly-hatched larvae of *E. summana* reported in this study are the smallest compared to those of other groupers (Leis 1987). Larvae of *E. fuscoguttatus* in this study are relatively larger than those reported by Kohno et al. (1990). Since egg sizes are similar in both studies, other factors controlling larval size must have been observed. Kohno et al. (1990) attribute intraspecific variation in size and relatively gentle growth curve to errors in sampling time.

Heavy mortalities have been reported during the early larval stages of *Epinephelus* species (Ukawa et al. 1966, Tseng and Ho 1979, Tseng and Chan 1985, Hussain and Higuchi 1980, Chen et al. 1977, Lin et al. 1986, Kohno et al. 1990). This could be due to failure of the larvae to start feeding and/or learn to feed (Kohno et al. 1990). Mouth width establishes the upper limit of prey size (Hunter and Kimbrell 1980) and unavailability of correctly-sized food organisms contributes to mortality during the first feeding stages (Hussain and Higuchi 1980).

Larval settlement in *Epinephelus* species takes place at a fixed size or development stage which occurs at about 25 mm rather than after a given period (Leis 1987). Time to reach that size vary from 30 to 40 days. The largest *Epinephelus* larva reported from field samples is 27 mm (Heemstra 1974) while settled individuals as small as 22.40 mm have been reported (Aboussouan 1972).

After larval settlement, however, variation in growth of *E. fuscoguttatus* individuals results in a wide range of body sizes which seems to be characteristic of *Epinephelus* species. Growth rates vary among species, different rearing experiments and within rearing experiments for a given species (Mito et al. 1967, Leis 1987). Variability in sizes offer hindrances in the management of mass-produced *Epinephelus* larvae. Cannibalism occurs as smaller-sized individuals are automatically considered prey for the larger-sized individuals. Thus, this factor also limits the success of rearing groupers in the laboratory.

Conclusion. Among the four species that spawned naturally in the laboratory, only eggs of *E. summana* and *E. fuscoguttatus* were fertilized. Only *E. fuscoguttatus* was successfully reared to the juvenile stage. Based on our observations, *E. fuscoguttatus* is a better candidate than *E. summana* because of higher fecundity, fertilization success, and egg viability as well as its relatively larger egg, yolk, and larvae. These are characteristics which have direct correlation to survival and development of the individual or the species. To date, about 100 8-months old and 20 1.5-years old *E. fuscoguttatus* juveniles survive at SUML. Based from the approximate total number of eggs released per spawning night, survival rate is less than 0.01%. More studies are thus needed to improve this percentage. However, the fact that the four species can spawn naturally in the laboratory points positively to future breeding endeavors.

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