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Induced breeding and embryogenesis of vermiculated spine foot, *Siganus vermiculatus,* in captivity

A. Anuraj*, P. P. Suresh Babu, Tanveer Hussain, R. Siju, Jayasree Loka, Mahendra Pal, N. Anjulekshmi, K. S. Aneesh and Boby Ignatius
ICAR-Central Marine Fisheries Research Institute, Kochi-682 018, Kerala, India.

*Correspondence e-mail: anurajarsicar@gmail.com
ORCiD: https://orcid.org/0000-0002-8893-9324

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Original Article

Abstract

*Siganus vermiculat*us, commonly known as the vermiculated spinefoot, is one of the most promising mariculture species within the Siganidae family due to several advantageous characteristics. The present study investigated the broodstock development, induced breeding, and embryonic development of the *S. vermiculatus*. Wild-caught specimens (211-325 g) were collected from Kali estuary (14 °51′ 0″ N and 74 °8′ 0″ E), Karwar, Uttara Kannada and reared in marine cage farm conditions for six months (April to September 2021). Mature brooders were selected through cannulation, with breeding induced using LHRH-a (20 µg/kg) administered intramuscularly. Spawning occurred during the third quarter of the lunar cycle, approximately 20 h, 15 min. post-injection. Fertilized eggs were spherical, demersal, adhesive, and transparent, measuring 0.56 ± 0.03 mm. Embryonic development proceeded through characteristic teleost patterns, beginning with meroblastic discoidal cleavage resulting in a 2-cell stage at 18 min. post-fertilisation. Subsequent development included blastula formation, yolk syncytial layer, epiboly, embryonic shield, axis establishment, somite formation, optic bud and otic vesicle development, and cardiac function initiation. Embryonic movements began at 16 h 15 min., with hatching occurring between 22 h 39 min. and 23 h 55 min. at 32 ppt and 29.5±0.4 °C. Newly hatched larvae measured 1.9 ± 0.11 mm in total length with an initial yolk volume of 0.015±0.003 mm^3 and oil globule volume of 0.004±0.001 mm^3. This detailed characterization of *S. vermiculatus* embryonic development contributes valuable information to teleost developmental biology and provides essential knowledge for optimizing hatchery protocols, potentially enhancing aquaculture production of this commercially important species.

Keywords: *Siganus vermiculatus, siganidae, induced breeding, embryogenesis*

Introduction

External mode of fertilization is the common reproductive strategy in aquatic environments (Giese and Kanatani, 1987). The vast majority of fish species, exceeding 95%, employ external fertilization as their primary reproductive strategy (Fitzpatrick, 2020, Goncalves and Oliveira, 2011). Fishes belonging to the rabbitfish family, Siganidae exhibit external fertilization and pelagic larval development. These fishes exhibit a broad distribution throughout the Indo-Pacific realm, extending from Africa's eastern coastline to Polynesia, and spanning from Australia's northern regions to southern Japan (Herre and Montalban, 1928) and the Mediterranean region (Ben-Tuvia, 1966). The species belonging to Siganidae inhabit estuarine waters to coral reefs (Kohno *et al.,* 1988) and 15 species belonging to rabbit fishes have been reported from Indian waters (Murugan and Namboothri, 2012). Nearly all species of rabbit fishes spawn demersal eggs except *Siganus argenteus* (Lacson and Nelson, 1993).

Broodstock development, breeding and seed production in captivity of several rabbit fishes have been achieved in various parts of the world (Durray, 1998; Anuraj *et al.*, 2021). Although gametogenesis is achieved in captivity, oocyte maturation and ovulation in females, and spermiation in males require exogenous hormonal therapies to facilitate manual or volitional spawning (Berlinsky *et al.*, 2020). These reproductive manipulations are essential for consistent seed production in controlled environments. *Siganus vermiculatus* or vermiculated spinefoot is one of the fastest-growing fish among siganids and grows faster in captivity compared to other rabbit fishes while also displaying tolerance to a wide range of salinity, temperature and pH (Gundermann

et al., 1983). *S. vermiculatus* has been induced to spawn in captivity with hormones (Popper and Gundermann, 1976; Popper *et al*., 1976; Avila, 1984; Anuraj *et al*., 2019; 2021), demonstrating the feasibility of captive breeding for this species. The standardized captive breeding technology has significant implications for both commercial aquaculture (in terms of species diversification in marine and brackishwater environments) and conservation efforts.

Spawning in rabbit fishes can be related to the lunar cycle (Hara, 1986; Rahman *et al*., 2003; Takemura *et al*., 2010). Induced spawning in *S. vermiculatus* has been reported on or around the first quarter (Popper *et al*., 1976; Popper and Gundermann, 1976; Avila, 1984; Anuraj *et al*., 2019) and after the full moon (Anuraj *et al*., 2021) of the lunar cycle. Comprehensive documentation of the embryonic development of *S. vermiculatus* remains limited even though commercial technology for seed production is available. Basic ontogeny studies are essential for evaluating the variations in development in various environmental conditions. The present study was aimed at comprehensively documenting the embryonic development of *S. vermiculatus* from fertilization through hatching to the yolk-sac larval stage.

Material and methods

Fish collection, broodstock development and sex determination

Fishes (211-325 g) were collected from the wild from Kali estuary (14° 51′ 0″ N and 74° 8′ 0″ E) located in the northern part of Uttara Kannada district, Karwar, Karnataka. The fishes were caught using traps, gill nets and shore seines. The collected fishes were reared in a circular galvanised iron cage (6 m diameter x 3 m depth) over six months (April to September 2021) in a marine cage farm of Karwar Regional Station of ICAR-CMFRI @ 2 number/m^3. The fishes were fed twice daily with enriched pellet feeds (40% crude protein) @ 2% bodyweight of stocked fishes. In addition to pellet feed, squid meat cut into small pieces was also fed to the fish once every 3 days. After this rearing period, the sex and maturity of the fishes were determined using a polyethene cannula. The fishes were anaesthetized using clove oil (Omm Laboratories, Karnataka, India) at a dose of 10 µL / L of seawater before cannulation (Anuraj *et al*., 2021).

Brooder selection and induced breeding

The hydrated females and running males (274-455 g, 25 numbers) were transported to the hatchery complex of Karwar RS of ICAR-CMFRI and quarantined for seven days. After the quarantine period, fishes were reared inside a recirculatory tank of 5-ton capacity filled with 32 ppt dechlorinated seawater. After an acclimation period of 15 days, a female fish (375 g, 22 cm) with an average ova diameter above 400 µm and a male fish (345 g, 20.6 cm) with running milt were introduced into a 500 L cylindrical FRP spawning tank filled with 32 ppt dechlorinated seawater to a height of 60 cm. The fish were fed with similar feed in quarantine and recirculatory tanks. The brood pair consisting of 1 male and 1 female fish were injected with LHRH-a (Samarth Life Sciences Pvt. Ltd., Mumbai, India). LHRH-a @ 20 µg/kg was injected intramuscularly below the dorsal fin, twice (at 24 h intervals) to the female brooder and the male brooder was administered hormonal injection at the time of the second injection of female. As *S. vermicualtus* eggs are demersal and adhesive, ceramic tiles were placed to facilitate the attachment and shifting of eggs to other tanks (Fig. 1).

Embryonic development

To study the embryonic developmental stages, a total of 300 adhesive fertilized eggs were collected from ceramic tiles and incubated in a 500 ml glass container filled with filtered dechlorinated seawater of 32 ppt at 29.5±0.4 °C. Photographs of 30 samples were taken before the eggs were placed in the container. Sub-samples of 10-15 eggs were photographed at five-minute intervals till hatching. Photomicrographs and measurements were taken with the Zeiss Axio Scope A1 microscope attached to the Jenoptik ProgRes C3 digital camera fitted to the microscope. The duration of embryonic development stages from fertilized egg to hatching and important characteristics during these stages were also recorded.

Fig 1. Breeding setup with ceramic tiles as substrate for egg attachment at the bottom of the FRP tank

Endogenous reserves

The yolk globule volume was calculated by the formula for a prolate spheroid V= $\pi/6$ x l x w^2, where 1 is yolk sac length and w is yolk sac width (Blaxter and Hempel, 1963). The volume of the oil globule was computed from the formula V= $\pi/6$ x d^3, where d is the oil globule diameter (Bagarinao, 1986).

Results and discussion

The male and the female fish swam closely and touched each other with their body in the tank after the second dose of hormonal inducement. In paired fishes, the male and the female may position their genital pores close and synchronize the release of eggs and sperm (Breder and Rosen, 1966). During spawning, female fish exhibited touching and nudging behaviour as reported in *S. canaliculatus* (McVey, 1972) and *S. vermiculatus* (Anuraj *et al*., 2021). The lunar period is one of the environmental cues for the reproduction of fishes (Giese and Kanatani, 1987) and moonlight intensity is said to influence the spawning of rabbit fishes (Takemura *et al*., 2010). However, the spawning was observed in the present study during the third quarter of the lunar cycle after 20 h 15 min. of the second dose of injection and lasted for 1 30 h at 30±0.2 °C. Several authors have reported varying lunar phases and the time of spawning after inducement in *S. vermiculatus* (Popper and Gundermann, 1976; Avila, 1984; Anuraj *et al*., 2019, Anuraj *et al*., 2021). This might be due to the nature of hormones, doses of hormones, brooder conditions and environmental conditions.

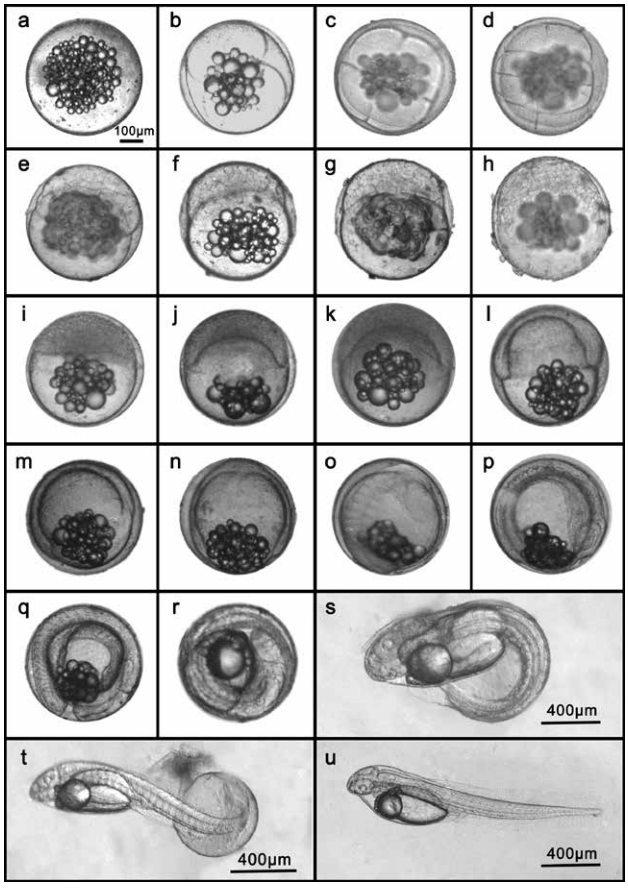

Fig 2. Egg development of from fertilized egg to newly hatched larvae

The eggs were found adhered to the ceramic tiles and sides of the tank; with a thin adhesive coating on the exposed surfaces of the chorionic membrane (Avila, 1984). The fertilized eggs were spherical, demersal, adhesive, and transparent and measured 0.56±0.03 mm (n=30) in diameter (Fig. 2a). Other studies also reported that siganids eggs are demersal, strongly adhesive, small, and spherical with many oil globules (Leis and Rennis, 1983; Tabugo *et al*., 2012), except those of *S. argenteus,* which are free-floating and non-adhesive (Burgan and Zseleczky, 1979; Luchavez and Carumbana, 1982). The egg sizes are said to vary with species (Avila, 1984) and salinity of incubating media (Westernhagen and Rosenthal, 1975). Several oil globules (7-15 numbers) were observed in fertilized eggs which were found to coalesce into one before hatching (Anuraj *et al*., 2021). The egg was typically centro-lecithal, featuring a centrally positioned yolk mass encircled by a thin cortical cytoplasmic layer (Avila, 1984).

The various embryonic development stages and their characteristics from fertilized egg (zygotic stage) to yolk sac larvae are depicted in Fig. 2 and Table 1. a meroblastic discoidal cleavage resulted in 2 cell stages or 2 blastomeres (Fig. 2b) ending the zygotic stage (fertilized egg) and formation of two blastomeres, in 18 min. after fertilization. Subsequent cell divisions proceeded in an asynchronous and irregular pattern across the newly formed blastomeres, accompanied by a progressive reduction in cell size. Following the first cleavage, several parallel and perpendicular cleavages resulted in the formation of 4 blastomeres, 8 blastomeres, 16 blastomeres, 32 blastomeres, and 64 blastomeres (Fig. 2b-g). All the cleavage furrows are vertically oriented, incompletely undercut blastodisc and formed blastomeres till the 32-cell stage and a horizontal cleavage in the 64-cell stage (Kimmel *et al*., 1995). The cell divisions observed in this study were almost similar to those observed by Avila (1984) in *S. vermiculatus* and a reduction in the size of blastomeres was observed in subsequent divisions from the first division (Shields *et al*., 1997).

Successive cell divisions occurred, culminating in the formation of the 128-cell stage which signified the initiation of the blastula period (Kimmel *et al*., 1995; Cucchi *et al*.,

2012), with a raised blastodisc positioned above the yolk mass. Subsequent cellular divisions became increasingly difficult to distinguish due to the diminishing cell size and increasing cellular density. The late blastula stage was characterized by the formation of a flowery appearance of blastomeres was observed (Fig. 2h). In *Lethrinus lentjan*, Gomathi *et al.* (2021) reported that asynchronous cell division was observed after the blastula period and the blastomeres formed were accumulated around the animal pole resembling a flowery appearance. A cap-like structure observed with differentiation of blastodisc with yolk by syncial layer called as yolk syncial layer (YSL) was evident (Fig. 2i). The initial observation of the YSL in teleost fishes was documented by Agassiz and Whitman (1884), with Wilson (1889) later providing more comprehensive descriptions of this structure. YSL is a distinctive characteristic present in teleost, which serves in embryonic pattern formation and morphogenetic processes (Carvalho and Heisenberg, 2010). Further development during embryogenesis was characterized by the flat growth of blastodisc over the yolk and doming of YSL towards the animal pole, also referred to as the dome stage (Fig. 2j). This stage further progressed with thinning and spreading of both YSL and the blastodisc over the yolk cell resulting in a uniform thick blastoderm initiating epiboly (Kimmel and Law, 1985). This also signals the transition from the blastula stage to the gastrula stage (Avila, 1984).

Blastoderm migration of over 25 % yolk can be termed as 25 % epiboly (Fig. 2k). Blastoderm migration of over half of the yolk can be termed as 50 % epiboly (Fig.2l). The migration progressed to cover 2/3 of the yolk (75% epiboly) (Fig. 2m) and germ ring was visible. The appearance of germ ring varied from other species (Hall *et al.*, 2004; Kimmel *et al.*, 1995; Warga and Kimmel, 1990) but was similar to *S. vermiculatus* (Avila, 1984). The yolk was completely

Table 1. Timing and characteristics of embryonic stages in from fertilized eggs to yolk sac larvae

Stage of development	Time from fertilization (in h: min)	Figure	Characteristics
Fertilized Egg	00:00	Fig. 1a	Spherical, transparent, demersal, adhesive, 0.56 ± 0.03 mm.
2-cell Stage	00:18	Fig. 1b	1 Meroblastic cleavage, vertically oriented cleavage furrow, 2 blastomeres
4-cell Stage	00:35	Fig. 1c	2 cleavage, vertically oriented cleavage furrow, 4 blastomeres
8-cell Stage	00:57	Fig. 1d	3 cleavage, vertically oriented cleavage furrow, 8 blastomeres
16-cell Stage	01:20	Fig. 1e	4 cleavage, ve vertically oriented cleavage furrow, 16 blastomeres
32-cell Stage	02:18	Fig. 1f	5 cleavage, vertically oriented cleavage furrow, 32 blastomeres
64-cell Stage	02:36	Fig. 1g	6 cleavage, horizontal cleavage furrow, 64 blastomeres, blastomere dimensions decreased substantially, resulting in markedly diminished cell size
128-cell Stage	03:24	Fig. 1h	Initiation of blastula stage, flowery appearance
Early Blastula	04:35	Fig. 1i	YSL became discernible, forming a critical boundary between the developing blastodisc and the underlying yolk mass
Late Blastula	05:50	Fig. 1j	The yolk syncytial layer exhibited pronounced upward curvature toward the animal pole, establishing the characteristic dome stage of embryonic development.
25% epiboly	06:27	Fig. 1k	25% yolk invasion of the blastoderm, gastrula stage
50% epiboly	07:15	Fig. 1l	50% yolk invasion of blastoderm
75% epiboly	08:42	Fig. 1m	75% yolk invasion of blastoderm
100% epiboly	09:35	Fig. 1n	100% overgrowth of blastoderm over yolk
Somite	10:05	Fig. 1o	The antero-posterior axis of the embryo was established, and somites were visible.
Head formation	11:18	Fig. 1p	Optic buds visible
Otic placode	13:48	Fig. 1q	Paired otic vesicle appearance; heartbeat was observed
Tail twitching	16:15	Fig. 1r	Tail elongates and touches head, tail twitching noticed
Tail flapping	19:50	Fig. 1s	Vigorous movement resulted in the detachment of the tail from the yolk, tail flapping against the head
Chorion rupture	21:55	Fig 1t	Synchronized movements of the head and tail regions, head emerges out first and larvae free from the egg case
Yolksac larvae	22:39	Fig 1u	Hatchling with a yolksac and oil globule

engulfed during the completion of epiboly (100% epiboly) (Kimmel *et al.*, 1995) and the embryonic shield became visible (Fig. 2n) although the time of appearance differed (Hara *et al.*, 1986). The anteroposterior axis of the embryo was established and the extension of the head fold opposite to the tail bud was observed along with the appearance of somites (Fig. 2o). Somites were also visible in *S. guttatus* after completion of epiboly (Hara *et al.*, 1986) and these are said to give rise to the axial skeleton and the skeletal muscle of the trunk (Stickney *et al.*, 2020). Later stage an optic bud (rudimentary eye vesicle) appeared on each side of the cephalic end (Fig. 2p). A paired placode of otic (auditory) vesicles appeared and heartbeat was observed signalling the formation of the embryonic heart (Fig. 2q). Similar developmental sequences in zebrafish, where otic placode formation closely preceded the onset of cardiac function (Kimmel and Law, 1985) and formation of optic lenses (Hara *et al.*, 1986). The optic lens and auditory capsule were distinguishable after 13 h and 50 min. after fertilisation in *S. vermiculatus* (Avila, 1984).

The embryo started twitching movement after 16 h 15 min. The twitching originated in the tail region towards the head (Fig. 2r). The frequency of these movements gradually increased, resulting in detachment of the tail from the yolk mass. Hatching was initiated through vigorous jerking movements, with the tail flapping as the head pressed against the egg membrane until rupture occurred (Fig. 2s). Complete hatching was achieved through synchronized movements of the head and tail regions, culminating in the larva's full emergence from the egg case with head coming out of the chorion first (Fig. 2t). This series of events were similar to Avila (1984) but with a decreased duration starting from blastula period. In the present study, hatching commenced at 22 h 39 min and continued until 23 h 55 min under conditions of 32 ppt salinity and 29.5±0.4 °C, corresponding closely with observations reported by Anuraj *et al.* (2021) and Popper *et al.* (1976) at similar temperatures. Depending on temperature (22 to 30 °C) and locality, the incubation period of other siganid fish is reported to be in the range of between 18 to 35 h after fertilization (Duray, 1998).

The newly hatched larvae (yolk sac larvae) were pelagic and measured 1.9 ± 0.11 mm in total length with an initial yolk volume of 0.015±0.003 mm^3 and oil globule volume of 0.004±0.001 mm^3 (Fig. 2u). The yolk sac is oval with a single oil globule protruding at the anterior portion. The gut forms a straight tube, the eyes remain unpigmented, the mouth is still unformed, and the primordial fin extends across the full trunk of the larvae. The larger-sized larvae at hatch when compared to other reports in *S. vermiculatus* (Popper *et al.*, 1976; Avila, 1984)) might be due to the larger egg size (Anuraj *et al.*, 2021). The endogenous reserves of larvae depend on the size of larvae at hatch (Blaxter and Hempel, 1963) and generally carnivorous fishes are said to have more endogenous reserves to support their more energy-intensive and complex development (Shirota, 1960).

Conclusion

The present study provides a comprehensive account of the spawning behaviour, egg morphology, and embryonic development of a siganid species, *S. vermiculatus* following hormonal inducement. The observed spawning behaviour and induced breeding during the third quarter of the lunar cycle expands the established breeding window beyond the previously reported first quarter and full moon phases, creating additional opportunities, that could significantly increase hatchery seed production and aquaculture of this species. This study also contributes to a better scientific understanding of developmental patterns in fertilized eggs of *S. vermiculatus*, enabling interventions in environmental parameters during critical periods to improve hatching rates and embryo manipulations. The study also provides comparative data within the Siganidae family and contributes to the broader understanding of teleost embryology.

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Author contributions

Conceptualization: AA; Methodology: AA, SB, TH; Writing Original Draft: AA, SB; SR Data Analysis: AA, SR, AKS, SB; Supervision: TG; Data Collection: SR, MP, AN; Supervision: JL, BI.

Data availability

The data are available and can be requested from the corresponding author.

Conflict of interests

The authors declare that they have no conflict of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

Ethical statement

No ethical approval is required as the study does not include activities that require ethical approval or involve protected organisms/ human subjects/ collection of sensitive samples/ protected environments.

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References

Agassiz, A. and C. O. Whitman. 1884. On the development of some pelagic fish eggs, Preliminary notice. *Proc. Am. Acad. Arts Sci,* 20: 23-75.

Anuraj, A., P. P. Suresh Babu, J. Loka, B. Ignatius, B. Santhosh, K. R. Ramudu, S. M. Sonali, K. Srinivas Rao, P. Dube, N. Kumbhar, S. Joseph and I. Joseph. 2021. Induced breeding and larval rearing of vermiculated spinefoot, *Siganus vermiculatus* (Valenciennes, 1835) in indoor conditions. *Aquaculture,* 539: 736600.

Anuraj, A., P. P. Suresh Babu, J. Loka, K. Srinivas Rao, P. Dube, S. M. Sonali, H. B. Pramila, N. Kumbhar, I. Joseph and B. Ignatius. 2019. First report on induced spawning of *Siganus vermiculatus* in India. MFIS T & E Ser., Series (239). p. 11-13.

Avila, E. M. 1984. Hormone-induced spawning and embryonic development of the rabbitfish, *Siganus vermiculatus* (Pisces: Siganidae). *The Philipp. Sci.*, 21: 75-108.

Bagarinao, T. 1986. Yolk resorption, onset of feeding and survival potential of larvae of three tropical marine fish species reared in the hatchery. Mar. Biol. 91: 449-459.

Ben-Tuvia, A., 1966. Red Sea fishes recently found in the Mediterranean. *Copeia.,* 1966 (2): 254-275.

Berlinsky, D. L., L. W. Kenter, B. J. Reading and F. W. Goetz. 2020. Regulating reproductive cycles for captive spawning. In: T. J. Benfey, A. P. Farrell and C. J. Brauner (Eds.), *Fish Physiol.*, 38: 1-52.

Blaxter, J. H. S. and G. Hempel. 1963. The Influence of Egg Size on Herring Larvae (*Clupea harengus* L.). *ICES J. Mar. Sci.*, 28: 211-240.

Breder, C. M., Jr. and D. E. Rosen. 1966. Modes of reproduction in fishes. Natural History Press for the American Museum of Natural History, New York. 941 pp.

Burgan, B. G. and K. A. Zseleczky. 1979. Induced spawning and early development of the rabbitfish, *Siganus argenteus* (Quoy and Gaimard), in the *Philippines. Silliman J.,* 26 (2 and 3): 163-171.

Carvalho, L. and C. P. Heisenberg. 2010. The yolk syncytial layer in early zebrafish development. *Trends Cell Biol.*, 20 (10): 586-592.

Cucchi, P., E. Sucré, R. Santos, J. Leclère, G. Charmantier and R. Castille. 2012. Embryonic development of the sea bass *Dicentrarchus labrax. Helgol. Mar. Res.*, 66 (2): 199-209.

Duray, M. N. 1998. Biology and culture of siganids. Aquaculture Department, Southeast Asian Fisheries Development Center, Iloilo, Philippines, 110 pp.

Fitzpatrick, J. L. 2020. Sperm competition and fertilization mode in fishes. *Philosophical Phil. Trans. R. Soc.* B, 375: 0074. https://doi.org/10.1098/rstb.2020.0074.

Giese, A. C. and H. Kanatani. 1987. Maturation and spawning. In: A. C. Giese, J. S. Pearse and V. B. Pearse (Eds.) Reproduction of Marine Invertebrates, Vol. 9. Blackwell Scientific/ Boxwood Press, Palo Alto/Pacific Grove, CA, p. 251–329.

Gonçalves, D. M. and R. F. Oliveira, 2011. Hormones and sexual behavior of teleost fishes. In: D. O. Norris and K. H. Lopez (Eds.) Hormones and Reproduction of Vertebrates: Fishes, Vol. 1. Academic Press, London, p.119–147.

Gomathi, P., R. Siju, M. K. Anil, G. P. Ambarish, S. Surya, B. Raju, P. K. Raheem, B. Ignatius, and A. Gopalakrishnan. 2021. Embryonic and larval development of pink ear emperor, *Lethrinus lentjan* (Lacepede, 1802) under captive conditions. *Aquac. Res.,* 52 (11): 5857–5869.

Gundermann, N., D. M. Popper and T. Lichatowich.1983. Biology and life cycle of Siganus vermiculatus (Siganidae, Pisces). *Pac. Sci.*, 37: 65-180.

Hall, T. E., P. Smith and I. A. Johnston. 2004. Stages of embryonic development in the Atlantic cod *Gadus morhua. J. Morphol.* 259 (3): 255-270.

Hara, S., H. Kohno and Y. Taki. 1986. Spawning behavior and early life history of the rabbitfish, *Siganus guttatus*, in the laboratory. *Aquaculture,* 59 (3-4): 273-285.

Herre, A.W. and H. R Montalban. 1928. The Philippine siganids. *Philipp. J. Sci.,* 35(2): 151-185.

Kimmel, C. B., W.W. Ballard, S. R. Kimmel, B. Ullmann and T. F. Schilling. 1995. Stages of embryonic development of the zebrafish. *Dev. Dyn.*, 203 (3): 253-310.

Kimmel, C. B. and R. D. Law. 1985. Cell lineage of zebrafish blastomeres: III. Clonal analyses of the blastula and gastrula stages. *Dev. Biol.,* 108 (1): 94-102.

Kohno, H., S. Hara, M. Duray and A. Gallego. 1988. Transition from endogenous to exogenous nutrition sources in larval rabbitfish Siganus guttatus. Nippon Suisan Gakk, 54 (7): 1083-1091.

Lacson, J. M. and S. G. Nelson. 1993. Genetic distances among fishes of the genus *Siganus* (Siganidae) from the Western Pacific Ocean. *Mar. Biol.*, 116: 187-192.

Leis, J. M. and D. S. Rennis. 1983. The larvae of Indo-Pacific coral reef fishes. New South Wales University Press, Sydney; University of Hawaii Press, Honolulu, 269 pp.

Luchavez, J. A. and E. E. Carumbana. 1982. Observations on the spawning, larval development, and larval rearing of *Siganus argenteus* (Quoy and Gaimard) under laboratory conditions *Silliman J.,* 29 (1–2): 1-15.

McVey, J. P. 1972. Observations of the early-stage formation of rabbitfish *Siganus fuscescens* (should be *S. canaliculatus*) at Palau Mariculture Demonstration Center. *South Pac. Isl. Fish. News l. (Noumea, New Caledonia),* 6: 1-12.

Murugan, A. and N. Namboothri. 2012. Fin fishes of the Gulf of Mannar: A field identification guide. Dakshin Foundation, Bengaluru, p. 72–84.

Popper, D. and N. Gundermann. 1976. A successful spawning and hatching of *Siganus vermiculatus* under field conditions. *Aquaculture,* 7 (3): 291-–292.

Popper, D., R. C. May and T. Lichatowich. 1976. An experiment in rearing larval Siganus vermiculatus (Valenciennes) and some observations on its spawning cycle. *Aquaculture,* 7 (3): 281-290.

Rahman, M. S., M. Morita, A. Takemura and K. Takano. 2003. Hormonal changes in relation to lunar periodicity in the testis of the forktail rabbitfish, *Siganus argenteus. Gen. Comp. Endocrinol.*, 131 (3): 302-309.

Shields, R. J., N. P. Brown and N. R. Bromage. 1997. Blastomere morphology as a predictive measure of fish egg viability. *Aquaculture,* 155 (1-4): 1-12.

Shirota, A. 1970. Studies on the mouth size of fish larvae. Nippon Suisan Gakk. 36 (4): 353-368.

Stickney, H. L., M. J. F. Barresi and S. H. Devoto. 2000. Somite development in zebrafish. *Dev. Dyn.*, 219 (3): 287-303.

Tabugo, S. R. M., J. P. Sendaydiego, E. A. Requieron and M. D. Dimalen. 2012. Embryonic developmental stages in cultured rabbitfish (Siganus guttatus, Bloch 1787). *Int. Res. J. Biol. Sci.*, 1 (8): 65-70.

Takemura, A., M. S. Rahman and Y. J. Park. 2010. External and internal controls of lunar-related reproductive rhythms in fishes. *J. Fish Biol.*, 76 (1): 7-26.

Warga, R. M. and C. B. Kimmel. 1990. Cell movements during epiboly and gastrulation in zebrafish. *Development,* 108 (4): 569-580.

Westernhagen, V. H. and H. Rosenthal. 1975. Rearing and spawning siganids (Pisces: Teleostei) in a closed seawater system. *Helgoland Mar. Res.*, 827(1): 1-18.

Wilson, H. V. P. 1889. The embryology of the sea bass. *Bull. U.S. Fish Comm.*, 9: 209-277.