THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Reproductive Biology of *Tetraodon cutcutia* (Pisces: Tetraodontidae) from Meleng River in Jorhat District, Assam

P. Karmakar
Department of Life Sciences, Dibrugarh University, Assam, India
S. P. Biswas
Department of Life Sciences, Dibrugarh University, Assam, India

Abstract:
The fresh water puffer fish, Tetraodon cutcutia shows sexual dimorphism during breeding season. The gonado-somatic ratio (GSR) was increased from March and reached its peak in June (4.832±0.56) and progressively decreased after words till January in case of female. The maximum ova diameter (1.09±0.35mm) was recorded in July and the minimum (0.32±0.12mm) in February. The absolute fecundity ranged from 447.48 to 1540.08 and that of the relative fecundity varied from 68.79 to 166.25. The number of egg increases as the fishes gain the weight and length. T. cutcutia breed once in a year during summer season (May, June, July).

Keywords: *Sexual dimorphism, GSR, puffer fish, Assam*

1. Introduction

The study of gonadosomtic ratio (GSR) is very essential for a better understanding of the reproductive biology of fish. It is an efficient indicator of the functional state of the gonads (Vazzoler, 1996). GSR also determines the period of greatest reproductive intensity, evidencing the reproductive strategy of each species. These vary within a species, and even within a sex, and may occur in most taxa (Gross, 1996). Fecundity represents the reproductive potential and estimation of abundance of a population. Estimation f fecundity and GSI of a fish is essential for evaluating the commercial potentialities of its stock, life history, practical culture actual management of the fishery (Lagler, 1956; Doha and Hye, 1970).

Puffer fish, also called blowfish, toadfish, swellfish, globefish and balloon fish, belongs to the order Tetraodontiformes, have about 120 species and mostly found in the tropical seas (Torda *et. al*., 1973). The fresh water puffer fish, locally known as *Gangatop*, is a common trash fish in the Brahmaputra valley. The species is considered as a potential aquarium fish and hence an attempt has been made to study certain aspects of the reproductive biology of the fish.

2. Materials and Methods

Samples were collected from Meleng River near Neematighat in Jorhat District, Assam during April 2011 through March 2013. A total of 285 species were dissected for the study of reproductive biology. Before dissecting out the gonads, the length and weight as well as colouration pattern and other morphological features of males and females were recorded and the gonads, after recording their weight to nearest 0.01gm in an electronic balance were preserved in 5% formalin for further studies.

Sex determination of target fish species was confirmed as per the description given by Talwar and Jhingran (1991). Similarly, maturity cycle and maturity stages were determined following the key as outlined by Kesteven (1960) and Hopkins (1979). In order to study the condition of the gonads, the maturity index or gonadosomatic ratio (GSR) was calculated according to the following formula - GSR= weight of gonad (g) × 100/ total body weight (g). The percentage of mature fish that is of stage IV to VI was examined for each length group of both sexes, and the 50% maturity or M_{50} has been determined as per Hodgkiss and Mann (1978). Measurements of ova diameter were taken from random sub-samples of formalin preserved ovary (anterior, middle and posterior part of individual ovary and ova-diameter measurements were taken with the help of an ocular micrometer. The diameters of ova along whatever axes they lay parallel to the graduation of the micrometer, were measured to ensure random nature of the readings and unbiased values as suggested by Clark (1934). Finally, absolute fecundity was calculated by using the following formula: F=N×Gg-1 (Grimes and Huntsmen, 1980), where, F=fecundity, N= no. of eggs in sub sample, G=total weight of ovary and g=weight of the sub sample. Similarly, relative fecundity was estimated by simply dividing absolute fecundity by total length; between fecundity and body weight and between fecundity and ovary weight were calculated and log to log relationship in the form given below (Bagenal, 1978):

Log F= log a+ b log X, Where, F=fecundity; X=variable factors like body length, weight. etc, and **a** and **b** are constants.

3. Result and Discussion

- *Sexual dimorphism:* The fully ripe females have bulging abdomen where as males have cylindrical body shaped, greenish yellow body colour. Males have two round black circles on the posterior end of the lateral sides of the body and yellow colour blotches are found on the dorsal side of the body. The urinogenital opening enlarges. In case of female, the dorsal surface of the body is blackish in colour and black dotted circles are present on the both lateral side of the body. During breeding season dark reddish colour outline is found on the caudal fin of the fish and dark reddish colour circle is found in the both eyes of the fish. Females are relatively larger than the males. The large size of mature female appears to be very common in fishes. This occurs because of the pressure for greater fecundity of the females (Pauly, 1994; Vazzoler, 1996), because the reproductive success of females increases directly with size (Conver, 1984).
- *Maturity Stages:* The gonads are elongated organs lying inside the body cavity. The five (5) different maturity stages were identified depending on the morphological structure of the gonad, space occupied in the abdominal cavity by gonads and the diameter of the ovarian eggs. It is observed that all the maturity stages are found mainly from February to October of the year. Spawning appears to be completed during rainy months. Richter (1982) reported that the freshwater puffer *Carinotetraodon* spawned in algal clumps, however the eggs to be scattered primarily over rocks. In Japan, however, the marine puffer fish, *Fugu pardalis* is known to spawn in sea grass beds (Fujita, 1962). Arai and Fujita (1988) recently described spawning in an aquarium by *Canthigaster rivulata*, which released their gametes together above the substratum.

Figure 1: Lateral view of Tetraodon cutcutia (female)

Maturity stages	Testis	Ovary
Stage I (Immature)	Testes very thin, thread or ribbon like, translucent, no sperm	Ovaries are very thin, transparent, eggs very small,
Stage II (Maturing)	Slight increase in volume and weight, opaque to transparent in colour, testes small, whitish, trace of sperm.	Ovaries become thicker & small, slight pinkish or yellowish in colour, weight increases, eggs visible under microscope. Right ovary is larger than the left ovary.
Stage III (Developing)	Testes enlarged & rounded, marked increase in volume weight, opaque to transparent, whitish in colour.	Ovaries occupies about one-third of the body cavity, pinkish to yellowish in colour, diameter of the ova increases, ovaries are rounded.
Stage IV (Mature/ripe)	Testes whitish, creamy, soft flabby, full of sperm, testes are very large, some extruded with pressure.	Eggs enlarged and can be seen with naked eye, blood vessels distinct, deep yellowish or orange eggs are found, extruded with slight pressure, ovaries having loose walls, ripe and translucent eggs
Stage V (Spent)	Testes shrinking, flaccid, whitish to translucent and very thin.	Ovaries flaccid, shrinking, shows reduction in weight and volume, yellowish, wrinkled, large eggs having disappeared.

Table 1: Maturity stages of T. cutcutia

Figure 2: Lateral view of Tetraodon cutcutia (male)

- *Maturity index:* The gonadosomatic ratio (GSR) or maturity index had been calculated for male and female separately. In the present study the GSR of the female increased from March to July and reached maximum in June (4.832±0.56) and then decreased from July onwards reaching its minimum (0.16±0.02) in January. In case of male, the GSR increases from January to July and reached highest on the month of July (1.975±0.44) and again decreased and minimum value is found on the month of January (0.38±0.18). In case of *Lagocepheus sceleratus* (Gmelin, 1789) the monthly variations in GSR suggest that the spawning takes place during summer season (May, June, July) for both sexes. This finding fully complies with those of Kotb (1998) and Sabrah et al. (2006).

Month	Male	Female
Jan	0.38±0.18	0.16±0.02
Feb	0.478±0.21	0.306±0.14
Mar	0.546±0.31	1.332±0.87
Apr	0.744±0.34	2.054±0.85
May	0.853±0.43	3.018±0.94
June	1.651±0.63	4.832±0.56
July	1.975±0.44	3.026±0.47
August	1.072±0.34	1.221±0.35
Sept	0.998±0.76	1.132±0.47
Oct	0.897±0.02	0.835±0.39
Nov	0.45±0.12	0.762±0.29
Dec	0.49±0.21	0.754±0.23

Table 2:Monthly maturity index (GSR) of T. cutcutia

- *Size at first maturity and M_{50}:* Stages IV and V gonadal maturity had been considered as a mature gonad. The 50% maturity stage of male is at the length group 4.5-5.5 cm (44.45) and in case of female it is on the length group of 6-7.5 cm (52.38). Length at first maturity and determination of $M_{50.}$ There was a close relationship between maturity and the length of the fish.

Length group (cm)	Sex	Immature % (Stage I)	Maturing % (Stage II)	Mature/Ripe % (Stage III & IV)
2.5-3.5	Male	100	-	-
3.5-4.5	Male	33.33	41.67	25
4.5-5.5	Male	22.22	33.33	44.45
5.5-6.5	Male	6.67	33.33	60
6.5-7.5	Male	-	7.14	92.86
3-4.5	Female	100	-	-
4.5-6	Female	70.59	29.41	-
6-7.5	Female	14.29	33.33	52.38
7.5-9	Female	-	16.67	83.33
9-10.5	Female	-	-	100

Table 3: Percentage of maturity (M_{50}) in different length groups of T. cutcutia

- *Ova diameter:* The progressive change observed in the intra-ovarian diameter for a period not less than a year can give an idea of the spawning periodicity of the fish studies (Biswas, 1993). The mean ova diameter is highest in June (1.93±0.43) and lowest is in February (0.32±0.12). Measurement of ova-diameter and their frequency polygon distribution at different months in a year was a common method in determining the maturity cycle of the fish (Macer, 1974). The eggs of puffer fishes (*Fugu, Sophoeroides, Tetraodon*) are typically spherical, 0.85-1.41mm in diameter, contain a dense cluster of small oil globules and are covered with an adhesive coating (Welsh and Breder, 1922; Schreitmuller, 1930; Munro, 1945; Uno, 1955; Fujita, 1962; Leis, 1984;).

Month	Range of OD	Mean OD
Jan	--	--
Feb	0.1-0.5	0.32±0.12
Mar	0.4-0.9	0.6±0.17
Apr	0.5-1.2	0.82±0.19
May	0.5-1.4	0.86±0.32
June	0.5-1.7	1.93±0.43
July	0.6-1.5	1.09±0.35
Aug	0.5-1.3	1.05±0.43
Sept	0.6-1.2	0.7±0.37
Oct	0.4-1	0.65±0.26

Nov	--	--
Dec	--	--

Table 4: Monthly mean ova diameter of T. cutcutia

- *Fecundity:* Absolute fecundity of the fish ranges from 447.48 (±44.72) to 1540.08(±350.93). In all the individuals the number of eggs increased as the fish gained weight and length. Again, relative fecundity ranged from 68.79 (±27.18) to 166.25 (±54.76). In *Lagocephalus sceleratus* the relative fecundity was estimated as 780.8±171.8 eggs g-1 (Aydin, 2011).

Month	Mean body length (cm)	Mean body weight (g)	Mean ovary weight (g)	Mean absolute fecundity	Mean relative fecundity
Jan	4.74±1.07	4.91±1.104	0.03±0.001	Spent	Spent
Feb	5.86±1.19	5.99±1.05	0.018±0.008	447.48±44.72	76.34±11.37
Mar	6.34±1.88	8.12±2.58	0.098±0.018	555.85±48.66	75.13±27.03
Apr	6.27±1.64	7.64±2.08	0.054±0.04	619.08±82.28	87.22±26.98
May	6.157±1.26	8.135±3.57	0.138±0.19	609.382±68.80	84.714±28.21
June	6.81±1.62	9.89±5.39	0.364±0.48	1118.15±134.101	142.55±53.36
July	6.55±1.36	10.81±5.63	0.607±0.61	1540.08±350.93	166.25±54.76
Aug	6.614±144	10.014±4.72	0.539±0.52	925.98±68.69	115.85±64.49
Sept	6.64±1.05	11.59±5.03	0.658±0.56	700.04±196.22	68.79±27.18
Oct	5.81±0.92	6.52±1.29	0.034±0.005	521.8±40.09	75.81±14.03
Nov	6.69±0.92	7.72±0.89	0.02±0.01	Spent	Spent
Dec	5.33±0.93	5.36±0.98	0.03±0.01	Spent	Spent

Table 5: Absolute and relative Fecundity of T. cutcutia

4. References

1. Arai, H and S. Fujita (1988): Spawning behaviour and early life history of the sharp nose puffer, Canthigaster rivulata, in the aquarium. Japan. J. Ichthyol., 35(2): 194-202.
2. Aydin, M. (2011): Growth, reproduction and diet of puffer fish (Legocephalus scleratus Gmelin, 1789) from Turkey's Mediterranean Sea Coast. Turkish J. Fish. Aquatic Sciences. (11): 569-576.
3. Bagenal, T. B. 1978. Aspects of fish fecundity. In: S.D. Gerking (Ed) Ecology of Freshwater fish Production. Blackwell Scientific Publications., Oxford: 75- 101.
4. Biswas, S. P. (1993): Manual of Methods in Fish Biology. Delhi, South Asian Publisher. Pvt. Ltd. 157p.
5. Clark, F. N. (1934): Maturity of the California sardine (Sardinia caerulea), determined by ova-diameter measurement. Fish. Bull. Sacramento, 42: 1-49.
6. Conver, D.O. (1984): Adaptive significance of temperature-dependent sex determination in a fish, Am, Nat., 123,(3) pp 297-313.
7. Doha, S. and M. A. Hye (1970): Fecundity of Padma River Hilsa hilsa (Ham). Pak. J. Sci., 22: 176-178.
8. Fujita, S (1962): Studies on the life history and culture of common puffer fishes in Japan. Nagasaki Pref. Fish. Res. Stn. Rep., (2), 121p. 40pls. (In Japanese).
9. Grimes, C. B. and G. R. Huntsman (1980): Reproductive biology of the vermilion snapper, Rhomboplites aurorubens from North Carolina and South Carolina. Fish. Bull., 78: 137-146.
10. Gross, M.R. (1996): Alternative reproductive strategies and tactics; diversity within sexes. Tree, 11(2), 92-98.
11. Hodgkiss, I. J. and H. S. H. Mann (1978): Reproductive biology of Saratherodon mossambicus (Cichlidae) in plover clove reservoir, Hong Kong. Environ. Biol. Fish, 3 (3): 287-292.
12. Kesteven, G. L. (1960): Manual of Field Methods in Fisheries Biology. FAO Manual of Fisheries Science, No. 1. 152 p.
13. Kotb, S. A. (1998): Biochemical studies on toxicity of Pleurancanthus scelertus (El-Karad) in the Red Sea. PhD. Thesis, Faculty of Sciences, Department of Biochemistry. Alexandria University, Egypt.
14. Lagler, K. F.(1956): Freshwater Fishery Biology. 2nd ed. W.M.C. Brown Company, Bubuque, Iowa, 541 p.
15. Leis, J. M. (1984): Tetraodontioidei: Development Pages 447-450 in H. G. Moser and W. J. Richards, eds. Ontogeny and systematic of fishes. Special Publication No. 1, American Society of Ichthyologists and Herpetologists. Allen Press, Kansal, ix-760p.
16. Macer, C. T. (1974): The reproductive biology of the horse mackerel, Trachurus trachurus (1) in the North Sea and English Channel. J. Fish. Biol., 6: 415-435.
17. Munro, I. S. R. (1945): Post larval stages of Australian fishes. No. 1. Mem. Qld. Mus., 12: 136-153.
18. Pauly, D. (1994): On the sex of fish and the gender of scientists. A collection of essays in fisheries science. Chapman and Hall, Fish and Fisheries Sciences 14, 250p.
19. Richter, H. J (1982): Spawning Somphongs' puffer Carinotetraodon somphongsi. Trop. Fish Hobbyist, 31: 8-25.
20. Sabrah, M. M., A. A. El-Ganainy and M. A. Zaky (2006): Biology and toxicity of the puffer fish Lagocephalus sceleratus (Gmelin, 1789) from the Gulf of Suez, Egyptian J. Aquatic Research, 32: 283-297.

21. Schreitmuller, W (1930): Kugelfishes. Das Aquarium, Berlin, Jan.: 12-16, Feb.: 20-26. (In German).
22. Torda,T. A., E. Sinclair and D. B. Ulyatt (1973): Puffer fish (Tetraodotoxin) poisoning: Clinical record and suggested management. Med. J. Aust., 1: 599-602.
23. Talwar, P. K. and A. G. Jhingran (1991): Inland Fishes of India and Adjacent Countries. Oxford & IBH Publishing House, New Delhi.
24. Uno, Y. (1955): Spawning habit and early development of a puffer Fugu (Torafugu) niphobles (Jordan and Schneider). J. Tokyo. Univ. Fish., 41: 169-183.
25. Vazzoler, A. (1996): Biologya da reproducao de peixes teleosteos: Teoria e Pratica. Maringa-PR, Nupelia, 169p.
26. Welsh, W. W. and C. M. Breder, Jr (1922): A contribution to the life history of the puffer fish, Sopheroides maculaus (Schneider). Zoologica (N. Y.) 2: 261-276