



FECUNDITY OF THE FRESHWATER INDIAN KILLIFISH *APLOCHEILUS LINEATUS* (VALENCIENNES, 1846) FROM THE RIVER TAMBAPARANEI OF TIRUNELVELI DISTRICT

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ABSTRACT

It was observed in the present study that *A. lineatus* had an asynchronous ovary with multiple spawning. The peak spawning period coincided with the northeast monsoon during October - November and the southwest monsoon in April - June. At an early age, the females were bigger than males. But once they attained maturity the males became larger than the females. Almost both the males and the females were recorded in equal numbers in all the months. The minimum size at maturity for male and female *A. lineatus* was 30mm and 35mm respectively.

INTRODUCTION

Reproduction is one of the most important fundamental activities of organisms. The future of its race, its survival, and its successful colonization mainly depend on the reproductive success of a species. Reproduction in most teleost species is a cyclical or seasonal phenomenon and is limited to a relatively brief time span (Selman and Wallace, 1989; Misra, 1994). Ecologically sustainable use of fisheries resources requires a thorough knowledge of the reproductive biology of the species concerned. The reproductive cycle and spawning season of a fish could be ascertained by analyzing a number of reproductive and body indices such as gonad somatic index (GSI), condition factor (K), fecundity, size at first maturity (L_{p50}), hepatosomatic index (HSI), length and weight-frequency distribution, and sex ratio.

Fecundity indicates the reproductive potential of a fish. The estimation of fecundity and its relationship with the body measurements gives an idea about the clutch size of fish species (McDowall and Eldon, 1997). Fecundity was calculated for killifishes in *Notobranchius guentheri* (Haas, 1976), *Epiplatys bifasciatus* (Gumaa, 1982), *Fundulus luciae* (Kneib, 1978; Hardy, 1978), *Lucania goodie* (Foster, 1967; Fuller, 2001; 2002), *Fundulus diaphanus* (Chippett, 2003), *Fundulus heteroclitus* (Taylor *et al.*, 1977; Heins and Baker, 1988; Able and Hagan, 2003) and *Millerichthys robustus* (Castaneda *et al.*, 2013; 2016; 2017; 2018) and in *Aplocheiloides* killifish (Furness, 2016, Furness *et al.*, 2015) and *Lucania* killifish (Berdan *et al.*, 2021).

A perusal of available literature indicates that not much work has been done in the native killifish *Aplocheilus*

lineatus. In order to identify the spawning period of the killifish, *Aplocheilus lineatus* several reproductive and body indices such as GSI, condition factor (K), size-frequency distribution, fecundity, sex ratio, and size at first maturity were estimated from August 2020 to June 2022.

MATERIALS AND METHODS

Collection and maintenance: Live specimens of *Aplocheilus lineatus* were collected fortnightly at random for 24 months (August 2020 to July 2022) from river Tambraparani at Tirunelveli, Tamil Nadu (8.44° N and 77.44° E). Fish were collected using a scoop net with a mesh size of 2mm in diameter. Mutilated, injured, and diseased fishes were discarded immediately after collection.

The fishes were transported to Animal Health Research Unit, Department of Zoology, Periyar Arts College, Cuddalore in large plastic oxygenated containers and were stocked in outdoor cement tanks. The fishes were then segregated into juvenile, immature and adult based on their total length (TL) and were reared separately. During the period of acclimatization, the fish were fed *ad libitum* twice a day with live feeds such as mosquito larvae, tubifex worms and *chironomus* larvae. Hatchlings and fry were fed with plankton (*Daphnia* and *Moina*) collected from local ponds. Water quality parameters like DOC, pH, hardness and water temperature were carefully monitored during acclimatization. The stocking tanks were provided with aquatic plants (*Vallisneria*, *Ceratophyllum*) which prevented the fish from jumping out of the water.

Morphometric measurements

In the laboratory, data such as standard length (SL), total length (TL), live body weight, stages of sexual maturity, liver weight, and length and weight of gonads were recorded for each fish. The length measurements were taken to the nearest mm and weight to the nearest mg. Sex was identified by microscopic and macroscopic examinations.

Fecundity: Fecundity was calculated using the gravimetric method described by Bagenal (1978) from all the dissected mature, maturing and regressed ovaries of *A. lineatus*. The eggs in the dissected ovaries were counted using a dissection microscope.

Absolute fecundity: The total number of mature eggs present in the female before spawning was calculated by counting all the eggs in an ovary since the size of the ovary is small. However, if the ovary was big, the following formula was used (Biswas, 1993):

Absolute fecundity AF (Total no. of eggs/fish) = (n G) / g

Where n is the number of eggs in the sub-sample, G is the weight of the ovary and g is the weight of the sub-sample.

Relative fecundity: Relative fecundity (RF) of a fish denotes the number of eggs per unit weight or length of the fish. This was calculated using the formula (Biswas, 1993):

Relative fecundity, RF (no. of eggs / g live fish) = Total no. of eggs/weight of fish.

Batch fecundity was estimated by counting the number of hydrated oocytes within their follicles in the ovary (Abaunza *et al.*, 2003).

The number of vitellogenic oocytes in the ovary prior to spawning was calculated using the gravimetric method (Bagenal, 1978, Ma *et al.*, 1998):

Potential fecundity F_p (No. of eggs/fish) = $(W_o / W_s) * N$

Where W_o is the weight of the ovary, W_s is the weight of the sample taken for counting, and N denotes the number of vitellogenic oocytes in the sub-sample taken for counting. The term W_o / W_s is defined as the raising factor.

The number of eggs (fully developed cells) spawned in the current season was calculated by the following formula:

Realized fecundity F_R (no. of fully developed eggs/fish) = $F_p - N_{Res}$

Where N_{Res} is the number of oocytes resorbed due to atresia.

Relative potential fecundity was calculated by the following formula:

Relative potential fecundity RF_p (no. of vitellogenic oocytes/g live fish) = $F_p / (W - W_g)$

Where F_p is the potential fecundity, W is the weight of the fish and W_g is the weight of the gonad.

Relative realized fecundity was calculated by the following formula:

Relative realized fecundity, RF_R (no. of fully developed eggs / g live fish) = $F_R / (W - W_g)$

Where F_R is the realized fecundity, W is the weight of the fish and W_g is the weight of the gonad.

The relationships between the fecundity and other meristic parameters were analysed by plotting the respective values in a scatter diagram and calculating a regression equation (Biswas, 1993),

$$Y = a + b x$$

Where, Y is a dependent variable, i.e., fecundity, x is an independent variable, i.e., fish length, fish weight, ovary length and ovary weight, a and b are the constants; or in the logarithmic form,

$$\log Y = \log a + b \log X.$$

The correlation coefficient (γ) was calculated using Stat view 5.0 for each relationship.

RESULTS

The absolute fecundity of *A. lineatus* ranged from 225 - 254 eggs in an ovary of 0.025g to 0.032g weight for a fish of 36mm SL to 315 - 377 eggs in an ovary of 0.045g to 0.055g weight for a fish of 52mm SL and 1.95g live weight. The relative fecundity (number of eggs per gram body weight) was less than absolute fecundity in fishes having a body weight more than unity. The relative fecundity of *A. lineatus* was always more than absolute fecundity when the fish was less than 1gm in body weight. The absolute fecundity of *A. lineatus* was 215 eggs/fish and the relative fecundity was 226 for a fish weighing 0.95g. The relationship between live body weight and gonads of *A. lineatus* is given in tables 1 and 2.

The seasonal variations in the fecundity parameters such as absolute fecundity, relative fecundity, potential fecundity, relative potential fecundity, realized fecundity and the relative realized fecundity are illustrated in Figure 1. It is observed that absolute fecundity was maximum during October-November-December-January i.e., 416 ± 19 , 364 ± 25 , 245 ± 15 and 198 ± 10 respectively. Once *A. lineatus* started spawning there was decline in absolute fecundity during February and March i.e., 51 ± 5 and 110 ± 7 . Table 3 shows the relationship of fecundity with size during the pre-breeding, breeding and post-breeding months. The mean diameter of the ova varied from 85µm to 935µm in *A. lineatus*. It is inferred from the above data that *A. lineatus* is a multiple spawner having extended breeding. In the laboratory conditions *A. lineatus* laid 9 - 12 eggs in each clutch. Each batch consisted of three clutches of a total of 25 - 45 eggs. It also laid eggs at the interval of 30 days (Inter Spawn Interval).

The data of the month-wise relationship between fecundity and standard length is given in Table 3. During the breeding months (October-November-December-January) the number of eggs present in a 34mm SL fish was 125 ± 5 whereas the fish of the same size had only 75 ± 9 eggs during post-breeding months (February-March-April-May). The same trend was observed in all length groups. Similarly a fish of 56mm SL had an ovarian index of 0.048 ± 0.003 , a dobriyal index of 0.363 ± 0.041 , and an absolute fecundity of 425 eggs/fish during the peak breeding period, whereas fish with the same standard length had an ovarian index of 0.018 ± 0.003 , a dobriyal index of 0.212 ± 0.023 and an absolute fecundity of 125 eggs/fish during the post-breeding period. These data also indicated the pre-breeding period (September, November, February, March), breeding period (October, December, April, June) and post-breeding period (August, January, May, July) of *A. lineatus*. From the observed data, it could be inferred that *A. lineatus* is a multiple batch spawner.

The minimum fecundity was 75 eggs for an immature fish of 30mm TL. Fecundity increased smoothly with the increase in total length and the largest fish had nearly 6 times as many ova as the smallest one and this relationship was found to be significant. The regression relationship between fecundity and total length of *A. lineatus* could be expressed in logarithmic form as:

$$\text{Log } F = 1.149 + 1.726 \text{ Log } \text{TL}; p < 0.001$$

Where F represents fecundity and TL is the total length in cm. Fecundity increases at the 1.726^{th} power of length.

The relationship between fecundity and total length in *A. lineatus* can be expressed in the following regression equation:

$$F = -100.376 + 65.542 \text{ TL}$$

In small (34 - 36mm SL) ripe females, fecundity ranged from 215 - 227 eggs/fish and increased progressively with increase of fish length to a mean of 425 - 478 oocytes/fish in specimens of 50 - 60mm SL. The relationship between fecundity and standard length can be expressed in the following regression equation:

$$F = 71.455 + 4.369 \text{ SL}$$

This can be expressed in logarithmic form as follows:

$$\text{Log } F = 0.684 + 1.054 \text{ Log } \text{SL}; p < 0.001$$

Where F is the fecundity and SL is the standard length in cm.

Fecundity of *A. lineatus* ranged from 115 eggs in a fish of 0.85g body weight to 473 eggs in a fish of 2.23g body weight. The average number of eggs produced by *A. lineatus* increased with an increase in weight, but there was considerable overlapping in the ranges (0.825g to 2.475g). The relationship between fecundity and live body weight of *A. lineatus* is expressed in the following regression equation:

$$F = 186.2 + 0.058 \text{ FW}$$

Where F is the fecundity and FW is the fish weight in g.

In the logarithmic form the relationship between fecundity and fish weight can be expressed as:

$$\text{Log } F = 0.995 + 0.456 \text{ Log } \text{FW}; p < 0.001$$

Fecundity in *A. lineatus* showed increasing trend as a function of the length of the ovary. The relationship between the fecundity and the ovary length of *A. lineatus* could be expressed in the following regression equation:

$$F = 82.688 + 19.348 \text{ OL}$$

Where F is the fecundity and OL is the ovary length in mm.

The above relationship between fecundity and ovary length is expressed in the form of a logarithmic equation as follows:

$$\text{Log } F = 1.462 + 0.982 \text{ Log } \text{OL}; p < 0.001$$

The number of eggs in *A. lineatus* ranged from 125 in 0.018g ovary weight to 475 eggs in an ovary of 0.053g. The number of eggs per gram weight of the ovary varied from 6944 to 8960 with an average of 7485 eggs. The relationship between the fecundity and weight of ovary of *A. lineatus* could be expressed as:

$$F = 212.197 + 1.962 \text{ OW}$$

Where F is the fecundity and OW is the ovary weight in mg.

The relationship between fecundity and ovary weight can be expressed in logarithmic form as:

$$\text{Log } F = 1.928 + 0.35 \text{ Log } \text{OW}; p < 0.001$$

During the pre-breeding and breeding months the fecundity, relative fecundity, gonadosomatic index, ovary weight, standard lengths were positively correlated with one another ($p < 0.001$). The standard length of *A. lineatus* was negatively correlated with relative fecundity and gonadosomatic index during the breeding season.

DISCUSSION

In the present study, the fecundity of *A. lineatus* differed with the size of the fish. The fecundity ranged from 125 - 525 eggs/fish. In many species, fecundity was directly related to female size (Travis, 1983; Choeng *et al.*, 1984). Chippett (2003) reported that the fecundity of *Fundulus diaphanus* was in the range of 250 - 400 (Carlander, 1969). Cooper (1983) observed that the size of the eggs of *Fundulus diaphanus* was larger than 2.0mm. Schmeidler and Brown (1990) reported the existence of a significant linear relationship between fecundity and standard length of all mature females in *Fundulus zebrinus*. The batch fecundity of *Fundulus heteroclitus* for a 70mm SL female was 94 eggs (Kneib, 1986). One large female of *Fundulus luciae* (30mm SL) had 21 ripe ova (batch fecundity). *A. lineatus* spawned her eggs on the plants and attached them with the help of an adhesive filament of the chorion. The males released the milt and fertilized the eggs as and when they were released from the female papilla in clusters of 3 - 5 eggs. It was reported that among *Fundulus diaphanus* (Richardson, 1939; Chippett, 2003), *Millerichthys*

robustus (Castanedo *et al.*, 2013; 2016; 2017;2018) and in Aplocheiloidei killifish (Furness, 2016, Furness *et al.*, 2015) and *Lucania* killifish (Berdan *et al.*, 2021) the male fertilized the eggs when they hang from the female papilla.

Hubbs and Mosier (1985) reported that the fecundity of *Gambusia gaigei* was size-dependent with large females

producing 50 or younger in peak months and fewer than 30 in late summer. Guma'a (1982) reported that the absolute fecundity of *Epiplatys bifasciatus* (total number of embryos in a brood on the nursing portion of the ovary) ranged between 15 and 35, depending on the size and the stage of maturity of the fish.

Table 1: *Aplocheilus lineatus*: Relationship between live weight and ovary size.

Live body weight (g)	Standard length(mm)	Total length(mm)	Wt of ovary(g)	Length of ovary(mm)
0.393± 0.015	30±2	35±2	0.009±0.001	6
0.512± 0.013	32±2	37±2	0.011±0.003	6
0.598±0.011	35±2	39±2	0.012±0.004	6
0.635±0.015	37±2	43±2	0.015±0.002	8
0.783±0.012	38±2	45±2	0.015±0.003	8
0.852±0.002	39±2	47±2	0.022±0.002	9
0.889±0.012	40±2	50±2	0.027±0.003	9
1.252±0.024	44±2	53±2	0.031±0.004	10
1.357±0.012	45±2	55±2	0.025±0.003	10
1.639±0.011	47±2	58±2	0.021±0.002	10
1.753±0.002	51±2	60±2	0.025±0.004	12
2.213±0.003	52±2	63±2	0.015±0.003	12
2.324±0.016	54±2	65±2	0.028±0.005	12

X ± SE of 75 observations

Table 2: *Aplocheilus lineatus*: Relationship between live weight and testis size.

Live body weight (g)	Standard length(mm)	Total length (mm)	Wt of testis (g)	Length of testis (mm)
0.357± 0.014	30±2	35±2	0.005±0.001	6
0.478± 0.013	32±2	37±2	0.005±0.002	6
0.512±0.011	35±2	39±2	0.007±0.001	6
0.585±0.012	37±2	43±2	0.006±0.003	6
0.825±0.012	38±2	45±2	0.008±0.002	6
0.935±0.012	39±2	47±2	0.006±0.003	6
0.989±0.012	40±2	50±2	0.008±0.002	8
1.275±0.024	44±2	53±2	0.009±0.003	8
1.379±0.012	45±2	55±2	0.009±0.003	8
1.735±0.013	47±2	58±2	0.012±0.005	10
1.879±0.003	51±2	60±2	0.008±0.003	10
2.353±0.016	52±2	63±2	0.005±0.002	10
2.475±0.003	54±2	65±2	0.009±0.001	12
2.675±0.015	58±2	68±2	0.009±0.001	12
2.753±0.014	60±2	70±2	0.015±0.003	14
3.125±0.012	62±2	73±2	0.009±0.002	14

X ± SD of 75 observations

Table 3: *Aplocheilus lineatus*: Relationship of fish length and weight with GSI, Ovarian index, Dobriyal index during the pre- breeding, breeding and post- breeding periods.

PERIOD	Standard Length (mm)	N	Live Fish Weight(g)	GSI (%)	Ovarian Index (%)	Dobriyal Index(%)
Pre-breeding	25±4	132	0.43±0.015	1.953±0.310	0.018±0.003	0.217±0.017
	30±4	252	0.50±0.017	2.203±0.311	0.026±0.004	0.222±0.023
	35±4	158	0.68±0.012	2.406±0.271	0.029±0.003	0.247±0.031

	40±4	164	0.97±0.029	2.733±0.235	0.035±0.002	0.255±0.035
	45±4	197	1.58±0.031	2.927±0.213	0.042±0.006	0.285±0.042
	50±4	16	2.15±0.011	3.304±0.311	0.045±0.003	0.327±0.035
	55±4	10	2.22±0.012	3.567±0.324	0.043±0.004	0.335±0.034
	60±4	5	2.45±0.023	4.020±0.342	0.048±0.003	0.365±0.024
Breeding	25±4	129	0.45±0.023	1.520±0.125	0.017±0.001	0.215±0.035
	30±4	145	0.67±0.015	1.937±0.117	0.024±0.002	0.257±0.041
	35±4	162	0.79±0.023	2.232±0.310	0.029±0.002	0.271±0.035
	40±4	190	1.07±0.019	2.336±0.112	0.031±0.005	0.292±0.015
	45±4	111	1.75±0.017	2.500±0.261	0.039±0.002	0.327±0.017
	50±4	157	2.13±0.025	3.970±0.117	0.044±0.002	0.342±0.027
	55±4	40	2.25±0.032	4.133±0.121	0.048±0.003	0.363±0.041
	60±4	7	2.48±0.150	4.357±0.234	0.051±0.002	0.389±0.243
Post breeding	25±4	100	0.48±0.021	3.125±0.175	0.025±0.002	0.247±0.021
	30±4	158	0.55±0.051	4.000±0.121	0.031±0.002	0.280±0.025
	35±4	135	0.62±0.320	2.097±0.231	0.022±0.002	0.235±0.037
	40±4	193	0.85±0.021	1.765±0.235	0.021±0.003	0.247±0.035
	45±4	332	1.03±0.025	2.136±0.415	0.028±0.001	0.280±0.041
	50±4	212	1.65±0.019	1.515±0.129	0.028±0.002	0.292±0.043
	55±4	14	1.69±0.013	0.975±0.025	0.018±0.003	0.212±0.023
	60±4	25	2.03±0.025	1.232±0.032	0.022±0.003	0.225±0.004

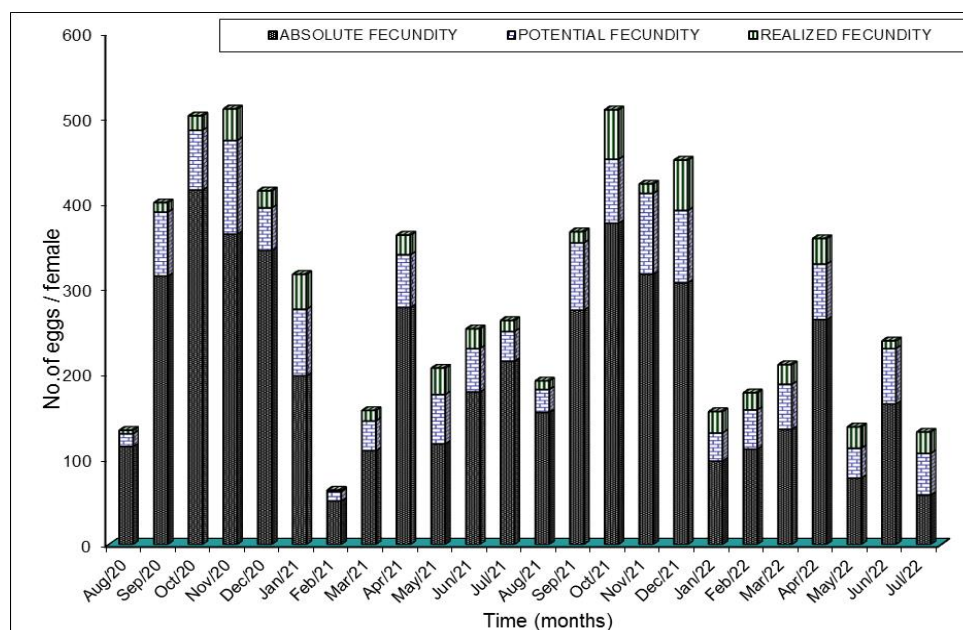


Figure 1: *Aplocheilichthys lineatus*: Comparative representation of different fecundity estimates of brood fishes sampled monthly from August 2020 to July 2022.

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