Fisheries and Aquaculture Journal
ISSN: 2150-3508

Mathialagan et al., Fish Aqua J 2017, 8:3
DOI: 10.4172/2150-3508.1000223

Research Article OMICS International

Maturation and Reproductive Biology of Reba Carp *Cirrhinus Reba* (Hamilton) in Lower Anicut Reservoir, Tamil Nadu, India

Mathialagan Ramasamy[1] **and Sivakumar Rajangam**[2*]

[1]*Department of Life Science, MASS College of Arts & Science, Chennai Salai, Kumbakonam-612501, India*

[2]*Department of Zoology, Government Arts College (Autonomous) Kumbakonam-612002, Tamil Nadu, India*

[*]**Corresponding author:** Sivakumar Rajangam, PG and Research Department of Zoology, Government Arts College (Autonomous) Kumbakonam-612002, Tamil Nadu, India, Tel: +91 94433 32332; Fax: 0435-2442977; E-mail: sivaera@gmail.com

Received date: August 24, 2017; **Accepted date:** September 13, 2017; **Published date:** September 21, 2017

Copyright: © 2017 Mathialagan R, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Examine the reproductive biology of *Cirrhinus reba* at Lower Anicut reservoir and analyses of gonads assessed by ovarian cycles. Sex ratio, size at first maturity, GSI, macroscopic appearance ovary, fecundity, oocyte diameter and histological examination suggested as a result of spawning season. Males were 59.4% and females 40.6% suggested the ratio significantly deviated from expected 1:1. Size at 50% maturity estimated from logistic model curve 132 mm in males and 148 mm for females. GSI was represented increasing the development of gonads in both sexes until it was ripe and spent. During spawning, increasing the length and weight of gonads from immature to ripe, maximum values of GSI for both sexes (3.43%-3.49% to 4.09%- 6.01%) were obtained during April to August with peak in July. Size-frequency distribution of ova diameter were clearly indicate maximum values of diameter enclosed during July to corresponding microscopic stages of gonads. Five stages of oocyte development were determined through histological examination can be divided immature, maturing, matured, ripe and spent. Total length, weight, gonad length and weight were significantly related to fecundity. Stocks of *C. reba* were enclosing females spawn once a year during spawning season. Following, the implications for management and controlled feral carp stocks were discussed in detail.

Keywords *C. Reba* sex ratio; Size at first maturity; GSI; Fecundity; ova diameter; Macroscopic; microscopic examination of gonads

Introduction

Investigating the reproductive biology of commercially downtrodden fishery can provide some useful information on reproductive uniqueness to expand sound biological assumption for unambiguous stock assessment and management purposes [1]. Beginning the management standpoint is very essential for fish resources and stock that uneven nature and it defined remain more or less unwavering whole time distribution [2]. Following, the reproductive stock assessments should be made for each stock (sexes) separately. Several taxonomical assessments of the stock variables of fish species, along with studies that the species genetic and migratory characteristics to provide the information are used to define the stock structure of such species [3]. Generally, there is only sparse information on reproductive-related characteristics of the species and even fewer data were available for developing more timely estimates of these characters for current stock and reproductive assessment. To date, reproductive variables (e.g. sex ratio and maturity) pertaining to the population used to assessments based research were conducted over three decades ago. Besides, both scientific and management consensus have recommended that all nations strive to limit further increases in fishing effort [4].

Determination of reproductive maturity and development of fecundity is rudimentary in fishery biology. Owing, the importance of these parameters is full of life in fish populations [5] and commonly estimate for economic significance of species. As well, the most suitable way to determine the reproductive cycle in female fishes to observe the seasonal and developmental changes in gonads [6-8]. Subsequently, the maturation cycle has been described by morphological changes of gonads undergo to attain full growth and ripeness. However, methods to identifying spawning seasons of fishes are reviewed who recommended that the histological studies while expensive, time consuming, most reliable yield and to focusing a clear objectives on spawning cycles [9]. Like, the histological examination is well thought-out essential to detecting the details within the maturation cycles in females [9,10]. In current years, there has been an ever-increasing interest for culture aspects in Reba carp *Cirrhinus reba* both commercial and angling species in India and adjacent Asian countries. Although there is much anecdotal evidence of the moribund stocks and there has been no obvious and complete meticulous investigation on biology and reproductive stock structure. Resembling, *C. reba* is one of the most popular food fish and it's widely distributed in India, Bangladesh, Pakistan, Nepal, Burma and Thailand [11,12]. Yet, the wild population of this species on the way out due to heavily harvested. To date, no qualitative and quantitative unique stocks are available in this riverine system. However, the catches of this species have turned down in recent years due to increase its range of fishing mortality etc. [13]. For that reason, the feasible management seems to be very necessary of this species. Hitherto, the biological research information is most vital one to meet the diminishing state of the *C. reba*. Besides, being important noted studies on fisheries biology can expressed in reproductive survey have largely favoured to collection of all information on the wild captured fishes [14].

Furthermore, the reproductive biology i.e. length at first maturity, sex ratio, GSI, fecundity and spawning are among the important aspects of reproductive biology of fishes. To explain the variations in the level of populations as well as make efforts to increase the amount

of harvest and recruitment stocks are great value in management of fishery and also very essential part of aquaculture practices. Because, the low down reproductive rate and direct relationship between stock structures of the fish populations are very extremely susceptible [15]. Consequently, the populations could be depleted under high fishing mortality on mature fish or by any substantial fishing on immature age. These results are implied that the population is going to vulnerable intense fishing activities in the sampling (reservoir) region. The reproductive information which is vital one for stock assessment and fishery management of this species is still unknown. Some of them authors were reported that observations on the spawning of *C. reba* in Cauvery and Bhavani waters were estimated fecundity of this species from India [16-18]. Presently, there is a little and incomplete information was available in reproductive biological status of *C. reba* in different waters. However, no specify and completely published evidences on reproductive biology and histological background in this sampling region. As an outcome of the information on reproductive history through comparison with previous studies and also the foremost occurrence of some temporal changes in these traits are detailed discussed. On the other hand, the deficient in the earlier period of research information (GSI, fecundity, reproductive cycle, growth and mortality) are uncertainty resource and fishing pressures at highly risk and it possibility to sustainable exploitation. Besides, the results obtained in this study, this is my part of doctoral research work. Finally, the main objective of this study was providing the first and complete reproductive information of *C. reba* in Cauvery (Kollidam) waters. Collectively, the results presented here are intended to provide fishery biologists more timely information and ultimately make stronger stock assessment efforts.

Materials and Methods

Study site

Biological data was collected from a branch of Cauvery, specifically Kollidam (Coleroon) river, Lower Anicut was selected for the present study (Figure 1) which is located 11° 08' 03" N latitude and 79° 27' 05" E longitude. The Kollidam river flows from west to east forming located in northern boundary of this block flanking at Tamil Nadu. The present fishery occupies a prominent one and totally 395 (225 males and 170 females) individuals of *C. reba* were collected.

Sample collection

Specimens were kept chilled in ice box immediately after capture and brought to the laboratory for further examinations. Total length (TL) were obtained using a measuring board and recorded to nearest mm while the total weight (TW) was measured [19] using an electronic balance (DIGI' Arts maximum=500 g to d=0.05 g) and recorded nearest to g. Specimens were identified morphologically using scientific literature relevant to the group with original descriptions [20]. Further, species identification using tissue samples (i.e., caudal fin) were collected and stored in sterile eppendorf tubes containing absolute ethanol, sealed with parafilm kept at room temperature for DNA analysis [21]. After that, sex was determined by gross examinations of the stomachs. At the same time juveniles, males and females were differentiated and data were recorded after dissecting out the gonad. Specimens were preserved in 5% formalin for future examinations. Damaged specimens were rejected.

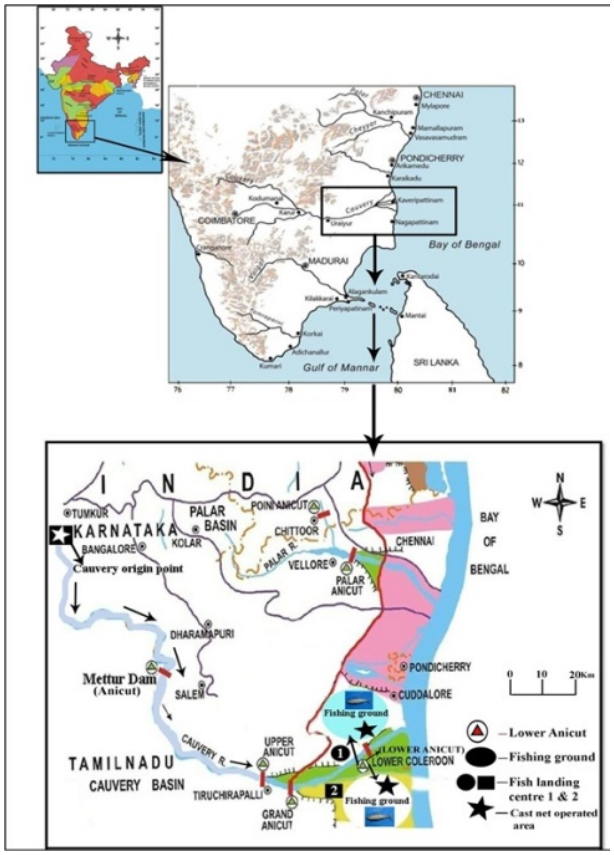

Figure 1: Map showing the geographical location of sampling area in Lower Anicut, Tamil Nadu

Reproduction

Specimens were eviscerated in order to determine sex and collect the gonads for further analysis. After examination of the gonads, fishes were classified in different sexes, length and shape of the gonads and colours are also recorded. Sex ratio of *C. reba* were studied using Chi-square test (x^2), following the equation [22] assuming that the ratio of both sexes in the population. Gonado Somatic Index (GSI) was calculated [23] whereas, the GSI was expressed as GSI = (GW/TW) × 102 while, GW- gonad weight and TW- total weight. Following, the relation between length and maturity in each size class was demonstrated on a logistic diagram to estimating the total lengths at 50% (Lm_{50}) maturity. The proportion of mature fish gonads were observed by following the description [24]. The size at first maturity was determined by one of the author [25] and also directly compared to plotting the percentage of mature fish against their length. The size at which 50% of the females were matured (with developed ova in ovary) was considered as the size at first maturity. Subsequently, the gonads were cleaned, weighed and preserved in 10% neutral buffered formalin. The fecundity was estimated by counting the number of mature ova (opaque and larger in size) from known weights of sub samples collected from the anterior, posterior and middle portions of the ovaries and calculating the total number of mature ova [26].

Where,

F=annual fecundity; C=number of eggs counted in sample; S=weight of sample (g) and OW=weight of the ovary (g).

The relationships between fecundity with total length, total weight, gonad length and gonad weight were obtained by plotting as a scatter-plot by linear regressions. To determine the spawning season and breeding periodicity, the ova diameter progression was recorded (an ocular micrometre was used for measurement of diameter) with plotting the mean percentage frequency of ova of diameter in mature ovaries, against different months [27]. Small and transparent ova were considered as immature, medium sized and less transparent were considered as maturing and the large opaque ova were considered mature or ripe. The histological analysis was performed in order to analyse and assess the reproductive characteristics of this species. Determine the individual stage of sexual maturation and annual reproductive cycle of fishes, because it provides more consistent than visual inspection of the reproductive organs [28]. The sexual stage and histological interpretations is most appropriate way to determining the spawning cycles of the ovary. Thus, the present study has followed that the histological guidelines presented [26].

Results

Monthly variations of sex ratio were referred as 395 specimens whereas 225 males and 170 females. The percentage of males ranged from 48.0% (July 2011) to 66.0% (October 2010) and 33.4% (August 2010) to 51.7% (July 2011) in females while, the minimum and maximum sex ratio was ranged from 1:0.50 and 1:1.06 ($P>0.05^{*}$ and $P>0.01^{**}$) whereas, the overall sex ratio was examined 1:0.69 ($P>0.01^{**}$). To conclude, the percentage of males was 59.4% and females 40.6%. Generally, the sex ratio was approximately 1:1. However, the present study suggested that the ratio were highly significant and deviated from the expected 1:1 ratio (Table 1).

Months	Male	Female	Total samples	% of Male	% of Female	Ratio M:F	Chi square
Apr-11	20	17	37	60	40	01:00.7	1.2**
May	18	15	33	60	40	01:00.7	1.2**
June	19	15	34	58.6	41.4	01:00.7	0.5*
July	19	15	34	48.3	51.7	01:01.1	0.125*
August	19	14	33	63.3	36.7	01:00.6	2.1**
September	18	12	29	60.7	39.3	01:00.6	1.3**
October	20	10	30	66.6	33.4	01:00.5	3.3**
November	18	13	31	62	38	01:00.6	1.2**
December	19	14	33	57.5	42.5	01:00.7	0.47*
Jan-12	18	16	34	60	40	01:00.7	1.2**
February	19	16	35	61.2	38.8	01:00.6	1.125*
March	18	13	31	62	38	01:00.6	1.2**
Total	225	170	395	59.4	40.6	01:00.7	25.94**
Significant at P>0.05* and P>0.01**							

Table 1: Monthly variations of sex ratio of *C. reba.*

The size at first maturity was studied based on the various size groups of individuals. The logistic curves describing that the relationships between proportions of maturity in each length interval were estimated for both sexes. The LM 50% sexual maturity was observed in both sexes while, large and white testes and brownish ovaries were defined as mature and also increased the GSI values of gonads. The proportions of 50% maturity were estimated and attain 132 mm in males and 148 mm in females were found (Figure 2a and 2b).

In males, (testes) stage-I immature were recorded low during August (10%) and gradually increased with peak in October (80%). Stage-II maturing during October (16%) were gradually increased with attained high level during December (45%) and gradually decreased during January (36%) to March (21%). Stage-III mature fishes were recorded from December (26%) onwards with peak during March (87%). The stage-IV ripe gonads were noticed high during April (80%), June and July (100%). Finally, the spent or resting stage-V were observed between August (60%) to September (30%).

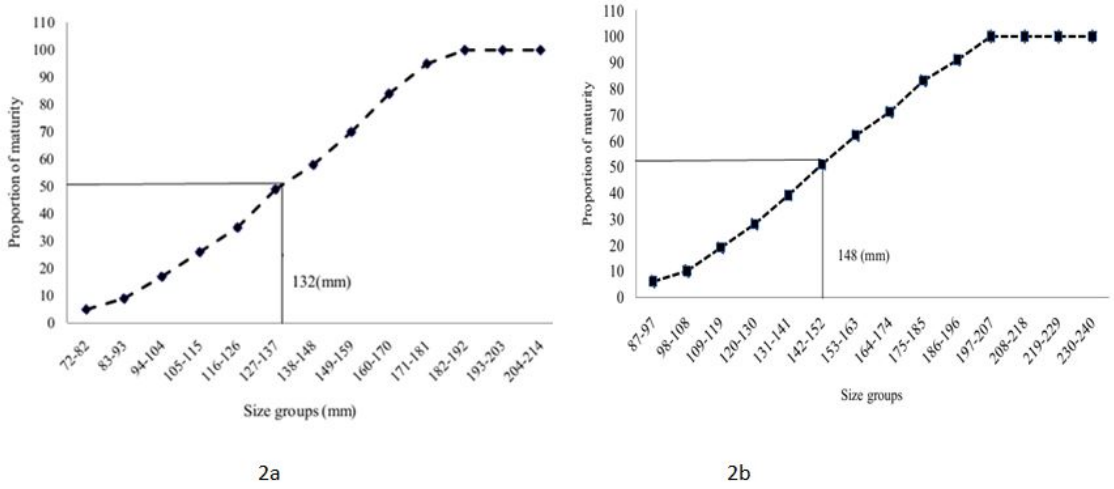

Figure 2: a) Percentage composition with proportions of size at first maturity of male *C. reba*. b) Percentage composition with proportions of size at first maturity of female *C. reba.*

The gonado somatic index (GSI) was represented as increasing the progressive development of gonads in both sexes until gonads were ripe and index was high during spawning and low for the period of spent. Increasing weight from immature to ripe gonad condition (GSI) was illustrated in Figure 3. The maximum values of GSI for both sexes were obtained during April to August with peak in July. Following, the monthly changes of reproductive activity in male *C. reba* are shown in Figure 4a. Gonads were categorized into five developmental stages based on the observations of the nature and their abundance.

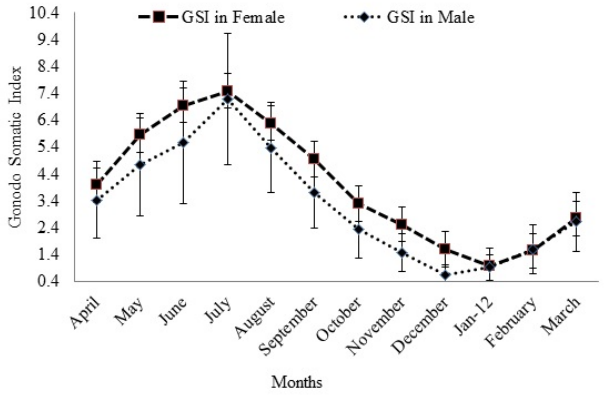

Figure 3: Monthly variations of Gonado Somatic Index (GSI) of *C. reba.*

Description of the maturity stages of ovaries was illustrated in Figure 4b. In females stage-I ovaries were recorded low during August (12%) and high during October (80%). Stage-II were encountered small level during October (19%) whereas large during December (40%). High percentage stage-III recorded during March (90%) and low in the month of April (12%). Following, the stage-IV was evidence high during June and July with this condition increased dramatically (100%) low during April (89%). Subsequently, stage-V was documentation high during August (71%) and low in September (24%). Finally, the GSI values were low for both sex ovaries with assigned to Stages I–II, reflecting their immature status and gradually increased, the ovaries allocated Stages III–IV, coinciding with maturation of the ovary.

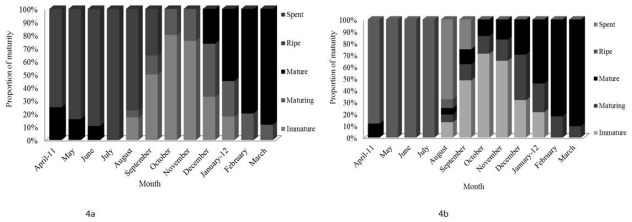

Figure 4: a) Monthly proportion of maturity stages of testes (spermatogenesis) of *C. reba*; b) Monthly proportion of maturity of testes (oogenesis) of *C. reba.*

A total of 63 ovaries with distinct modes of size-frequency distribution of oocytes were used to estimate fecundity. The fecundity was estimated ranging from 91321 to 337389 (TL-from 137 mm and TW 61.2 g to 235 mm and 157.5 g) and also the average was referred as (Mean ± SD) 256420.8 ± 67659.47. Further, the relative fecundity was estimated against to total length and total weight of the body, total length and total weight of gonads were shown in Figure 5a and 5d and Table 2.

Variables	Log values of Fecundity	'b' value of Fecundity	r	r^2	P
Total Length-Fecundity	Log F=1.156 + 1.981 Log TL	F=14.322*L1.981	r=0.726	r^2=0.684	P<0.05
Total Weight-Fecundity	Log F=3.322 + 1.143 Log TW	F=2098.94*L1.143	r=0.785	r^2=0.625	P<0.05
Gonad Length-Fecundity	Log F=2.963 + 1.315 Log GL	F=918.34*L1.315	r=0.760	r^2=0.652	P<0.05
Gonad Weight-Fecundity	Log F=3.352 + 1.032 Log GW	F=2249.06*L1.032	r=0.792	r^2=0.638	P<0.05
b-Slope; r-Correlation co-efficient; r^2-Regression co-efficient; P-Probability					

Table 2: Regression models of different variables for fecundity of *C. reba.*

On the basis of monthly variation, distinct groups of ova were observed during ripe condition in Figure 6. The size-frequency distribution of ova shows that diameter were clearly indicate that equally distribution of the gonads, with maximum values of ova diameter (1.693 ± 0.087) covered during July to corresponding the shown in microscopic stages of gonads.

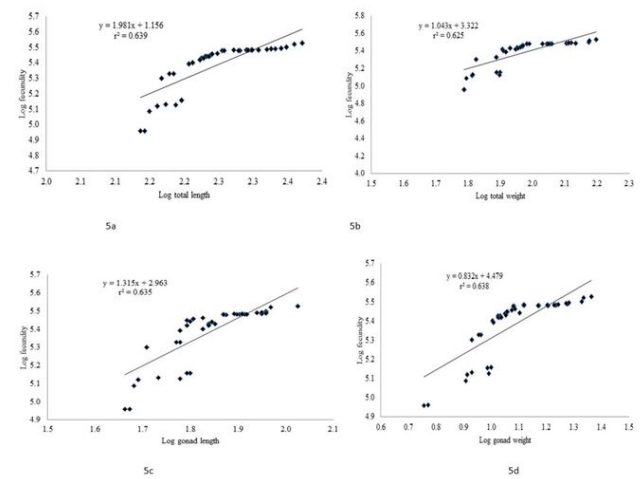

Figure 5: a) Logarithmic relationship between total length and fecundity of *C. reba.* b) Logarithmic relationship between total weight and fecundity of *C. reba.* c) Logarithmic relationship between gonad length and fecundity of *C. reba.* d) Logarithmic relationship between gonad weight and fecundity of *C. reba.*

The morphological examinations of gonads of *C. reba* were consisted into five stages in order to year around collection of the gonads. Further, the gonads (testes and ovary) were taking photographs for the purpose of morphological differences of gonads (Table 3 and Figure 7a and 7b). Subsequently, the histological analysis of gonads to assess the changes in reproductive state of Reba carp throughout the study period.

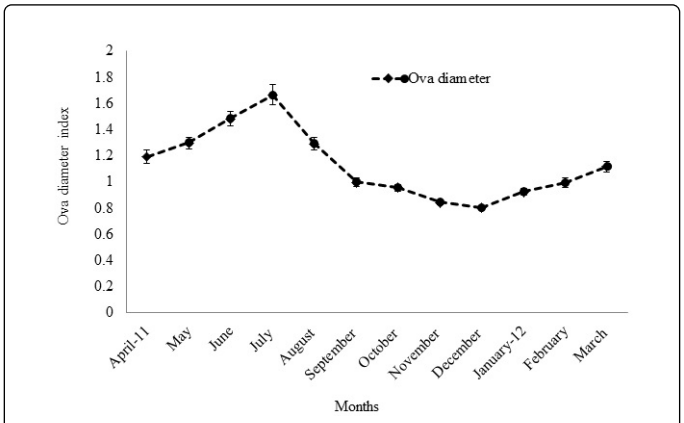

Figure 6: Monthly variations of ova diameter (μm) of *C. reba*

The reproductive cycles were noticed as five stages viz., stage-I (Immature), stage-II (Maturing), stage-III (Mature), stage-IV (Ripe) and stage-V (Spent) in both sexes. The gonads were segregated i.e., anterior, middle and posterior parts showed for histological variations in different maturity stages were covered in Figures 8 and 9. Firstly, all the parts of testes showed similar stages of development in a particular stage. Immature and resting phase (Figure 8a) were covered the numerous spermatogonia and small seminiferous lobules was observed. Spermatogonia were large containing central nucleus with distinct nucleolus.

Following the slow mitotic activity was occurring in maturing phase and spermatogonia were started dividing and transformed into sperm mother cells (Figure 8b). Intense spermatogenesis was treated during latter part of developing phase. Spermatogonia decreased and numerous primary and secondary spermatocytes were visible. The primary spermatocytes were smaller than spermatogonia whereas the secondary spermatocytes, were smaller than primary with clump chromatin material (Figure 8c). In pre-spawning phase, blood capillaries became conspicuous; the seminiferous lobules were larger in size and full of sperm. Spermatogonia were few and all stages of spermatogenesis can be seen in various lobules (Figure 8d). In spawning phase, the semiferous lobules became empty for the reason that of release of sperms (Figure 8e). During spent, the empty and collapsing seminiferous lobules were observed which contain residual or unexpelled sperm.

Development stages	Category	Testes	Ovary
Stage-I	Immature	Testes very small, thread-like, elongated, whitish in colour, not easy separate from viscera, small opaque and lack of milt in the transverse section.	Ovaries are ribbon-like, fleshy white-colour, occupy one-half of body cavity and oocytes are not visible.
Stage-II	Maturing	It is larger than immature testes, white with shining colour and slightly produces dilute milt when squeezed.	Ovaries light brownish colour and occupy to some extent of the body cavity. Mass of small invisible eggs to naked eye.
Stage-III	Mature	Testes large, ivory whitish colour, vascularization heavy in back of them and exteriorly dorsal blood vessels are present. Males are easily identified by reddish genital opening and oozing of milt with slight pressure is applied on abdomen.	Ovaries long, dark-brownish and fill almost whole of the body cavity. Ova big, brown or light greenish colour while, when seen under the microscope.
Stage-IV	Ripe	Testes are firm with elongated, bulged, creamy white colour with free-flowing milt. It occupies 20-80% length of body cavity. Sometimes testes were white with blood spot nature.	Brownish ovary, large and fill entire body cavity. Eggs pass out slight pressure applied on abdomen. In breeding the mature ova increase size with accumulation of yolk. The ova maximum attained the micro-divisions (µm). Gonad / genital aperture often inflamed.
Stage-V	Spent	Testes are grey (dull white) with bloody, flaccid, loosely and rubbery as the regress to resting.	The ovary light pinkish, flaccid, blood-spot. A water-like fluid passing out on pressing. The larger ova completely discharged or sometimes with black spots. Some residual oocytes visible, ovary wall thickened and wrinkled.

Table 3: Macroscopic gonad descriptions of *C. reba.*

In resting phase, the ovary showed nests of oogonia and immature oocytes in the stage I and II were occur (Figure 9a and 9b). In maturing stage the ovary contains numerous oocytes in different stages of development. In mature ovaries, the cytoplasm with largest oocytes is full of yolk granules and lipid droplets. In addition the just prior to spawning the hydration process continues awaiting ovulation, when the follicular epithelium surrounding oocyte breaks and egg is released. Further the POF (Post Ovulatory Follicles) undergo a rapid degeneration. Following the large number of small, clear vacuoles called yolk vesicles, appeared in the periphery of the ooplasm. However, the yolk vesicles increased in number and fill the entire part of the ovary (Figure 9c). So far, the yolk granules were fused form larger globules of egg yolk (Figure 9d). In pre-spawning phase, a large number of ova and ripe eggs were observed in the ovary Figure 9e. Some atretic follicles were also observed in this stage. In spawning phase, ripe ova come out by rupturing of follicular epithelium (Figure 9e). In spent phase, the ovary showed atretic and discharged follicles along with oocytes.

Discussion

The results point out that the *C. reba* undergoes a specific seasonal fluctuation and its gonadal conditions. At the outset, sex ratios can have an idealistic impact when predicting the species population point of reproductive output. Besides, the faster growth rate leads increasingly to less loss from predation and this might influence the sex ratio. In the present species, sex ratio in favour of males during the spawning has been reported and also no sexual dimorphism was occurred. The reasons probably may be ecologically or genetically or both [29]. Males are usually predominating in the young fish, because they mature earlier but live less [30]. In the younger groups, the males predominate because they tend to mature earlier than females. Following, the females are suspected to leave the spawning grounds more rapidly than male counterparts [31]. In natural environment the optimum sex ratio is 1:1 but it may be far from this in particular age and size group.

Besides, an increasing sex ratio with body size has been documented, it possibly due to differences in mortality rates between sexes. Whereas, the growth differences were documented between sexes, sexual dimorphism and migration may also use influential factors [32]. However this study revealed that, seasonal variation of the population occurs only in the catch. The presence of more female individuals during most of the months may be due to the vulnerability of females. So far, the variability may perhaps due to factual differences in the composition of local populations or it may be an artifact of sampling strategies rooted by season covered or gear biases [33]. Though the differential habitat occupy by sex has been earlier observed in tropical fishes [34] with the possible reason of this species may also accordance. Following, the males are compared to females during peak period of spawning and such predominance could be due to migration of females to relatively deeper waters, therefore being less vulnerable during spawning or behavioural differences between two sexes. On the other hand, males live in shallow areas from where they are easily caught. Some earlier authors were incomplete reported about these aspects like, sex ratio in same species in different waters at various time were strongly accordance in the present work [16,17,32,35-37].

The size at first maturity was observed the males are attained at 50% sexual maturity was smaller than females. LM 50% is an important trait of life history necessary for successful fishery management, thus fundamental establishment means that avoid exploitation of young individuals and consequential reduction of spawning stock. Besides, most of the males reach maturity smaller and younger than females which explains the greater duration of life, the females mature later. However, observed relationship between first sexual maturities temperature based in every region. Hence, younger ages and smaller sizes at maturity are observed in warm waters, whereas in cooler waters both age and size at maturity are higher [38]. Subsequently, the

Logistic curve has been successfully used to estimate the size at 50% maturity for suggested that stock density, food, water and temperature may influence the growth of fish and further affecting the age at 50% mature [39]. The present study provides the information on size at first maturity of *C. reba* which is reported for the first instance in this sampling region.

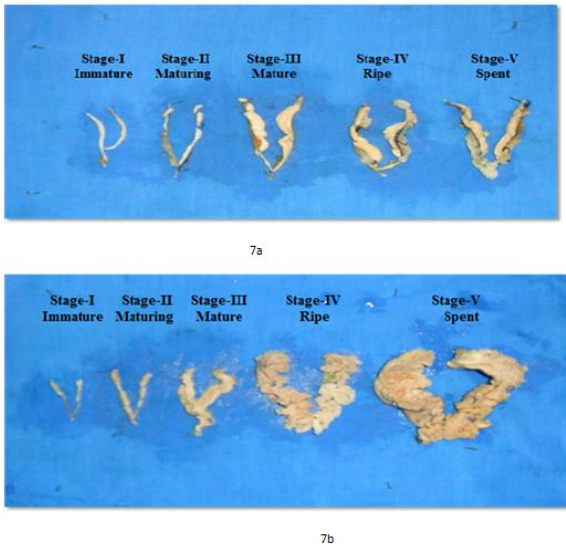

Figure 7: a) Morphological structure of testes of *C. reba*; b) Morphological structure of ovary of *C. reba.*

The gonado somatic index is predominantly helpful to identifying gonadal maturity and spawning season of any fish species. As expected, a clear relationship was apparent between GSI and reproductive stage in both sexes. However, it was observed that the species spawned once in a year with single spawning peak during July confirmed by GSI and ovarian cycle. Moreover, in this study females are larger than males with the ratio (male: female) increased based on the growth. Here, the changes of GSI and histological analysis of gonads, the ovary and testis were mature during April-August and regressed during September-December with peak in July [37]. The gonads were enclosed in different stages of seasonal maturation were also observed in this study. The present findings were supported some earlier authors in same species [40,36,41] to predictably the *C. reba* is an annual breeder with a single spawning period restricted to south-west monsoons extending from May to July in Assam and Bangladesh. One of the author's was reported that the species were spawned from June to August with a peak in June [32].

Authors were reported that the spawning period of *Cirrhinus mrigala* from June to August with a peak in July [42]. The mature ripe ovaries in females and also increase the values of GSI. In contrast, GSI values of testes showed that the mature stages are covered in the season of summer with increase in temperature and also raise the spermatogenetic activity whereas, the primary and secondary spermatocytes and spermatids were filled in the lumen of lobules. On the similar, the season of summer also comprises the preparation of pre-spawning phase. Hence, the GSI level of gonads was higher in summer than winter.

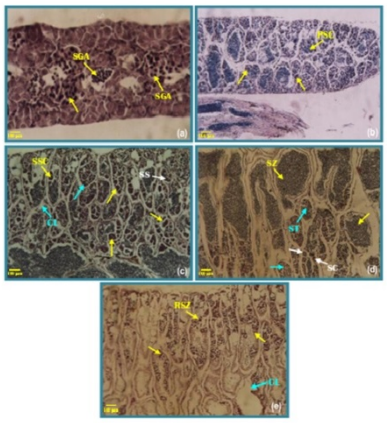

Figure 8: Microscopic stages of testes development in male *C. reba*; a) Stage-I: Tunica albuginea very thin covered externally with the help of mesothelium. Spermatogonia (SGA) indicated yellow colour arrow in immature testis; b) Stage-II: Arrangement of primary spermatocytes (PSC); c) Stage-III: Secondary spermatocytes (SSC-yellow colour arrow) of testicular cells in mature testis containing a prominent central lumen (CL-pink colour arrow) and peripheral sperm sinuses (SS-white colour arrow); d) Stage-IV: Spreading pattern of spermatozoa (SZ- yellow colour arrow) in a ripe/running ripe testis; spermatids (ST- pink colour arrow) and sertoli cells (SC-white colour arrow) also present; e) Stage-V: Residual spermatozoa (RSZ- yellow colour arrow) and large central lumen (pink colour arrow) is clearly shown. Scale bars =100 µm.

Besides, the seasonal factors of GSI might a greatly influence in the maturation of ovary by resulting the successive changes of the gonads and body weights [43]. On the other hand, declining GSI it gives clear indication for affected metabolism which in turn affects the reproductive potential due to some stressors [44]. From the final observation it is evident that the *C. reba* has only one breeding season of short duration running from April to August, with a peak in July. Similar observations have been made [16,17,40] from Cauvery, Bhavani River, Muzaffarnagar (UP) India and Bangladesh waters.

Gonad maturation during spawning low food availability in most of the fishes throughout to utilize somatic energy reserves, particularly rich protein content to need for reproductive growth [45]. This suggested that the fishes may store the energy required for spawning in the liver or viscera. It is likely that required energy for spawning might be derived from their diet instead of energy reserves [46]. In the tropical cyprinids, spawning seasons were associated with seasonal rains, flooding of rivers or monsoons and also alike in temperate cyprinids. One of the author's was [47] reported that the water temperature reaches to critical values during spring and final maturation stages of oocyte development are completed in carp may spawn, providing the appropriate spawning stimuli are require. Further, the photoperiod is also considered an important ultimate factor regulating the timing of spawning, recrudescence, spawning success, survival of the larvae, growth of juveniles, water level and quality, nutrients, temperature, breeding substrate and vegetation are also which may to determine the spawning success in cyprinids [47].

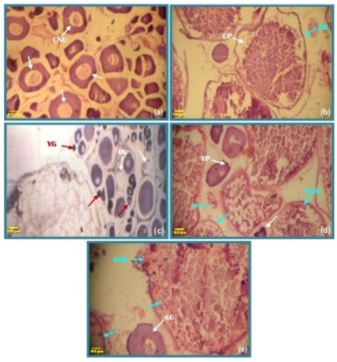

Figure 9: Microscopic stages of testes development in female C. reba; a) Stage-I: Peri-nucleolar stage shows that peri-nucleolar oocytes with large central nucleus (CNU-grey colour arrow); b) Stage-II: cortical alveoli stage, pink colour cytoplasm (CP-white colour arrow) and dark pink colour zona-radiata (ZR-grey colour arrow); c) Stage III: Ovaries were clear with densely packed oocytes in the cytoplasm and filled with yolk granules (YG- dark red colour arrow), cortical alveoli and oil vesicles (OV-red colour arrow); d) Stage IV: The number of peri- nucleolar oocytes (PNO-pink colour arrow) increase with oocytes size. Oocytes and yolk granules begin fuse with yolk plates (black colour spot- YP); e) Stage-V: POF- Post Ovulatory Follicles (pink colour arrow) are clearly visible. Spaces between oocytes filled with stromal cells (SC-white colour arrow) and irregular shape of cell and nucleus. Scale bars =100 µm.

Furthermore, the scatter diagrams were referred as linear relationship between fecundity and other variables. Similar findings were reported [18] in *C. reba* from Baigul reservoir (U.P) India and [48] in Labeo bata from Bangladesh waters. Some authors [35-37,40,41,49] were reported that the fecundity of an individual female varied likewise many factors including age, size, species and environmental conditions (such as food availability, water temperature and salinity) that support to the present findings. In addition, fecundity can be influenced by ecological factors through food. It may be suggested that fecundity differences were associated with temperature and infertile streams had lower egg production. However, the low fecundity was observed in related to fishing intensity and strong intra and inter-specific food competition [50]. It concluded and observed that fecundity increased also raise in total length, total weight and gonad weight.

Few authors [17,18,35,3749] were accounted that the maximum size of ova diameter were found in during peak of spawning which is in accordance with the present findings. During the study it was noted that the diameter of the egg varied with species. Like, the ovaries of same size of individuals contained various numbers of eggs this may showed due to environmental conditions and intake capacity of food by fishes. Authors are [51] reported that the lowest egg diameter was observed the uppermost number of eggs had employed the strategy of the force of number. Many eggs were laid by the least in abundance from the water. Another one important phenomena, absence of parental care may have contributed to low population from Cichlidae and Cyprinidae fishes; other factors that may be responsible are predation of the eggs besides some harsh environmental conditions to influence the size of eggs.

The ovarian or testis developmental stages documented through macroscopic examination were easier than other methods while, the large samples could be regularly examined. However, this technique may guide to erroneous categorization in reproductive analysis of fishes [52]. Further, the variation of GSI has been used for number species as a measurement of reproductive maturity. In this context, the author [53] was recommended that GSI is not precise indicator of gonadal activity. Hitherto, the spawning period to determine by four methods namely, macroscopic appearance of ovary, GSI, oocyte frequency distribution and histological examination was in good conformity [38], which suggested that the present research work of the spawning period (April to August) was reasonably assessed.

Subsequently, the gonad development and reproductive strategy have been described in many teleost fishes in a great endeavour to understand the time of course and energetic consequences of reproductive effort [54]. From the development of oocytes and microscopical histological examination, the present work was concluded that the *C. reba* belongs to synchronous type of oocyte development [54-56]. One of the author's [45] was reported the reproductive biology of carp around the world shows them to be a very adaptable species. This may be explained by a short lifespan during oocytes development, prior to spawning. As well, the present histological examination of the ovary was assessed by GSI and also validated through microscopical stages. Besides, the present study evidenced that the GSI, histological analyses, hydrating oocytes, new and old POF (Post Ovulatory Follicles) were suggests that the spawning season of *C.reba* was enclosed 1-5 (April-August) months and single spawning with peak during July. In this context, there are no more work were attempted in worldwide the same oocyte development, macroscopic and microscopic examination of the present species so far. However, this study was compared with other freshwater carps. Authors are [45] quoted that the spawning season of carp *Cyprinus carpio* can last 7 months to 9 months with peak during September. The only notable extremes being the season beginning one month earlier (August) and closing a month later (April). As a result, the spawning season does differ noticeably in duration across all sites. Further, the spawning might happen at some sites like autumn and even occurred in winter at one site. So, spawning season of carp populations in other temperate regions of the world is typically shorter 2 months to 4 months are differed. It can be concluded that the present work was confirmed by microscopically documented maturity stages with spawning period during summer (April-August) and peak in July. So far, no more studies have been conducted in complete aspects of reproductive biology of the present species while, it could not compared with the similar works. Furthermore, it might be evaluating the different observations were strongly accordance with the present work carried out. It is reasonably to think that the impact of *C. reba* fishery management is very low, since there are no one author could not attempt the complete biological aspect is still unknown. In this connection, the species abundance goes to decline in this sampling area [13] together at this time the reproductive capacity was low mean that the *C. reba* population should be closely disproportionately. Accordingly the proper monitoring of its reproductive possible and fishery management of its exploitation to conserve the sufficient reproductive potential must be addressed for future viewpoint work to reach sustainable development.

Conclusion

Based on conclude, the knowledge of reproductive biology of *C. reba* may also helpful in formulating regulations that can ensure their survival in our water ecosystems. One of the interesting realistic output of this study, the differences of sex ratio and size at maturity of both sexes, this species could be rapidly and accurately predicted using an 'easy-to-get' parameter. Following, the parameter could be a very useful biological indicator to evaluating the particular species with match up to others for forthcoming living. On the other hand, the general idea could be used as the life-history traits to indices of population feasibility. Hitherto, the pattern of GSI was concluded that the *C. reba* has an active short reproductive period. As a result, their reproduction in this reservoir shows slight differences from other reports, which might be reflected to diverse environmental conditions. Yet, the estimation of fecundity and total number of oocytes is often used. Further, this can be leading as shown from frequency distribution of the oocytes of *C. reba* while, proportion of the oocytes will mature and therefore it do contribute to reproduction process. To end with, a comprehensive foremost histological study were compared to other carps that should lead to a better understanding of these teleosts. So far, the major finding has been observed that the *C. reba* population was going to turn down at this catchment area. In the face of, this may not be possible for the reason and inability breeds most of them artificially.

Acknowledgement

We would like to thank Dr. S. Ajmalkhan, Emeritus Professor, CAS in Marine Biology Annamalai University, Parangipettai, Tamil Nadu for statistically standardizing the study of reproductive biology with practical comments for the present research work. We are very grateful to Dr. V. Ramaiyan, Former Director of CAS in Marine Biology, Annamalai University, for helping to improve this work with his extensive suggestions. During this work, one of the author Dr. R. Mathialagan express his thanks to UGC-RGNF, New Delhi-110058, for providing the financial assistance. Finally, we sincerely thanks to fishermen to collect the samples during the present work carried out.

References

1. Schaefer KM (2001) Reproductive biology of tunas. In: Block BA, Stevens ED (editors).
2. Fish Physiology, San Diego, CA. Academic Press pp: 225-270.
3. Sparre P, Venema SC (1998) Introduction to tropical fish stock assessment. FAO pp: 306.
4. Carvalho GR, Hauser L (1994) Molecular genetics and the stock concept in fisheries. Reviews in Fish Biology and Fisheries 4: 326-350.
5. Stocker M (2006) Report of the ISC-Albacore Working Group Stock Assessment Workshop, National Research Institute of Far Seas Fisheries, Orido.
6. Hunter JR, Macewicz BJ, Lo NCH, Kimbrell CA (1992) Fecundity, spawning and maturity of female Dover sole Microstomus pacificu with an evaluation of assumption and precision. Fishery Bulletin 90: 101-128.
7. Sivakumaran KP (1991) Studies on the Biology and Population Identification of Rastrelliger kanagurta (Curvier, 1817) (Pisces: Scombridae) from the Coastal Waters of India. Ph.D. Thesis, Annamalai University, Porto-Novo pp: 250
8. Karlou-Riga C, Economidis PS (1996) Ovarian atretic rates and sexual maturity of horse mackerel, Trachurus trachurus (L), in the Saronikos Gulf (Greece). Fishery Bulletin 94: 66-76.
9. Karlou-Riga C, Economidis PS (1997) Spawning frequency and batch fecundity of horse mackerel, Trachurus trachurus (L), in the Saronikos Gulf (Greece). Journal of Applied Ichthyology 13: 97-104.
10. West G (1990) Methods of assessing ovarian development in fishes: A review. Australian Journal of Marine and Freshwater Research 41: 199-222.
11. Davis TLO, West GJ (1993) Maturation, reproductive seasonality, fecundity and spawning frequency in Lutjanus vittus (Quoy and Gaimard) from the North West Shelf of Australia. Fisheries Bulletin 91: 224-236.
12. Jayaram KC (1981) The freshwater fishes of India, Pakistan, Bangladesh, Burma and Sri Lanka. Zoological Survey of India, Calcutta pp: 475.
13. Kapoor D, Dayal R, Ponniah AG (2002) Fish biodiversity of India. National Bureau of Fish Genetic Resources, Lucknow, India pp: 775.
14. Mathialagan R, Sivakumar R, Chandrasekar S, Rajasekaran N, Suresh K (2014) Diminishing fishery of reba carp Cirrhinus reba (Hamilton, 1822) in Lower Anicut, Tamil Nadu with reference to needful conservational measures. Indian Journal of Science and Nature 5: 215-221.
15. Ecoutin JM, Albaret JJ, Trape S (2005) Length-weight relationships for fish populations of a relatively undisturbed tropical estuary, the Gambia. Fisheries Research 72: 347-351.
16. Azadi MA, Mustafa MG, Naser A (1996) Studies on some aspects of population dynamics of Labeo bata (Hamilton) from Kaptai reservoir, Bangladesh. Chittagong University Studies Part II, Science 20: 13-18.
17. Rao NGS, Ray PO, Gopinathan P (1972) Observations on the spawning Cirrhinus reba (Hamilton) in the Cuavery and Bhavani Rivers. Journal of Inland Fisheries Society of India 4: 69-73.
18. Gupta S (1975) Some observations on the biology of Cirrhinus reba (Cuvier). Journal of Fish Biology 7: 71-76.
19. Khan IA (1986) Fecundity of Cirrhinus reba (Hamilton-Buchanan) from Baigul reservoir in Uttar Pradesh. Indian Journal of Animal Science 56: 711-716.
20. Arunachalam M, Raja M, Malaiammal P, Richard LM (2014) New species of Aborichthys (Cypriniformes: Balitoridae) from Arunachal Pradesh, India. Species 7: 33-47.
21. Talwar PK, Jhingran AG (1991) Inland fishes of India and adjacent countries. Oxford-IBH Publishing Co. Pvt. Ltd., New Delhi. AA. Balkema, Rotterdam pp: 541.
22. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: A laboratory manual. Cold Spring Harbor, Cold Spring Harbor Laboratory Press, New York.
23. Fisher RA (1970) Statistical methods for research workers. 14th Edition, Oliver and Boyd, Edinburgh.
24. Render JH, Thompson BA, Allen RL (1995) Reproductive development of striped mullet in Louisiana estuarine waters with notes on the applicability of reproductive assessment methods for isochronal species. Transactions American Fisheries Society 124: 26-36.
25. Pollard DA (1972) The biology of a landlocked form of the normally catadromous salmon-form fish Galaxias maculates (Yenys) with structure of the gonads. Australian Journal of Marine and Freshwater Research 23: 1-17.
26. Farmer BM, French DJW, Potter IC, Hesp SA, Hall NG (2005) Determination of biological parameters for managing the fisheries for Mulloway and Silver Trevally in Western Australia, Murdoch University, Murdoch Western Australia 6150, Fisheries Research and Development Corporation Report FRDC Project 2002/004 pp: 150.
27. Knuckey IA, Sivakumaran KP (1999) Spawning and Reproductive Characteristics of Blue Warehou in South-East Australian Waters. Final report to the Fisheries Research and Development Corporation. Project No. 96/142. Canberra, Australia pp: 53.
28. Hickling CF, Rutenberg E (1936) The ovary as an indicator of spawning period in fishes. Journal of the Marine Biological Association of the United Kingdom 21: 311-317.

29. Kjesbu OS, Solemdal P, Bratland P, Fonn M (1996) Variation in annual egg production in individual captive Atlantic cod (Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences 53: 610-620.
30. Lawson EO, Olagundoye AU (2011) Growth patterns, diet composition and sex ratios in giant African threadfin, Polydactylus quadrifilis from ologe lagoon, Lagos, Nigeria. International Journal of Agricultural Biology 13: 559-564.
31. Lawson EO, Jimoh AA (2010) Aspects of the biology of grey mullet, Mugil cephalus Lagos lagoon, Nigeria. AACL Bioflux 3: 181-193.
32. Ozcan G, Balik S (2009) Age and growth of Bassan barbel, Barbus pectoralis (Actinopterygii: Cypriniformes: Cyprinidae), under conditions of a dam reservoir. Acta Ichthyologica et Piscatoria 39: 27-32.
33. Hossain Y, Khatun MST, Jasmine S, Rahman M, Jahan S, et al. (2013) Life-history Traits of the Threatened Freshwater Fish Cirrhinus reba (Hamilton 1822) (Cypriniformes: Cyprinidae) in the Ganges River, Northwestern Bangladesh. Sains Malaysiana 42: 1219-1229.
34. Sivashanthini K (2008) Reproductive Biology of the Whipfin Silver biddy Gerres filamentosus (Cuvier, 1829) from the Parangipettai Waters (SE coast of India). Asian Fisheries Science 21: 127-145.
35. Macuiane MA, Kaunda EKW, Jamu DM, Kanyerere GZ (2009) Reproductive biology and breeding of Barbus paludinosus and Barbus trimaculatus (Teleostei: Cyprinidae) in Lake Chilwa, Malawi: Implications for Fisheries Management. African Journal of Aquatic Science 34: 123-130.
36. Narejo NT, Rahmatullah SM, Mamnur Rashid M (2002) Studies on the reproductive biology of freshwater spiny eel, Mastacembelus armatus (Lacepede) reared in the cemented cisterns of BAU, Mymensingh. Bangladesh Journal of Aquaculture and Tropical 17: 251-259.
37. Shendage AN, Mane UH (2006) Gonado somatic index and spawning season of cyprinid fish, Cirrhinus reba (Hamilton). Journal of Aquatic Biology 21: 127-123.
38. Lashari PK, Narejo NT, Laghari MY, Mastoi AM (2007) Studies on the Gonado somatic Index and Fecundity of a Carp Cirrhinus reba (Hamilton-1822) from Fishponds of District Jacobabad, Sindh, Pakistan. Pakistan Journal of Zoology 2: 95-98.
39. Liu KM, Hung KY, Chen CT (2001) Reproductive biology of the big eye Priacanthus macracanthus in the northeastern waters off Taiwan. Fisheries Science 67: 1108-1114.
40. Hargrave CW, Gido KB (2004) Evidence of reproduction by exotic grass carp in the Red and Washita rivers, Oklahoma, South West. Nature 49: 89-93.
41. Qazi ZH (2001) Induced breeding of the fish Cirrhinus reba, by pituitary gland extract and survival of Jawn in nursery ponds. Journal Asiatic Society of Bangladesh Science 27: 205-213.
42. Brewer SK, Rabeni CF, Papoulias DM (2008) Comparing histology, gonado somatic index and determining spawning condition of small bodied riverine fishes. Ecology of Fresh Water Fish 17: 54-58.
43. Chavan VR, Muley DV (2014) Study of gonado somatic index and fecundity of fish Cirrhinus mrigala (Hamilton). The Bioscan 9: 167-169.
44. Sunita K, Kulkarni KM, Gijare SS, Tantarpale VT (2011) Seasonal changes of gonado somatic index observed in the freshwater fish Channa punctatus. The Bioscan 6: 571-573.
45. Srivastava RK, Yadav KK, Trivedi SP (2008) Devicyprin induced gonadal impairment in a freshwater food fish Channa punctatus (Bloch). Journal of Environmental Biology 29: 87-91.
46. Sivakumaran KP, Brown P, Stoesse D, Giles A (2003) Maturation and reproductive biology of female wild carp, Cyprinus carpio, in Victoria, Australia. Environmental Biology of Fishes 68: 321-332.
47. Wu CC, Su WC, Kawasaki K (2001) Reproductive biology of the dolphin fish Coryphaena hippurus on the east coast of Taiwan. Fisheries Science 67: 784-793.
48. Hontela A, Stacey NE (1995) Cyprinidae. In: Reproductive Seasonality in Teleosts: A D Munro, A P Scott, T J Lam (Eds.) Environmental Influences, CRC Press, Inc, Boca Raton, Florida pp: 53-78.
49. Siddiqui AQ, Chatterjee A, Khan AA (1976) Reproductive biology of the carp, Labeo bata (Hamilton) from River Kali, India. Aquaculture 7: 181-191.
50. Sarkar UK, Negi RS, Deepak PK, Singh SP, Srivastava SM, et al. (2004) Captive breeding of vulnerable Indian carp Cirrhinus reba with Ovaprim for conservation of wild populations 4: 5-7.
51. Adeyemi SO, Akombo PM (2010) Inter and Intra-specific feeding competition among fish species in Gbedikere Lake, Kogi State, Nigeria. International Journal of Lakes and Rivers (In press).
52. Chioma G, Achionye N (2010) Fecundity, Condition Factor, Sex Ratio and Length-Weight Relationship of the Characidae (Tiger Fish) and Cyprinidae (African Carp) From a Reservoir in Ilorin, Nigeria. Asian Journal of Experimental Biological Science 1: 898-901.
53. Erickson DL, Harris MJ, Grossman GD (1985) Ovarian cycling of tilefish, Lophlatilus chamaeleonticeps, from the South Atlantic Bight. Journal of Fish Biology 27: 131-146.
54. DeVlaming VL, Grossman G, Chapman F (1982) On the use of the gonado somatic index. Comparative Biochemical and Physiology 73: 31-39.
55. Knuckey IA, Sivakumaran KP (2001) Reproductive characteristics and per-recruit analyses of blue warehou (Seriolella brama): Implications for the South East Fishery of Australia. Marine and Freshwater Research 52: 575-587.
56. Htun-Han M (1978) The reproductive biology of the dab Limanda ferruginea in the North Sea: Seasonal changes in the ovary. Journal of Fish Biology 13: 351-359.