

Histological Study of Ovarian Development and Sexual Maturity of Kutum (*Rutilus frisii kutum* Kamenskii, 1901)

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Abstract: Studies were conducted on the changes occurring in the ovaries of adult female Kutum, (*Rutilus frisii kutum* Kamenskii, 1901) in Bandar-e Kiashahr in Southwestern of Caspian Sea during a yearly cycle (between two spawning period) from October 2007 to April 2008. No abnormalities or pathological changes in the ovarian or body of the investigated fish were detected. The aims of this study were confirm to timing, frequency and duration of the spawning period, monitor changes in the gonado-somatic index (GSI), relate (GSI) to the histological characteristics of the ovaries and describe the stages of Kutum oocyte and ovarian development. Gonadal samples were taken monthly from the females stained with HandE stain and Samples were studied by light microscope. Different stages of oocyte development (nucleus changes, oocyte diameter and forming of yolk vesicle, yolk granules and lipid droplets) were surveyed. Six maturity stages were observed during our study that included: Virgin stage (I), Maturing virgin (II), Developing (III), Gravid (IV), Spawning (V) and Spent (VI). This study has shown that gonadosomatic index (GSI) began to increase in March and reached the highest value (29.47 ± 4.2) in April and then decreased sharply in early may. According to the results gonad development of Kutum was synchronous group.

Key words: Histology • Ovaries • Sexual maturity • Kutum • *Rutilus frisii kutum* Kamenskii • 1901
• Badare-kiashahr • Sefid-rood

INTRODUCTION

Histology studies currently in many biological phenomena such as fish reproduction to invent new and effective methods for increasing efficiency of broodstock, increasing fish production and ultimately increase efficiency and higher fish are predicted. Determine the peak period of spawning assessment and exploitation of fish, understanding the biological characteristics and life cycle of a species also supplies management and reconstruction is an important role [1] and [2]. Kutum species of economic and native of the Caspian Sea and the highest distribution in the south west coast of the Caspian Sea contain [3-5]. In Iranian coast, gathering and scattering of the fish heavily dependent on physical conditions such as temperature, Flows and marine food supplied [6]. Considering the histology science being young, so far except a few cases, comprehensive studies regarding ovarian Histology of

kutum is important to determine the independence of sexual, especially in the south west coast of the Caspian Sea highest allocation to these will, if is accepted. In this research, has been providing images of ovarian tissue levels of performance ovulation and reproductive pattern of kutum of the Caspian Sea Power intensify. In generally ovaries of bony fish in the bag as containing the channel exit duct and fish eggs bony fish as is comes in a single channel. Conduit between the outlet and cavity eggs on the outside leads to urinary tract. Course structure in the ovary of fish is different. Common Carp family fish ovary of a pair of symmetrical, as tight to the bag shape, membrane bag and swimming bladder (Ceolomic) [7]. Ovarian tissue graft wall and smooth muscle cells and formation of oocytes and the internal wall surface of the dilatation of ovarian germinal pages are known to be produced [8]. Studies about the physiological actions performed, indicated that the characteristics and behavior of uniform environmental conditions are comply with

fish [9]. Especially the timing, frequency and spawning duration, growth and fecundity rate large size and age dependence of the environment [10]. Therefore, changing the environmental conditions, many fish for adaptation to new situations, to stability in the generation of the necessary reactions show that the reaction to this collection with latter time changes in different tissues of the body and limbs, including the ovaries is the cause. Since the complexity of most biological problems to obtain oocytes and oocytes for the required artificial amplification and is defined manufacturing processes more oocytes consider the knowledge of biology and ovarian development is especially important. Therefore, this study investigated growth and ovarian development of kutum in the Caspian Sea area through Bandar-kiyashahr Histology studies are able to provide the necessary and appropriate strategies for optimum utilization and maintain supplies of this valuable species harvested step.

MATERIALS AND METHODS

This review of the number of kutum with number 64 in linear groups - was a different age. Sampling of fish at the same time started catching bony fish (15/8/1386) south-west coast of the Caspian Sea started in Bandare-kiyashahr, this catching performed until bony fish (15/1/1387) continued. Samples after the month in cooperation with the patrol unit to protect local fishing and marine resources and the market were prepared. In order to collect samples and record broodstock biometric data status and kutum, in Sefid Roud River is dispatched to field operations was acting. Quantitative indices of precision 1 mm, 0/01 gr and 1 gr evaluation and registration forms and booklet were biometry. Samples in specific small-scale special compartment specific studied for laboratory studies and determination of age were kept. Weight factors, including weight in fish samples and case filling empty abdomen with scale Sartorius (1 gr) with 1 gram accuracy and precision Gonad weight 0/01 gr using digital scale model Acculab.V-200 (0/01 gr) were determined [11]. Fish with different stages of maturity in different seasons and months by the sea and the river and collect fresh fish length and weight were measured. Describe the body from the body of fish ovaries were carefully weighed and 1 gr were registered. Number of oocytes counted in each warm with oocytes available in one to two grams of the ovary is attached [12]. Find all oocytes in the counting of different size were counted [13] and determine the absolute count fecundity eggs, whole ovary weight were generalized.

AF = absolute fecundity, n = number of eggs in the following example, G = ovary weight, g = weight of the following examples [13].

$$AF = n \times G / g$$

Competition relative to reproduction absolute

AF = weight of total fish TW \times 100 is achieved [13].

$$RF = (AF / TW) \times 100$$

Gonadic Index or Relationship Gonadosomatic Gsi:

$$GSI = WG / WT \times 100$$

Weight of Gonad = WG, WT= total weight of fish, [13].

For measuring abdominal empty weight of total body weight after registering gonad and intestinal contents out and weighing was done.

$$WE = TW - O_{I \text{ and } G}$$

That this formula WE= abdominal weight empty, TW= total body weight, $O_{I \text{ and } G}$ = gonad and intestinal contents.

Fish age determined through samples taken from the scales between the scales available in the median line and Dorsal side is done. Find the circle-shaped scales (Cycloid) number 5 to number 10 from the area generally between Dorsal and Peripheral line were then collected scales the following two left eye and enlarge Find Nikon 40 \times and 20 \times by counting rings twilight age were determined [13].

Histology Study Methods: To evaluate the changes that are necessary ovaries fish as new tour with blades or net mesh (vetch), Sheyl or Kulham Gnad cached and has been forthright and fish out the body and kept in physiological serum solution. Gonad several cuts in each step of investigation was performed in each sample. Counting the number of oocytes at different stages is usually investigation (ovugony, raw eggs, pitted eggs, eggs with yolk and the eggs are done Atretic [14]. Gonad for tissue sampling sectors of beginning, middle and end of it was cut. After ~~ENIÇÑÍ~~ piece (biopsy) of Gonads an ovaries (from the beginning, the median ovarian end), for tissue samples for histology by the Fixed, International Institute of Biochemistry in sturgeon - Rasht sad steps, making clear, Paraffin be, Impression, cutting, coloring Hematoxilin and Eosin (H and E) and by Monte and

password were ultimately to Find histological analysis by optical microscope image of the samples was done. Sampling Gonads divided based on six-stage method based on appearance and size oocytes, follicular layer, cell wall, vacuoles, nuclear and Nucleolus stage diagnosis were investigating [15].

Egg diameter, using binuclear scaled, so that some spawning any fish in any repeat was on and put binuclear under was reading the numbers. Please note that the number of 10 randomly eggs of ovaries available on Lam, select and then average egg diameter for each repetition of the samples was calculated.

All information obtained in the Excel program and 11.05. SPSS and entered the study and analysis were. For statistical methods for testing a one-way ANOVA and Tukey test (T. Student), Spearman correlation coefficients and Kendal Pierson were used.

RESULTS

Considering the results obtained on tissue sections in three area elementary, middle and end ovarian Kutum significant difference in the frequency of oocytes do not show ($p > 0.01$). Investigations with regard to the stages of growth and independence ovarian kutum can be divided into six parts. These six stages are: first stage (stage Nucleolus Chromatin Find), the second stage (stage Nucleolus side), the third stage (stage vesicles yolk), fourth step (step seeds yolk), the fifth stage (maturity stage) stage six (stage eggs found), which described in Table 2 is shown. Minimum diameter of oocytes between $0/19 \pm 2 / 81$ to $0/23 \pm 3/48$ mm and maximum diameter of oocytes range between $2/14 \pm 19/52$ to $1/49 \pm 22/38$ mm was measured. Process of gradual changes Gonad weight index (GSI) kutum during

Table 1: Scale investigation for sexual Macroscopic fish Isochronal [16].

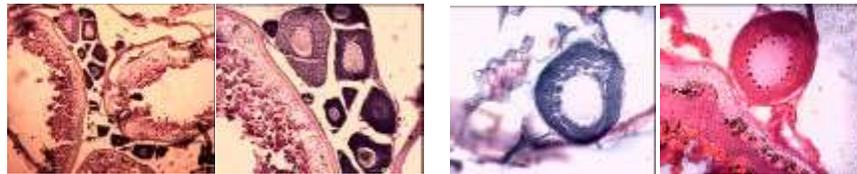
described stage	Degree of independence	stage
Sex organs very small and near the spinal column, testicles and ovaries transparent grayish color, eggs inenarrable eye disarm	minor (Virgin)	I
testicles and ovaries semi-transparent, gray, half or slightly more than half the abdominal length of enclosure, with a single particle between eggs visible, fish have spawning (in rest) in this class are put	investigation in immature (Maturing virgin)	II
ovaries and dark testicles, partial capillary blood red and occupies half the yard abdomen, eggs and armed non-eye visible as are scabrous	(Developing)	III
Sex organs courtyard filled abdominal testicles and white, poured liquid pressure of sperm and eggs completely round and some are semi-transparent and has	ready to spawning (Gravid)	IV
eggs and sperm of the current low pressure, semi-transparent eggs with some more egg on the outside are	in spawning (Spawning)	V
ovaries soften and Eggs depleted completely abdominal landscaping are discharged	has spawned (Spent)	VI

Table 2: Specification for investigating sexual microscopic kutum of the Caspian Sea. Degree of independence and described stage

discription	Maturity rate	stage
Find great in the center of the core oocytes and low value ovuplasm, Nucleolus several small nuclear and related disciplines Nucleolus Chromatin is highly basophile cytoplasm is dark blue in color and comes	Nucleolus Chromatin	I
oocytes Protoplasm side is growing, Chromatin material is visible inside the oocytes, Nucleolus to many small size and proximity internal layer nuclear membrane are put and vacuoles thin layer around the core made up of follicular intensity has decreased basophile oocytes.	Nucleolus	II
vesicles Find yolk oocytes increased in size, around a few core vesicles row is visible, follicular cells and increased thickness of layers formed radius, amount acidosis ovuplasm increased finds.		III
Nucleolus scattered in various parts of the core and the number of decreases, making the last stage of vacuoles reached, increased follicular layer and two layers of granulosa cell layer Teca radius is more specific. Acidophilic oocytes are completely.	seeds yolk	IV
more eggs and gonad mature oocytes and their diameter is increased, yolk accumulation found objects, with vacuoles also merged and formed a large vacuoles gives Intake oocytes and nuclear migration toward the animal pole we. Layer around the ovarian follicular developed.	maturity	V
found fish eggs found in their oocytes, Ron amount of ovarian follicles and oocytes non-empty view is normal. Find immature oocytes in this stage are visible.	Spawned or spent	VI

Table 3: Evaluation of some indices whitefish weight (gr)

standard deviation	average	at a maximum	at a minimum	Factor
36299/128	6425/256	654	23	Gonad weight
334/447	68/916	2141	280	Stomach empty weight
982/546	78/1175	2498	449	Total body weight



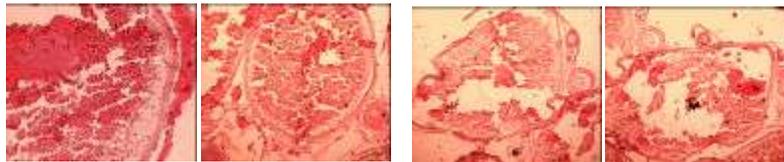
(A) (B)

Fig. 1: Oocyte in the A - phase I (of nucleus chromatin) 10 X & X20. And B - stage II (nucleus and side) 10X & X20. Eosin staining - hematoxylin



(A) (B)

Fig. 2: Oocyte in A - stage III 10X & X20 and B - stage IV (yolk grains) X & 40X20. Eosin staining - hematoxylin



(A) (B)

Fig. 3: Oocyte in A - Stage V (mature) and B - Stage VI (eggs found or spawned). Eosin stained - Hematoxylin

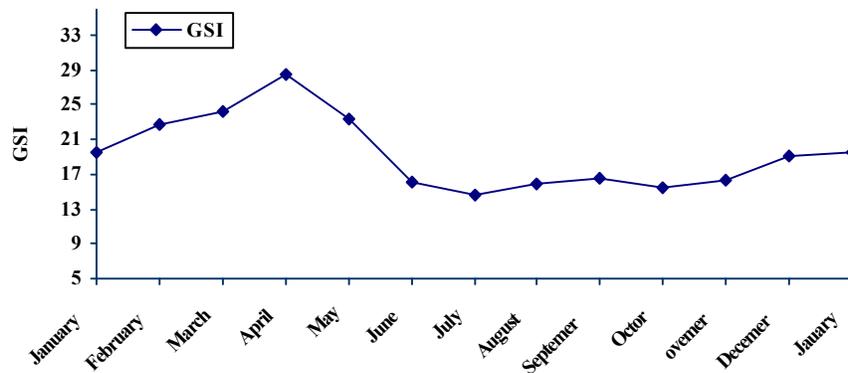


Fig. 4: Trend of average values Gonadic index (GSI) in the Caspian Sea Kutum at separately month

sampling showed that the index weight Gonad month of February with the process was gradual increase during the months March and April Find the significant increase demonstrated after the value of GSI be suddenly drop. Referring to Figure Number Five view is, between age and number of fish eggs in one gram of the ovary samples,

there are images in the year. therefore with increasing age of fish (which actually increase in weight and length it is with), the number of eggs in one gram sample decreases. This means the code, which parallels the growth of fish and increasing age, the amount of weight and diameter of oocytes in the ovary of fish, is added.

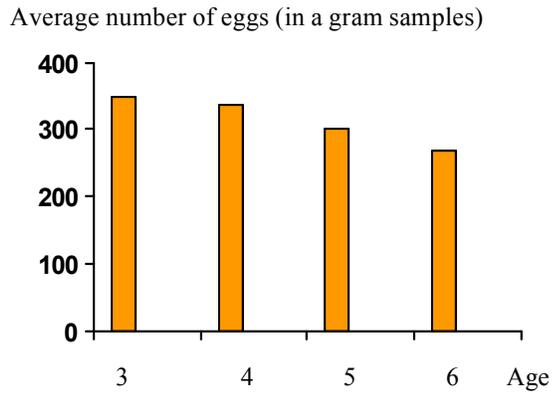


Fig. 5: The mean number of eggs in a Kutum samples at different ages

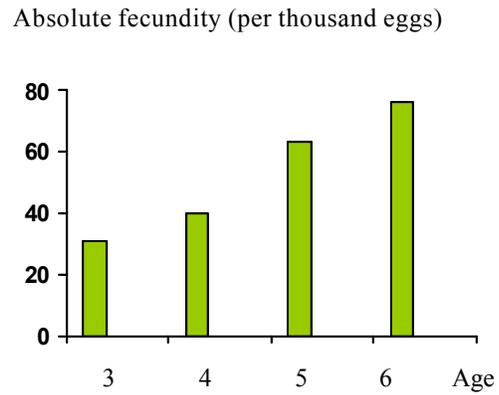


Fig. 6: Absolute fecundity rate of Kutum in different age groups

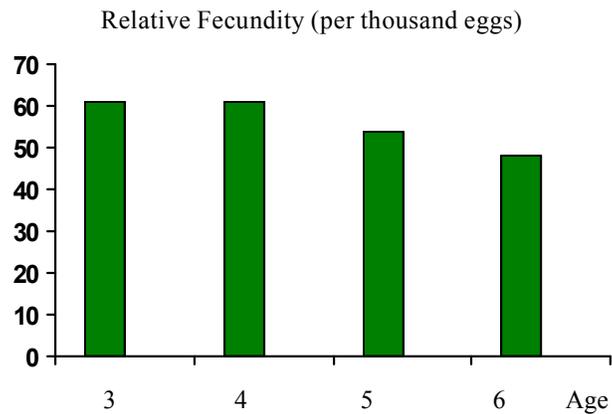


Fig. 7: Relative fecundity rate of Kutum at different ages

Figure 7 with a view, which is specified relative fecundity age fish (and biometric factors with length and weight) is inversely related to fish with increasing age, ie, relative rate of reproduction decreases. Considering the definition of competition and relative comparison with the absolute reproduction of fish in different age groups, the conclusion is that declare broodstock less than four years, more than the relative level of competition and rivalry in the absolute level of four years broodstock Go reproduction relative less than would be rivalry absolute.

DISCUSSIONS

Wide studies about structural changes in ovarian morphology in fish tissue and bone during ovogenesis process is done by different researchers [9]. Including seasonal changes can be the study ovarian tissue red mullet *Mullet surmuletus* southern coast of the UK [17], reproductive cycle and time of the annual reproductive Sea bass on Ovarian Morphology and Histology [18], Histology study and development stages fish ovarian bester sturgeon [19], macroscopic and microscopic stages of ovarian development in white sturgeon *Acipenser transmontanus* [20] and the independence of sexual sludge process based on fish *Tinca tinca* Histology [21] pointed out. This study showed that two types of fish bone spawning strategy are entitled. The first is seen as more fish trout and salmon at the same time that they are kind spawning (Synchronous) means oocyte pending a specified period of time or are outside the ovary, while fish such as fish stokehold spawning their type (Asynchronous) is that many hits and activities during different times of their reproductive and spawning will do. The other hand research conducted shows that postoperative spawning of fish sexual stage VI to stage II, which will open in stages, is attributed back. Viewpoints [13] microscopic diagnosis Gonad for the best and most reliable way to determine gender and diagnosis of early stage of investigating sexual addition for stage II and VI studies distinction Histology of ovarian exact path followed. Like other research in this kind of stage I (Chromatin Nucleolus Find) are large nuclear ovocyte Center and the amount is minimal ovoplasm, stage II (Nucleolus side) protoplasm oocyte is growing and to many and Nucleolus small size near the walls are put in domestic nuclear membrane [22]. Stage III (vesicles Find yolk) oocyte size is increased [23], stage IV (seeds yolk) Nucleolus scattered in various parts of the core decreases

and the number of them, stage V (mature) vacuoles merged with and vacuoles a large make up the core of immigration and we animal pole. Stage VI (eggs found) amount of empty follicles and immature oocytes can Find. Histology ovarian follicles in the structure of white fish with the results obtained in this investigation, similar to other bony fish are. In this study investigating changes in sexual glands (GSI) Article for white fish species in a significant increase for months January, February showed a peak of these changes was observed in March and April and in mid-April to June months (GSI) be suddenly drop. Diameter distribution in the oocytes during the sampling period was significant and the highest diameter oocytes in March and April months were observed in the months April to June diameter oocytes Reduces found. Diameter changes in fish oocytes advanced solution probably one important strategy in determining reproductive activities and their proliferation is [24]. One of the factors of growth and maturation in bony fish as was said environmental factors including light, air and water temperature, salinity and water and other parameters. Is that these factors, a series of conditions on the appropriate axis hypothalamus, pituitary and Gonad (HPG) this effect and the activation of this axis of growth and development is oocytes [25] and [26]. Since fish mainly with patterns and reproductive behavior or are scheduled to study the process of ovarian development and maturation stages of the studies investigating sexual Histology and Morphology of Endocrine and sexual ovaries be pending. In this way changes in the building level Morphology and structure of ovarian oocyte Referrals can be index and good in different stages of maturity in this species and other valuable fish species [27]. A histology Microscopic view of the whole ovary and trend curve changes (GSI) in Figure 4 also demonstrates that this article oocyte aÇ cleaning spawning short-term periods are abandoned. Therefore, white fish in terms of how spawning division [28] or group spawning suddenly Total Spawned oocytes and maturity in terms of division [29] that some ovarian component species they are discharged during a period (Synchronous). Usually set the evolution of this species of fish oocyte is clear and consists of two parts yolk is making and maturity [30]. It is necessary to declare in some oocyte the ovaries may be small at the same time be seen that this index spawning Rank or not because some small fish oocyte after ovarian spawning is the remaining gradually absorbed [31]. Most important economic

fish, are once a year and can be spawning with short reproductive season [32]. However number of stages of sexual development of fish close relationship with the white water temperature, salinity, water and physical-chemical factors the environment. Number of stages of reproductive development by making cuts ovarian tissue section and the study is achieved, the process can ovocyte diameter growth, ultimately to the exact pattern, time spawning and spawning Power range of fish species.

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