Annual Changes in the Ovarian Activity of the Catfish, *Mystus tengara* (Ham.) (Teleostei)

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Abstract Teleosts ovary undergoes regular changes in many species, but the breeding season and spawning reflex are varied. The ovarian histomorphology and annual changes and the spawning periodicity of this catfish were studied on the total of 78 individuals collected monthly in one year period of 1963-64. By gross morphology and its seasonal changes, especially on the changes in cell structure in this species, nine separate stages were distinguished to categorise the developing oocytes. As to the annual ovarian changes, seven phases of ovarian cycle was described based on the percentage and duration of different stages of developing oocytes, average diameter of oocytes and nature of ovarian wall—Immature Virgin (October to February), Preparatory Virgin (March-April), Maturing Virgin (April-May), Prespawning Virgin (June), Spawning (late June through August), Depletion (late August-September), and Recoupment (October-November). The studies revealed a close correspondence among gonosomatic index, ova diameter and water temperature. Discussion was made on some interpretations as to the origin of new crops of oocytes in teleosts ovary. In *Mystus* the new oocytes were found to be formed from dividing oogonia, which were transformed from stroma cells; the fish lacked a well defined germinal epithelium. As to the spawning periodicities, of which examples in teleosts were introduced, the present species revealed to produce two or more batches of eggs during the same season (April to August or early September) by finding that only two types of ova are present in ripe ovaries, immature and mature, the latter containing oocytes in late yolk stage and mature stage, and by examining gonosomatic index, ova diameter and histological nature of ova. Finally, relative and total fecundity in bony fish were discussed, and the present study suggested that the unshed ripe oocytes undergoing atresia must be counted in computing the total fecundity. It was estimated that in *Mystus* nearly 93% oocytes finally ripen and are shed.

It has been well established that in most teleosts the ovary undergoes fairly regular seasonal changes and it has been demonstrated in a score of oviparous and viviparous species by several investigators. The breeding season and the spawning reflex, however, vary interestingly in different species and various internal and external factors have been considered responsible for this variation in spawning behaviour. In this paper the ovarian histomorphology and annual changes and the spawning periodicity of the common Indian catfish have been illustrated.

Material and Methods

Monthly collections of the fish were made for one complete year (1963-64) and a total of 78 specimens were observed. Length and weight of each individual and ovaries were recorded and gonosomatic index was calculated from this data. Fixation of upper, middle and lower portions of the gonads was done usually in Bouin's fluid and Smith's bichromate-formol. Fixed material was cut at 6～10 micra and stained with iron-hematoxylin after Heidenhain and Delafield's hematoxylin, counterstained by eosin. Ova diameter measurements were made on Lanameter on random sampling basis.

Results

Gross Morphology

The paired ovaries of *M. tengara* range from 1.1 to 3.8 cm in length and occupy the posterior two-third of the body cavity suspended in it by thin transparent mesovaria, which are attached to the peritoneal covering of the swimbladder. The ovaries are usually of the same size and somewhat spindle-like with the posterior ends approximate together and joining to form the thick-walled oviduct. Beneath the peritoneal covering of the ovary lies the elastic and muscular tunica albuginea containing many blood vessels. The tunica is not lined with a well defined germinal epithelium. The connective tissue fibres along with the blood vessels of tunica are thrown into finger-like egg bearing folds, which project into the ovarian lumen from all sides (Fig. 3). Each fold encloses a large number of ova in various stages of development, along its border are seen scattered primordial germ cells, which repeatedly divide to form nests of oogonia. Interspersed or around these nests are present stroma cells, which are destined to form the follicular epithelium around the developing oocytes. In immature condition the folds are small leaving a wide central cavity in the ovarian sac (Fig. 3), while in ripe condition the central lumen is almost taut with mature eggs and the folds are inconspicuous (Fig. 5).

On the basis of changes exhibited in the nucleus and cytoplasm and the formation of yolk nine separate stages have been distinguished to categorise the developing oocyte viz.: (i) early chromatin-nucleolus stages (measuring around 0.04 mm), (ii) late chromatin-nucleolus stage (measuring from 0.06 to 0.09 mm), (iii) early perinucleolus stage (0.09 to 0.15 mm), (iv) late perinucleolus stage (0.15 to 0.25 mm), (v) early yolk vesicle stage (0.22 to 0.28 mm), (vi) late yolk vesicle stage (0.28 to 0.36 mm), (vii) early yolk stage (0.31 to 0.52 mm), (viii) late yolk stage (0.48 to 0.58 mm) and (ix) mature stage (0.58 to 0.72 mm). The cytohistological details of the developing oocytes will appear elsewhere.

Annual Ovarian Changes

According to the percentage and duration of different stages of developing oocytes, average diameter of oocytes and nature of ovarian wall, the ovarian cycle has been described in seven phases.

Phase I. Immature Virgin (October to February): The ovaries are at a maximum 20 mm long and 2.5 mm wide. The translucent and some what flaccid and reddish ovaries bear nearly 80% stage (iii) and 20% stage (iv) oocytes (Fig. 2), which are encased in the prominent ovigerous folds (Fig. 3). The folds appear to be more or less radiating towards the centre. A wide central lumen is clearly visible in a transverse section. The blood supply is poor and the capillaries are mostly confined to the ovarian wall. A large number of oogonia and earliest oocyte nests are present along the boundaries of egg bearing folds, surrounded by a large number of stroma cells. Oogonial divisions are occassionally seen during this phase. The gonosomatic index averages 4.1 and the mean ova diameter ranges between 136 and 156 micra (0.136~0.156 mm) (Fig. 1). The water temperature during this phase declines from 22℃ to 18℃.

Phase II. Preparatory Virgin (March-April): The ovary exhibits small voluminous increase. Slight increase in length is evident and they occupy nearly half of the body cavity. The colour is in transition from the reddish to opaque with the advancement of maturation of oocytes. The enlarging oocytes bear yolk vesicles and hence cause the folds

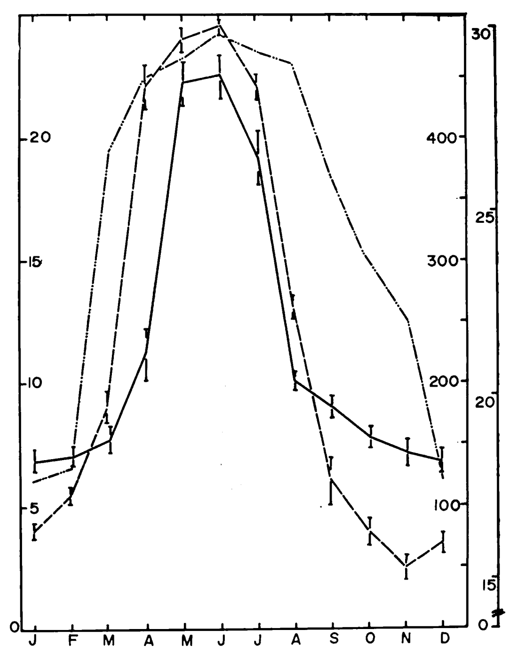


Fig. 1. Graphical analysis of the data pertaining to the gonosomatic index (left ordinate; dashed line), ova diameter (inner right ordinate; solid line) and water temperature (outer right ordinate; strokes and dots). Standard deviation given. Abscissa is months of year.

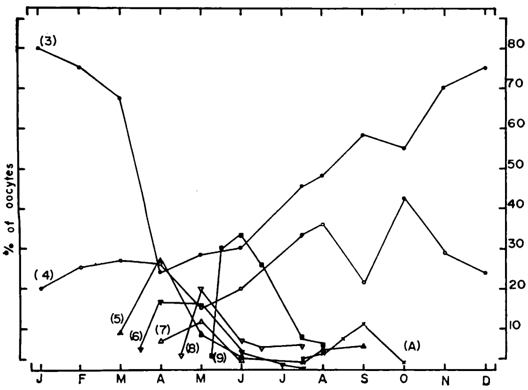


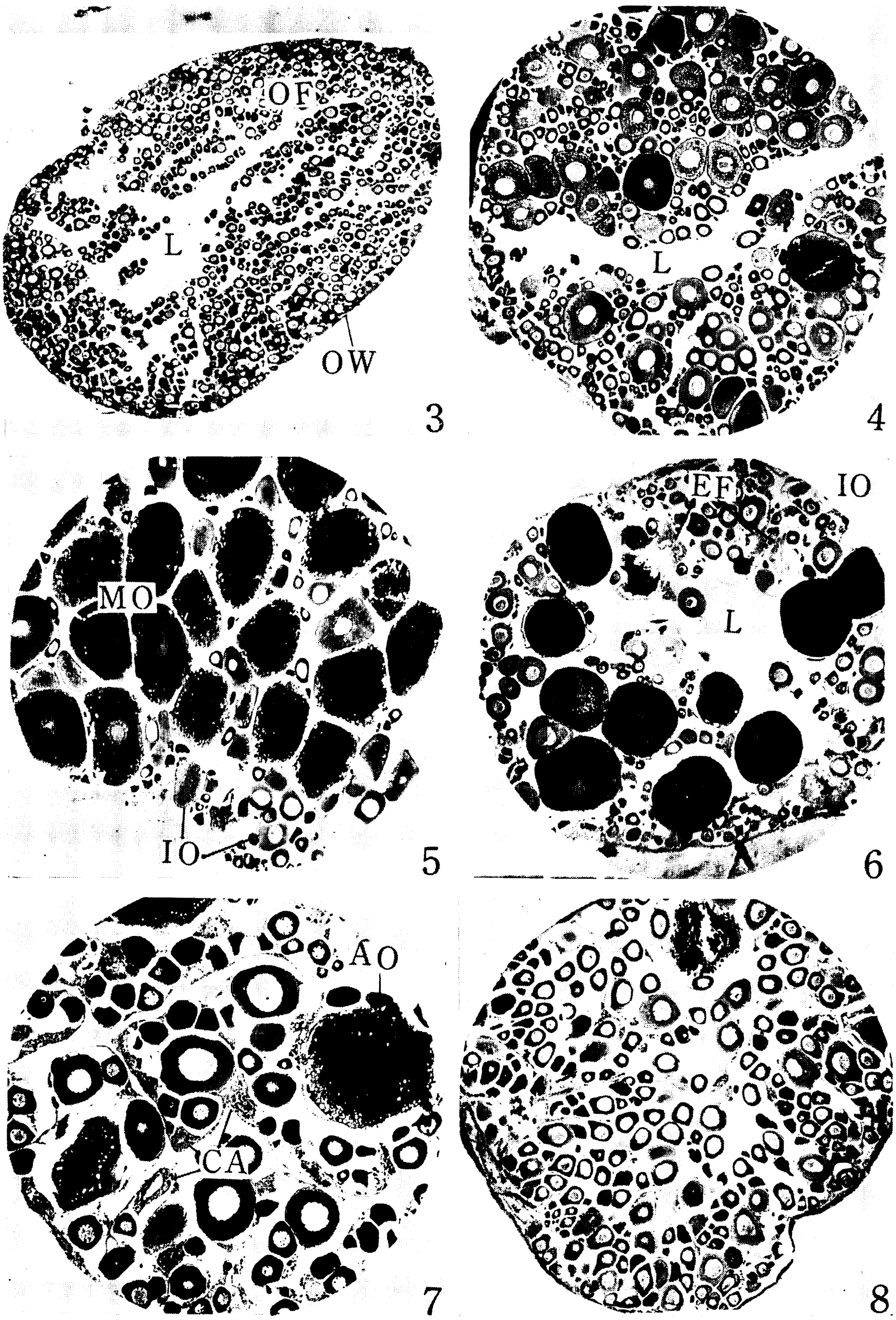
Fig. 2. Graphical analysis of the data on percentage and duration of different stages of oocytes. Abscissa is the months. Numerals in parentheses indicate the stage of the oocyte development. Each point represents a mean of countings made on 500 ova (Random sampling).

to widen and approximate more towards the centre narrowing the central lumen (Fig. 4). The ovarian wall is quite thick and measures at an average 0.025 mm. The vascular supply is better and enters the ovarian substance through the developing stroma. Oogonial divisions are abundantly encountered resulting in the augmentation of oogonial and early oocyte nests, which are situated along the boundary of folds. The oocyte population is composed of stages (iii), (iv), (v) and (vi) oocytes, their percentage being 24, 26, 26 and 23.8 respectively (Fig. 2). This composition varies but very little during the start and end of this phase. The gonosomatic index ascends up to 22 and the mean ova diameter around 0.224 mm (224 micra; Fig. 1). The water temperature rises to 28.6℃ (Fig. 1).

Phase III. Maturing Virgin (April-May): The ovaries become yellow in hue and measure about 32 mm in length and 9 mm in width. The ovarian wall stretches considerably. The developing oocytes can be seen from outside with naked eye. During this phase the maturing oocytes experience deposition of yolk and the weight, volume and colour of the ovaries change. The ovaries are sometimes so much expanded that the other visceral organs are badly displaced. The oocytes are suspended in a richly vascularised stroma. The lumen is fully obliterated by the maturing oocytes. The percentage of oocytes of stages (iii), (iv), (v), (vi), (vii) and (viii) is approximately 28.8, 15.5, 8.8, 15.5, 11.1 and 20 respectively (Fig. 2). A few abortive oocytes corresponding to stages (vi) and (vii) are also seen during this phase in various stages of degeneration. The gonosomatic index rises to about 23 and the mean ova diameter 0.44 mm (440 micra; Fig. 1). The water temperature further shoots up to 29.7℃ (Fig. 1).

Phase IV. Prespawning Virgin (June): The ovaries attain their maximum length of about 34 mm and width of 10.5 mm and are of typical dull yellow colour with lemon-skin-appearance. The oocytes are closely packed together obliterating the lumen and also making the folds indistinct. The well vascularised stroma is forced towards the periphery. The ripe yellow oocytes, which are 33.3 percent, acquire angular shapes due to pressure against each other (Fig. 5). The ovarian wall is very much stretched. It is noteworthy that the stages (iii) and (iv) oocytes increase to 30.66 and 20 percent respectively, while stages (v), (vi), (vii) and (viii) oocytes decrease to 2.6, 4, 2.6 and 6.6 percent respectively (Fig. 2). During this phase the ovary is found to bear occasionally a few atretic oocytes. Cases of burst atresia are sometimes observed. Burst atresia affects the maturing and mature oocytes on such a large scale that nearly 80% of the total yolky oocytes are damaged. The gonosomatic index is at the maximum being 24.12 and the ova diameter around 0.445 mm (445 micra; Fig. 1). The water temperature is also maximum in this month being 30℃ (Fig. 1).

Phase V. Spawning (Late June through August): During this phase the very much distended bag-like ovaries gradually commence shrinking in volume due to the shedding of mature oocytes and are about 30 mm long and 4 mm wide. The oocytes are shed in many batches and hence after the first expulsion of oocytes the rest of the oocyte population is loosely held in the ovarian lumen (Fig. 6). In such ovaries evacuated follicles are seen. Up to July stage (viii) oocytes totalling up to 5.4 percent transform into mature ones ready for shedding. The stroma



Figs. 3~8. T. S. of ovary in different phases: Fig. 3, phase I showing the lumen (L), ovarian wall (OW) and follicles (OF) ×16; Fig. 4, phase III showing reduced lumen (L) ×32; Fig. 5, phase IV showing immature (IO) and mature oocytes (MO) ×40; Fig. 6, phase V showing evacuated follicles (EF) and spent lumen (L) ×40; Fig. 7, phase VI showing atretic oocytes (AO) and corpora aterica (CA) ×105; Fig. 8, phase VII ×52.

is moderately developed and heavily vascularised. The atretic process is at its peak during this phase and the majority of the unshed mature oocytes and some stage (viii) oocytes undergoing atresia total nearly 4%. The percentage of mature oocytes gradually falls from 27.7 to 6.4, where as stages (iii), (iv), and (v) oocytes are 46, 33 and 2% respectively (Fig. 2). The transforming stages (vii) and (viii) oocytes total up to 2 and 6% respectively. The value of gonosomatic index decreases to 13.2 and the ova diameter to 0.201 mm (201 micra; Fig. 1). The water temperature also declines to 29℃.

Phase VI. Depletion (Late August-September): The spent ovaries are very much shrunken and blood shot. Their length and width are considerably reduced. Many a unshed mature oocytes are seen in a spent ovary undergoing atresia. The egg bearing folds are loose and the immature oocytes are embedded in them sparsely distributed (Fig. 7). Many evacuated follicles are seen at the surface of folds in ovaries which have just finished spawning. The evacuated follicles disappear rapidly and are not seen subsequently. The blood supply is at the maximum during this phase. The ovarian wall is thick and is traversed by many big blood vessels. Formation of new crop of oogonia starts during this period. The young oocytes corresponding to stages (iii), (iv) and (v) are between 46.2 and 57.3, 33.3 and 20.2, and 5.0 and 5.2 percent respectively, while the atretic oocytes total between 4.3 and 11.2 percent (Fig. 2). As a result of atresia corpora atretica, pregnant with yellow granules are seen along the vascular stroma quite abundantly (Fig 7). The gonosomatic index averages around 6 and the ova diameter 0.180 mm (180 micra; Fig. 1). There is further decline in the water temperature up to 26℃ (Fig. 1).

Phase VII. Recoupment (October-November): During this period new oocytes are formed in abundance and nests of oogonia and earliest oocytes dominate the general histological view of the ovarian substance (Fig. 8). The folded nature of the ovarian wall provides the clue for identifying the recouping spent ovaries. The central lumen gradually reduces and is finally totally obliterated. The subsequent phases of growth and maturation of the ovaries follow strictly the same course as shown by a virgin ovary. The values of gonosomatic index and ova diameter correspond closely to that of phase one ovary (Fig. 1). The water temperature is about 22℃ during this phase (Fig. 1).

Discussion and Conclusions

A perusal of figure one clearly reveals the close correspondence of the three curves pertaining to the gonosomatic index, ova diameter measurements and water temperature. It suggests a strong and close impact of the temperature cues upon the behaviour of ovary, which has earlier been supported by a number of workers like Craig-Bennett (1931), Gokhale (1957) and Sathyanesan (1962) in the fishes studied by them.

Regarding the origin of new crop of oocytes in the teleost ovary a number of interpretations have been offered. According to the first exemplified by *Zoarces* (Wallace, 1903), *Xiphophorus* (Bailey, 1933), *Neotoca* (Mendoza, 1943), *Gasterosteus* (Tromp-Blom, 1959), *Scomber* (Bara, 1960), *Oryzias* (Yamamoto, 1962), *Xenentodon* (Rastogi, 1966) and *Amphipnous* (Rastogi, 1967) new oocytes arise from the cells of germinal epithelium, whereas the second interpretation, forwarded by Bullough (1939) in *Phoxinus*, Craig-Bennett (1931) in *Gasterosteus* and Andreu and Pinto (1957) in *Sardina* suggests that the cells of the evacuated follicles divide mitotically to raise the new crop of oocytes every year. A third view held by Matthews (1938) and Yamamoto (1962) corroborates with the present findings in *Mystus* that the new oocytes are formed from dividing oogonia. The oogonia in turn are, however, formed by transformation of stroma cells in *Mystus*; it is noteworthy to mention here that this fish lacks a well defined germinal epithelium. A score of investigators like Clark (1934), Gokhale (1957), Bara (1960), Jhingaran (1961), Sathyanesan (1962) and Belsare (1962) used different micro- and macroscopic factors for the classification of the ovarian cycle during the year. The present classification follows Hjort's International Scale for Maturity Stages of Gonads and includes additional factors like the histological picture of ovary, percentage and duration of the oocytes, rise and fall in the gonosomatic index and ova diameter measurements.

Most of the studies on spawning periodicities on fishes are based chiefly opon maturation of intraovarian eggs and ova diameter measurements (Clark, 1934; Vladykov, 1956; Prabhu, 1956; Naumov, 1956; Andreu and Pinto, 1957; Sathyanesan, 1962; Jhinagaran, 1961 and Yamamoto *et al*, 1961~64). According to Marza (1938) the developing rhythm of oocytes may be classified into three groups, the total synchronism, group or partial synchronism and asynchronism. The first group in which all oocytes in an ovary develop synchronously includes *Oncorhynchus masou* Yamamoto *et al*, 1959), which spawns only once in life and dies soon after. The second category is common among fishes such as herrings (Hickling and Rutenberg, 1936) and speckled trout (Vladykov, 1956). In these species two groups of oocytes are sharply marked indicating spawning once a year with a short and definite season. The third category is most common among fishes such as sardines (Clark, 1934; Andreu and Pinto, 1957), goldfish (Yamamoto and Yamazaki, 1961) and medaka (Yamamoto and Shirai, 1964). In these species everal batches of oocytes in different stages are present indicating a long spawning season and several spawnings within a season. Prabhu (1956) concluded that spawning in different species is strictly periodic and distinguished four types of spawning, spawning once a year with a short spell (*Therapon*, *Mystus* and *Chirocentrus*), spawning once a year with a longer duration (*Pelates* and *Cypsilurus*), spawning twice a year (*Psammoperca*, *Therapon* and *Caranx*) and intermittent spawning throughout the year (*Stolephorus*). Jhingaran's (1961) study concluded on *Setipinna phasa* having many spawnings in a breeding season without showing any definite periodicity. This report contradicts that of Prabhu (1956). A ripe ovary of *Amphipnous cuchia* (Rastogi, 1967) bears only two groups of oocytes, indicating annual spawning with a short duration (May-July). *Xenentodon cancila* (Rastogi, 1966) ovary contains many batches of oocytes in different stages of maturity indicating that the fish has a spawning season of long duration (Feb.-Aug.) with many intermittent releases of ripe eggs. It is further strengthened by determining the values of gonosomatic index and ova diameter measurements. In the present species only two types of ova are present in ripe ovaries, immature and mature, the latter containing oocytes in late yolk stage and mature stage. It reveals that these eggs are spawned in two or more batches during the same season, which after examining the values of gonosomatic index, ova diameter curves and histological study of the mature ovaries is proved to be from April to August or early September. The rise in gonosomatic index and ova diameter is very slow from April to June and then there is a slow fall up to August (Fig. 1), which coincides with that of *Carassius auratus* (Yamamoto and Yamazaki, 1961) and the fish hence belongs to the type of asynchronism (Marza, 1938) and fourth category formulated by Prabhu (1956).

During this study the relative and total fecundity (Vladykov, 1956) in the light of the regular occurrence of ovular atresia of maturing and mature unspawned oocytes were also considered. Vladykov (1956) reported that the relative fecundity of wild brook trout is much higher than the total fecundity and only 56~61% of maturing oocytes ultimately ripen for spawning. The present study suggests that the unshed ripe oocytes undergoing atresia must also be counted in computing the total fecundity. Henderson (1963) in trout reported that 3.1% of the maturing oocytes underwent atresia and the rest matured and spawned. On the grounds suggested in this discussion Rastogi (1966~7) calculated total fecundity to be nearly 86% in *Xenentodon cancila* and 96% in *Macrognathus aculeatum* and 73% in *Amphipnous cuchia*. In the present species nearly 93% oocytes finally ripen and are shed.

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インド産 *Mystus tengara* の卵巣活動の年間変動. R. K. ラストギ・P. K. サクセナ. 1963～64 年の1カ年に全数 78 個体を毎月採集して, 卵巣重, 卵径, および組織を験して年間の変化を観察した. 本種の卵巣構造, 卵巣卵の構造 (と発育) より, 卵母細胞の発育段階を 1) 初期の染色質核仁期 (約 0.04 mm), 2) 後期の染色質核仁期 (0.06-0.09 mm), 3) 初期の perinucleolus 期 (0.09-0.15 mm), 4) 後期の perinucleolus 期 (0.15-0.25 mm), 5) 初期の卵黄嚢期 (0.22～0.28 mm), 6) 後期の卵黄嚢期 (0.28-0.36 mm), 7) 初期の卵黄期 (0.31-0.52 mm), 8) 後期の卵黄期 (0.48-0.58 mm), 9) 完熟期 (0.58-0.72 mm) にわけた. また, 卵母細胞発育程の割合, その期間, 大さ, 卵膜の状態を基準として, 卵

の発育周期を I) 未熟処女期 (10-2 月), II) 準備処女期 (3～4 月), III) 熟生処女期 (4-5 月), IV) 産卵前処女期 (6 月), V) 産卵期 (6 月末-8 月), VI) 放卵後期 (8 月下旬-9 月), VII) 恢復期 (10-11 月) の階級に分類した. 以上より, 熟度指数, 卵径, 水温変化に密な関連のあることを示した. 硬骨魚類における卵母細胞の新生についての3説を例示し, 本種では卵原細胞の分裂によって生じ, 後者は細胞間組織細胞の変生によって生ずることを示し, さらに, 本種では胚質表皮の不明瞭である点を認めた. 次に, 硬骨魚類における卵母細胞の発育周期性 (3 型) と産卵周期 (4 型) を例を以て説明し, 本種では未熟卵と熟卵の2種の卵群のみが存在し, 後者は後期卵黄期と完熟期の卵母細胞で組成されていることを明らかにし, これらの卵は産卵期内に2回あるいはそれ以上に放出され, また, 熟度指数, 卵径, 卵組織の観察により, 本種の産卵期は4月より9月初旬にわたるものであるとした. 熟度指数と卵径の上昇は4月より6月にかけて極めてゆるく, 以後, 8月に向ってゆるく下降する. 最後に, 相対および全放卵量につき, 生熟中および完熟卵の退化が正規におこる点を考慮し, また本種の実験結果より, 全放卵量の決定には, 退化しつつある放出されない完熟卵母細胞も計算に加うべきであると考えた. かくして, 本種では 93% の卵母細胞が最終的には生熟して放出されると計算した.

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