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Studying for Characterization of Ovarian Development and Associated Factors during the Breeding Migration of *Coilia nasus* in the Yangtze River

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Abstract: *Coilia nasus* is a typical anadromous migratory fish in the lower reaches of the Yangtze River. Every year, *C. nasus* cluster offshore and swim upstream along the Yangtze River into the tributaries and lakes in the middle and lower reaches of the Yangtze River to breed. In this study, female *C. nasus* collected as the subjects from Chongming section in Shanghai, Taizhou section in Jiangsu and Anqing section in Anhui. The ovaries were used for examining in tissue sections and researching of gene expression including follicle stimulating hormone receptor (*fshr*), luteinizing hormone receptors (*lr*), kisspeptin-1 (*kiss1*) and forkhead box l2 (*foxl2*) related to the reproductive development, and the estrogen (estradiol, E2) and progesterone (17α,20β-dihydroxy-4-pregenen-3-one, 17α,20β-DHP) were also analyzed in serum levels. The results showed that, first, the growth period of oocytes was small in stage II of ovarian development, in which both E2, 17α,20β-DHP levels and gene expression were low. Then, in stage Ⅲ, the growth period of oocytes were become large, as well as the yolk granules and oil droplets began to appear. Simultaneously, E2 and expression of *kiss1* and *foxl2* were significantly elevated. Finally, stage Ⅳ was the period of large accumulation of nutrients in oocytes, and 17α,20β-DHP level and expression of *fshr* and *lhr* were significantly elevated. The results have enriched the theoretical study of ovarian development in the natural population of *C. nasus*, supplemented the biological basis of *C. nasus* reproduction, and scientifically supported the study of *C. nasus* population ecology and resource conservation.

Keywords: *Coilia nasus*; ovarian development; organizational observation; sex steroid hormones; gene expression

1. Introduction

Ovarian development is mainly regulated by the endocrine system in fish. Estrogens and progestins, including estradiol (E2), 17α,20β-dihydroxy-4-pregenen-3-one (17α,20β-DHP), play essential roles in the regulation of ovarian maturation and development [1,2]. Studies have shown that E2 is produced in the follicular layer, and stimulates the hepatocytes to synthesize vitellogenin through estrogen receptor signaling and then release into bloodstream. During the maturation stage of the ovary, E2 makes the synthesis of 17α,20β-DHP in steroidogenic pathway, which induces the final maturation of the oocyte [3,4]. Oocyte maturates by 17α,20β-DHP through stimulation of maturation-promoting factor formation in oocytes [5]. A similar important role is played by genes in the regulation of ovarian development. Follicle stimulating hormone receptor (*fshr*) primarily mediates follicle stimulating hormone (*fsh*) functions in response to stimulation of ovarian follicle development and ovulation in female animals [6]. A member of the glycoprotein subfamily of the G-protein-coupled receptor superfamily, luteinizing hormone receptors (*lhr*) regulates reproduction in

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animals upon binding to luteinizing hormone (*lh*) [7]. In female fish, *fsh* is mainly involved in oocyte differentiation, development and yolk accumulation, while *lh* is involved in oocyte maturation and ovulation [8]. Kisspeptin-1(*Kiss1*) is a key regulator of the reproductive regulatory cascade with roles in regulating gonadotropin release and follicular maturation [9]. Kisspeptin is a peptide encoded by *kiss1*. It stimulates the release of follicle stimulating hormone and luteinizing hormone [10]. Part of the forkhead transcription factor family, forkhead box l2 (*foxl2*) is an upstream regulator of the P450 aromatase promoter and specific to the ovary. The main role of *foxl2* is to activate the expression of *cyp19a* in an effort to regulate estrogen levels and modulate ovarian development [11]. In vertebrates, the *foxl2* gene which encodes a highly conserved amino acid sequence is the initiating gene for ovarian development [12].

Coilia nasus (Clupeiformes: Engraulidae) is a small- moderate sized fish [13,14]. Reproductive migratory groups of *C. nasus* gather at the Yangtze River estuary in spring, and the clusters migrate anadromously into tributaries and lakes of the middle and lower reaches of the Yangtze River to finish reproduction [15,16]. Within the passage of migration time, the water temperature gradually increases, and the ovaries rapidly develop and mature. The spawning season of *C. nasus* is from June to October every year under natural conditions [17]. Xu et al. [18] showed that the oocytes in the ovaries of *C. nasus* at all developmental stages had obvious synchronization and belonged to the type of one- time spawning, and the ovaries could be divided into six developmental states from I-Ⅵ. Presently, more studies have been conducted on metabolic mechanisms [19], immunity [20,21], energy utilization [22–24], and key regulatory pathways in the liver and brain [25] during the reproductive migration of *C. nasus*, while relatively few studies have been conducted on factors related to ovarian development. In this study, , and investigating the changes of the factors related to the reproductive development of *C. nasus* at the hormone and gene levels. In order to understand the spatial and temporal characteristics of ovarian development, and investigate the changes of reproductive development at the hormone and gene levels, female *C. nasus* from Chongming section of Shanghai (CM), Taizhou section of Jiangsu Province (TZ) and Anqing section of Anhui Province (AQ) were chosen during the *C. nasus* migratory season in this study, futher observation of the ovaries at different developmental stages, levels of serum sex steroid hormones, expression patterns of genes related to the reproductive development of the ovaries were analysied. All of these enrich the study of *C. nasus* reproduction biology and support the conservation of *C. nasus* germplasm resources.

2. Materials and Methods

2.1. Survey Sample Areas and Methods

During the 2021 *C. nasus* migration season, three survey sample areas were set up in CM section (31° 29′ 52 ″ N, 121° 36′ 36″ E), TZ section (32° 12′ 14″ N, 119° 53′ 40″ E) and AQ section (30° 29′ 11″ N, 116° 59′ 39″ E), Figure 1. The specifications of the nets used in each segment of the river are shown in Table 1. During the survey period, except for bad weather such as typhoons, *C. nasus* surveys were carried out daily in TZ and AQ sections, while in CM section they were carried out at the right time according to the tidal conditions and synchronized with the measurement of water temperature data.

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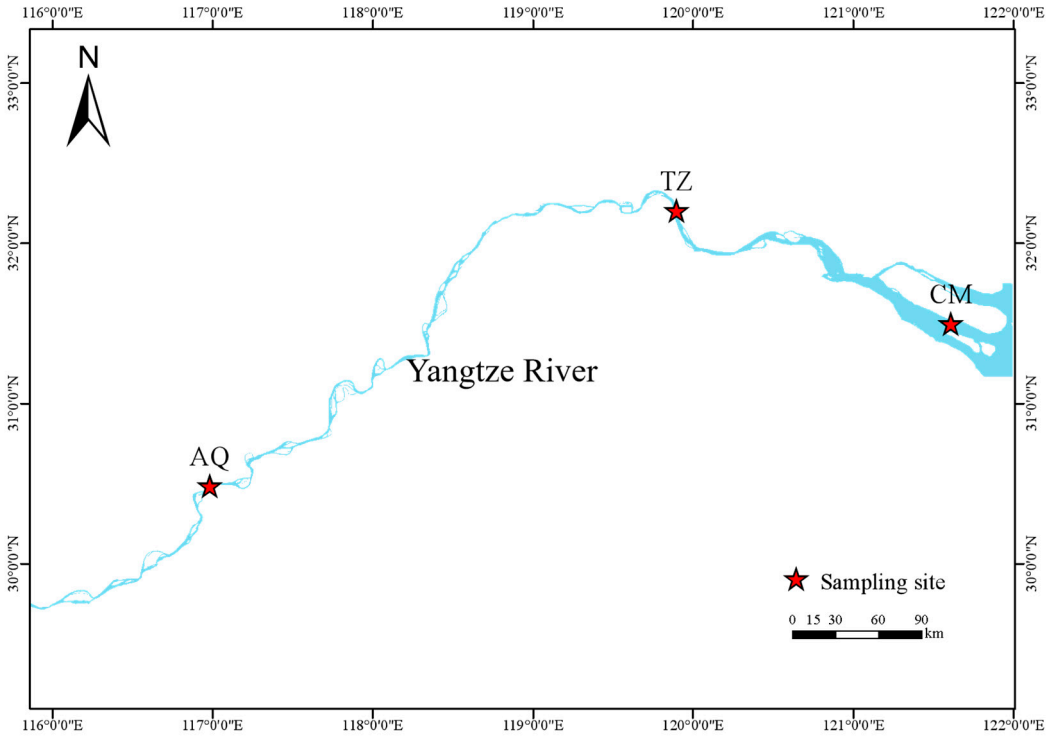

Figure 1. Sampling site of *C. nasus* in the Yangtze River. CM, Chong Ming; TZ, Tai Zhou; AQ, An Qing.

Table 1. Type and specification of the net used for collection of *C. nasus* samples.

Sampling area	Sampling point	Sampling period	Net type	Net secification
CM	31° 29′ 52″ N, 121° 36′ 36″ E	March- June	Throwing gill net	Length 150 m, height 12 m, mesh size 4 cm
TZ	32° 12′ 14″ N, 119° 53′ 40″ E	March- June	Gill net	Length 180 m, height 4 m, mesh size 4 cm
AQ	30° 29′ 11″ N, 116° 59′ 39″ E	April- July	Gill net	Length 180 m, height 4 m, mesh size 4 cm

2.2. Sample Collection and Processing

C. nasus samples were placed on ice trays to measure body length (accurate to 0.01 mm), body mass (accurate to 0.1 g) and other growth traits. The dissected male *C. nasus* was used for other experiments, while the females were visually identified according to the shape, size and color of the dissected ovaries, and the ovaries were weighed (accurate to 0.1 g) [18]. On the one hand, there was no obvious difference in appearance and morphology between the spermatogonial stages III and IV. On the other hand, the sample size of ovary stage V was relatively small due to the limitation of body length control conditions. Therefore, stage II, III and IV female *C. nasus* were selected for the study. One side of the ovary was preserved in 10% paraformaldehyde fixative for sectioning and observation, and the other side of the ovary was put into a freezing tube and preserved in liquid nitrogen, and then transferred to a refrigerator at -80℃ for the determination of gene expression. Meanwhile, the supernatant was collected after centrifugation of their corresponding blood samples and stored in liquid nitrogen, then transferred to -80℃ refrigerator for the determination of sex steroid hormones in serum. A total of 30 female *C. nasus* in stages II, III and IV in AQ section were selected for characterization of different developmental stages, and a total of 30 female *C. nasus* in stage IV in CM, TZ and AQ sections were selected for spatial characteristics analysis.

Ovarian tissue section preparation The paraformaldehyde-impregnated ovaries were dehydrated using an automatic dehydrator, and after dehydration, they were prepared into wax

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blocks by xylene transparency and paraffin embedding, after which they were subjected to paraffin sectioning with a slice thickness of 4 μm. Hematoxylin-eosin staining(HE) was performed on paraffin tissue sections, and the sections were sealed with neutral resin. The ovarian condition of development was observed under the microscope (Olympus BX51) and photographed. The following indicators were counted according to the HE section diagrams: size of oocytes and nucleus in stage II, III, IV. Additionally, number and percentage of oil droplets and yolk granules in stage III and IV.

Sex Steroid Hormone Measurement An enzyme-linked immunoassay (ELISA) was used to detect hormone levels in the serum of *C. nasus*. The basic principle of ELISA is to let the antibody bind to the enzyme complex, and then quantitatively detect it by color display. Serum E2 and 17α,20β-DHP indicator assay kit was purchased from Nanjing Sembega Biological Company, and all operations were carried out in strict accordance with the instructions of the kit.

Gene expression assays Primer sequences were designed using Primer Premier 5.0 software based on the sequence information of *fshr*, *lhr*, *kiss1* and *foxl2* genes from NCBI database (Table 2). Relative expression of the genes involved in development in the ovary using quantitative real-time PCR. The *C. nasus* β-actin gene was used as an internal reference, and the multiplicative relationship of the difference in expression between samples was obtained by a $2^{-(\Delta\Delta Ct)}$ mathematical operation between the Ct value of the internal reference gene and the Ct value of the gene to be examined. RNA was extracted using UNIQ-10 column Trizol total RNA extraction kit, the concentration and purity of RNA were detected by multifunctional enzyme labeling instrument, the electrophoresis results of RNA were detected by electrophoresis buffer, and reverse transcription was carried out on PCR instrument, and the reaction was prepared into 10 μl reaction mixture by mixing 5 μl of SybrGreen qPCR Master Mix, 0.2 μl of Primer F (10 μm), 0.2 μl of 5 μl SybrGreen qPCR Master Mix, 0.2 μl primer F (10 μm), 0.2 μl primer R (10 μm), 3.6 μl ddH2O and 1 μl cDNA were prepared into a 10 μl reaction mixture, and added into a 96-well plate. The reaction conditions were as follows: 94 ℃ predenaturation for 3 min, amplification for 45 cycles (95 ℃ denaturation for 15 s, 60℃ annealing for 30 s), and then put into the fluorescence quantitative PCR instrument (LightCycler480 II) for detection.

Table 2. Primer sequences used in the experiment.

Gene	Primers sequence(5′-3′)	Amplicon size(bp)
Coilia β-actin-F	GAGCCTCCGATCCAGACAGAG	
Coilia β-actin-R	CATGAAGTGTGATGTCGACATCC	
fshr-F	GATGCCAACCTCACATACCC	120
fshr-R	GAAGACGGGCTCCTCCAG	
lhr-F	ACCTCAGCAGCCTTCCCA	113
lhr-R	ATTACCGATGACAGCAGACCC	
kissr1-F	CTTGTTGGGCTTCTGGGTAA	228
kissr1-R	TGTCCTGTGGCGGAGTGA	
foxl2-F	CCGGCATGGTGAACTCTTAC	119
foxl2-R	GTTAGCGGAGGGGACAGG	

2.3. Data processing and analysis

Gonadosomatic index (GSI) (%) = weight of ovary (g) / weight of fish body (g) × 100%

The experimental data were analyzed statistically using SPSS26.0 software, and significant differences were analyzed by one-way ANOVA ($P < 0.05$ means significant difference), and the results were expressed as "mean ± standard deviation", and the data graphs were created by GraphPad Prism8.0 software.

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3. Results

3.1. Biological Characteristics of C. nasus

The GSI of stage I- V was 0.79%, 1.24%, 1.80%, 5.58% and 8.22% respectively from March to Julyn. In March, CM and TZ section were dominated by stage I and II, accounting for 97.06% and 99.34% respectively. In April, still for stage I and II, accounting for 89.61% and 92.21% respectively, while the AQ section were stage II and III, accounted for 49.01% and 45.26%. In May, in CM section was dominated in stage IV, accounting for 46.37%, and stage II and III in TZ and AQ sections, the accounte reached for 77.04% and 90.82% respectively. In June, CM and TZ section were mainly in stage IV and V, accounting for 75.79% and 83.87% respectively. Meanwhile, stage II and III in AQ section accounted for 91.94%. In July, stage III and IV in AQ section accounted for 51.31% and 47.38%, respectively (Figure 2). The average water temperatures from March to July showed an increasing trend, which were 15.47℃, 17.08℃, 21.87℃, 24.63℃ and 27.69℃, respectively.

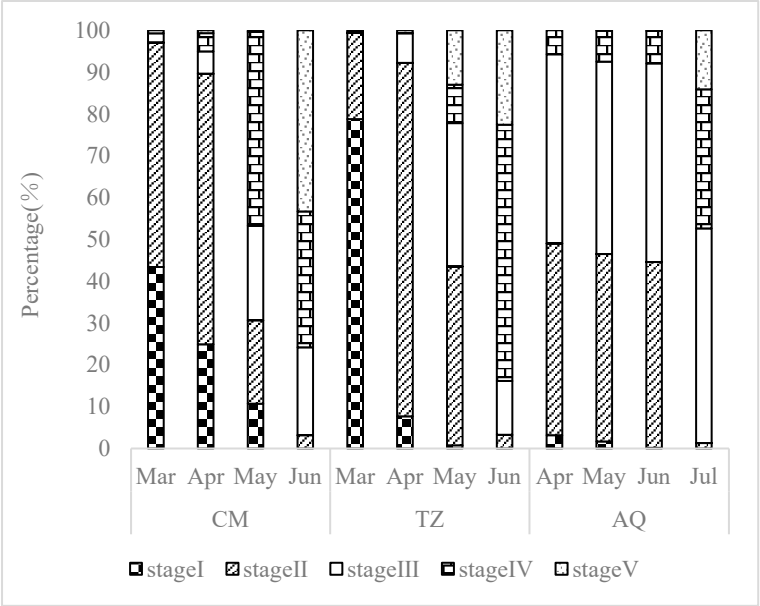

Figure 2. Spatio-temporal characteristics of ovarian development period in *C. nasus* breeding population.

3.2. Morphologic and Histologic Sections of the Ovary

The phases of the ovary are differentiated on the basis of the morphological and physiological characteristics of the oocytes during ovarian development, as well as the histological composition of the ovary itself [26], defined by the temporal phases of the germ cells that occupy more than half of the area in the ovarian tissue section or that are in the highest proportions [18].

3.2.1. Developmental Stage Characteristics of Ovarian Tissue Sections

The ovaries in stage Ⅱ were finely columnar, with inconspicuous blood vessels in the ovarian membrane and a slightly transparent light flesh-red to flesh-yellow appearance, with no oocytes visible to the naked eye. Histological observation showed that the oocytes were closely arranged, in the small growth phase of primary oocytes, dominated by the cells of the 2nd temporal phase, the oocytes of the 2nd temporal phase were round, oval or irregular polygonal, the cells had a long diameter of 78.90- 170.20 μm and a short diameter of 40.50- 120.30 μm, the cell nucleus had a diameter of 30.00- 70.10 μm, and the cytoplasm was strongly alkaline, stained with a The nucleus was 30.00- 70.10 μm in diameter, the cytoplasm was strongly basic and stained dark purple, the chromatin in the nucleus was finely linear, and the cytosol was surrounded by a single layer of follicular membrane (Figure 3 A1- A3).

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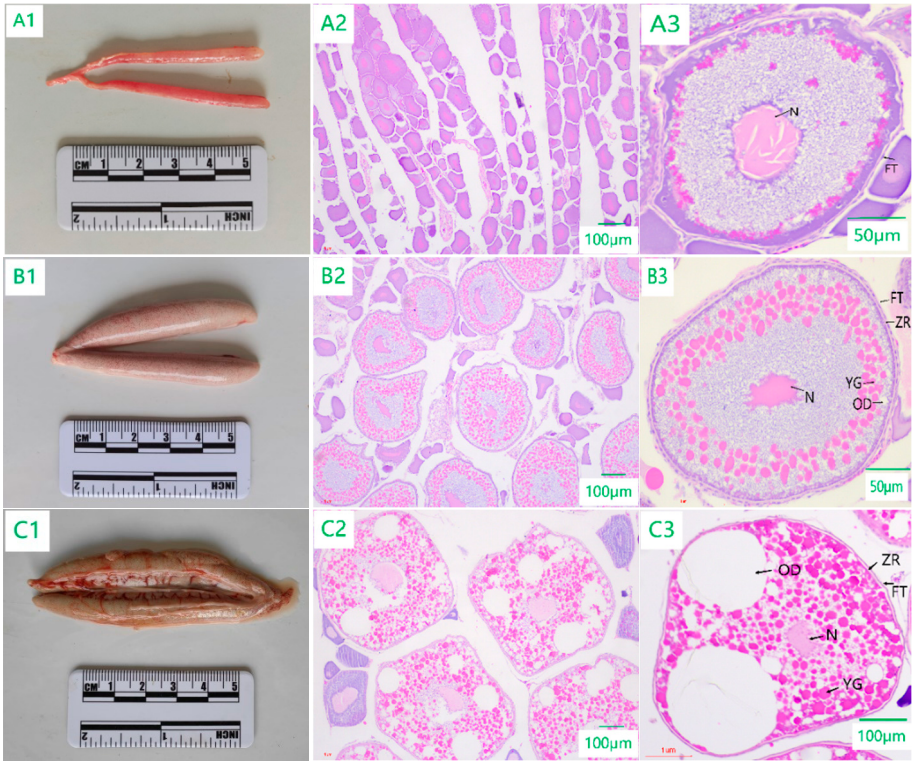

Figure 3. Morphological and histological characteristics of ovarian development of *C. nasus*. A1, B1, and C1 are the morphology of ovaries in stages II, III, IV. A2, B2, and C2 are the tissue section observation results of ovaries in stages II, III, IV. A3, B3, and C3 are oocytes in phases 2, 3, and 4. N: Nucleus; FT: Follicular membrane; YG: Yolk granule; OD: Oil droplet; ZR: Zona radiata.

The ovaries in stage III were enlarged in the middle and smaller at both ends, with blood vessels distributed on the ovarian membrane, had a flesh-colored to light greenish appearance, and the oocytes were macroscopical. Histological observation showed that the cells were in the large growth phase of primary oocytes, dominated by the cells of the 3rd temporal phase, with a cell diameter of 168.43- 338.69 μm and a nuclear diameter of 60.62- 110.50 μm. The cell volume increased, and oil droplets of different sizes appeared in the form of 10-43 droplets, which accounted for 2.05%- 5.44% of the area of the oocyte, and the yolk began to be deposited in the form of small granules, with a number of 130- 305 granules, accounting for 21.39%- 53.68%, and radial bands appeared in the periphery (Figure 3 B1- B3).

The ovary in stage Ⅳ was enlarged in size, with enlarged oocytes visible to the naked eye, greenish to grayish in appearance, with a thin and hyaline ovarian membrane and densely vascularized. Histological observation showed that yolk granules and oil droplets filled the cells, mainly the cells in the 4th temporal phase, with a cell diameter of 278.45- 525.33 μm and a nuclear diameter of 80.00- 140.58 μm. As the yolk granules accumulated, they accounted for more than 40.20% of the cell volume, and the number of oil droplets increased, and their size became larger, and they began to merge to form large oil droplets, which accounted for about 50.50% of the cell volume. At the later stage, it filled the cell, the nucleus was gradually invisible, and the nucleolus was distributed around the nuclear membrane (Figure 3 C1- C3).

3.2.2. Spatial Characteristics of Ovarian Tissue Sections

The yolk material of *C. nasus* was mainly yolk granules and oil droplets. The oocytes of stage Ⅳ in TZ section (342.41±56.43 μm) were significantly smaller than those in CM section (469.63±49.02 μm) and AQ section (413.15±53.40 μm), and oil droplets accounted for a larger proportion than yolk granules in the oocytes of the CM section and the AQ section, while yolk granules dominated in TZ section (Figure 4).

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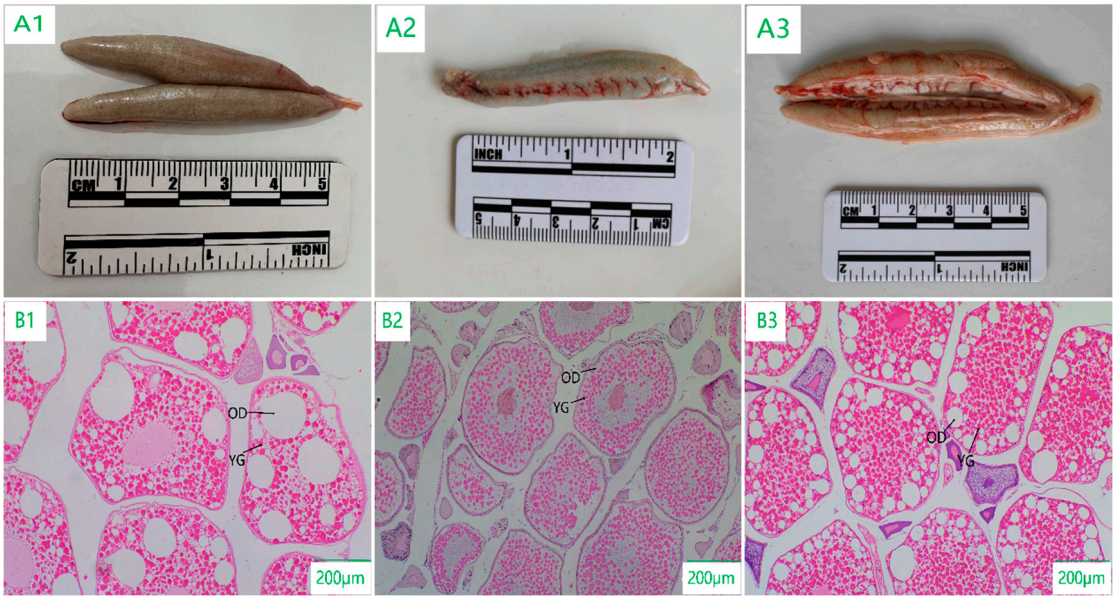

Figure 4. Morphological and histological characteristics in stage Ⅳ of ovarian development of *C. nasus* at different sampling points. A1, ovarian morphological characteristics at CM; A2, ovarian morphological characteristics at TZ; A3, ovarian morphological characteristics at AQ; B1, ovarian histological characteristics at CM; B2, ovarian histological characteristics at TZ; B3, ovarian histological characteristics at AQ; YG: Yolk granule; OD: Oil droplet.

3.3. Serum Neutral Steroid Hormone Levels

3.3.1. Characterization of the Developmental Phase of Sex Steroid Hormones

The levels of sex steroid hormones in *C. nasus* changed significantly throughout the process of ovarian development. E2 level in serum was significantly higher in stage III [(46.22±4.32) ng/l] than in stage II [(38.12±4.03)ng/l], and significantly lower in stage IV [(33.91±4.72)ng/l]. 17α,20β-DHP level was significantly higher in stage IV [(31.79±4.11)ng/l] than stage II [(24.48±1.25)ng/l] and stage III [(23.27±1.41)ng/l] (Figure 5).

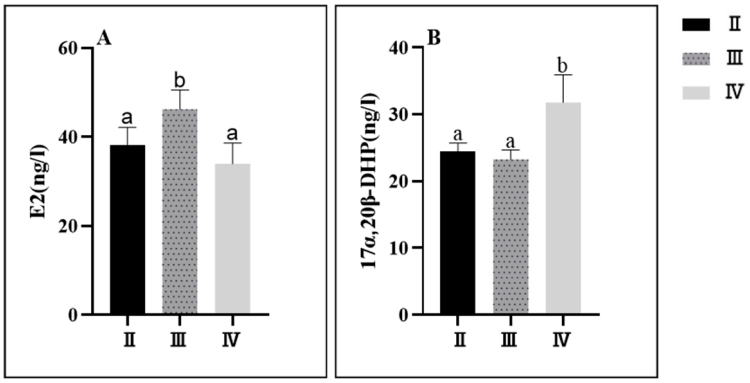

Figure 5. Serum sex steroid levels at different developmental stages of *C. nasus*. E2 level(A); 17α,20β-DHP level(B).

3.3.2. Spatial Characterization of Sex Steroid Hormones

Both estrogen and progesterone showed significant differences during the anadromous migration of *C. nasus*. The E2 level was significantly higher in TZ section than in CM section and the AQ section during stage IV, and the 17α,20β-DHP level was significantly lower in TZ section than in CM section and the AQ section.

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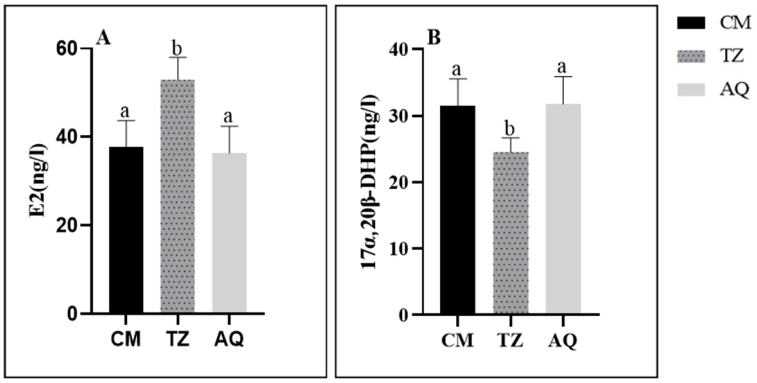

Figure 6. Serum sex steroid levels at different sampling points of *C. nasus.* E2 level(A); 17α,20β-DHP level(B).

3.4. Expression Patterns of Ovarian Development-Related Genes

3.4.1. Characterization of Developmental Stages of Gene Expression

The expression of *fshr*, *lhr*, *kiss1*, *foxl2* genes all changed significantly during the development of ovaries in *C. nasus*. There was no significant change in the expression of *fshr* and *lhr* in stages II and III, and it was significantly higher in stage IV. The expression of *kiss1* gene was significantly higher in stage III than in stages II and IV. The expression of *foxl2* showed an increasing change with the maturation of the ovary development, with the lowest expression level in stage II, and a significant increase in the expression of stage III and IV (Figure 7).

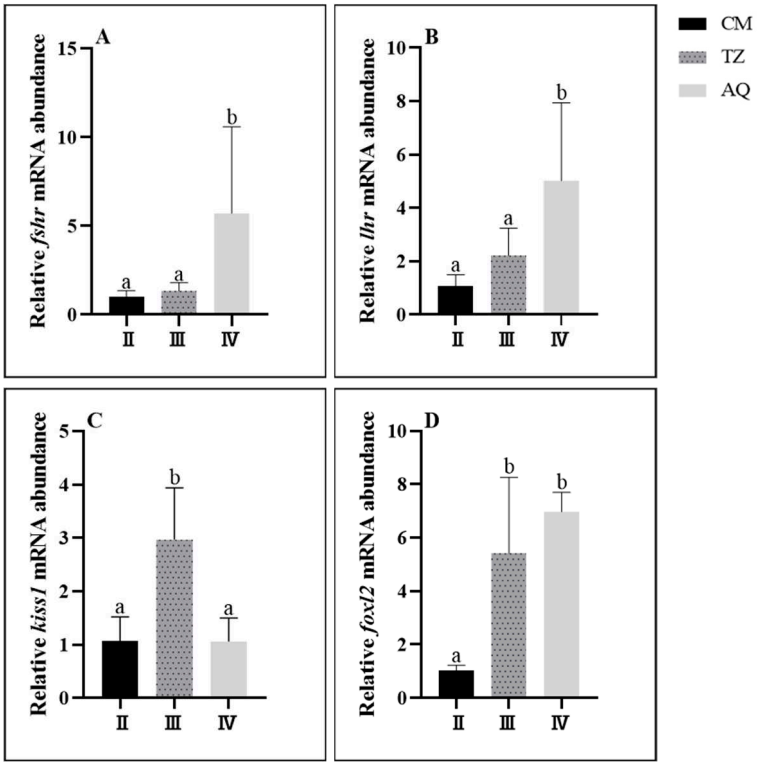

Figure 7. Ovarian gene expression levels at different developmental stages of *C. nasus*. *Fshr* mRNA level(A); *lhr* mRNA level(B); *kiss1* mRNA level(C); *foxl2* mRNA level(D).

3.4.2. Spatial Characterization of Gene Expression

The expression of *fshr* was significantly higher in TZ section than CM and AQ section, and the expression of *lhr* was significantly higher in TZ section than CM section. There was no significant difference in the expression of *kiss1* and *foxl2* (Figure 8).

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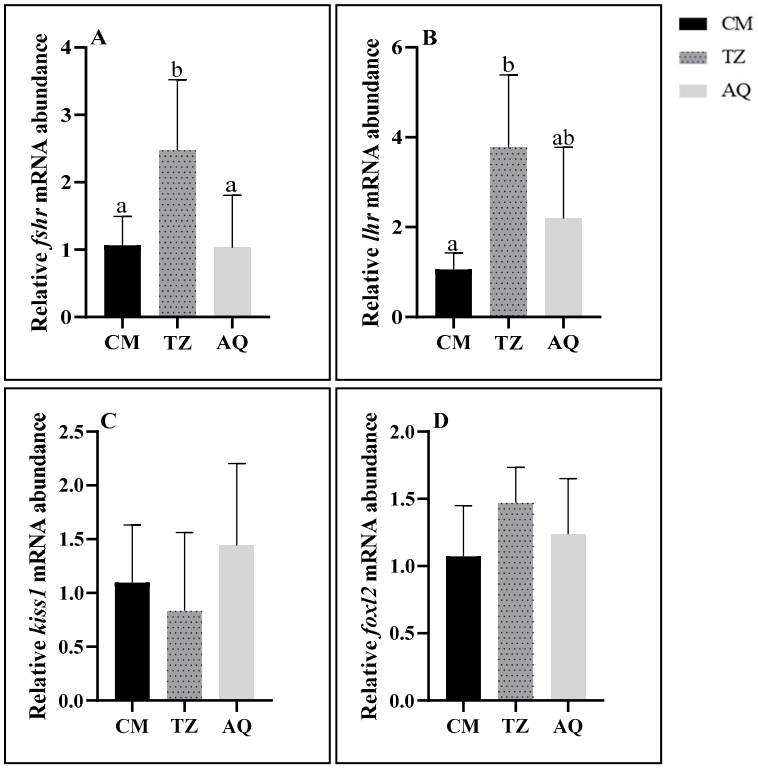

Figure 8. Ovarian gene expression at different sampling points of *C. nasus*. *Fshr* mRNA level(A); *lhr* mRNA level(B); *kiss1* mRNA level(C); *foxl2* mRNA level(D).

4. Discussion

4.1. Characterization of Ovarian Development in C. nasus

As a typical river-sea migratory fish, the anadromous migration of *C. nasus* is a seasonal reproductive activity [19]. *C. nasus* mostly lives in coastal and nearby waters, aggregates in the offshore waters of the Yangtze River estuary during spawning migration [15,23]. The increase of water temperature induces the pre-spawning migratory behavior of *C. nasus* and promotes its gonadal development and maturation [27,28]. The study analyzed the *C. nasus* in April-July 2018 of AQ sectionshowed that the main developmental stage of the ovaries was stage III, and the ovaries began to appear in June to stage V of *C. nasus* [29]. He et al. [30] investigated and studied *C. nasus* in AQ section from April to August 2005, found that the maturity coefficient of ovaries in stage II and III in April was 1.36% on average, in June, the maturity coefficient of ovaries developing to stages IV and V reached 6.66%. In this study, in the early stage of migration from March to April, the ovarian development of *C. nasus* was dominated by stage I and II, the proportion of stage III and IV increased in May. The number of *C. nasus* individuals in stage IV and V increased in June and July. Overall, the proportion of ovarian mature individuals and the coefficient of ovarian maturity increased with the passage of time and the increase of water temperature in all water segments. This was consistent with the results of historical studies. Yolk, as an energy-supplying substance for ovarian development in fish, has also attracted much attention in terms of changes in its content. Observation of tissue sections showed that the ovary in stage II was dominated by the 2nd time-phase oocytes, which were smaller, and no yolk material was produced, which was consistent with the histological characteristics in stage II of *Rhinobio ventralis* [31]. In addition, the ovary in stage III was dominated by oocytes in the 3rd temporal phase, which enlarged about onefold and began to show nutrients such as yolk granules and oil droplets, which was consistent with the findings of *Girella leonina* [32], and *Xenocypris microlepis* [33]. Moreover, ovaries in stage IV were dominated by time-phase 4 oocytes, which were almost full of nutrients such as yolk granules and oil droplets, with yolk granules becoming fuller and the area of oil droplets fused and becoming larger. Similarly, a large number of oil droplets

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synthesized into oil globules occurred in stage IV of *Girella leonina* [32]. The results of ovarian development and ovarian maturity coefficients in the present study were consistent with those of previous studies on *C. nasus* [17,34], reflecting the typical ecological characteristics of *C. nasus* in fattening and long-distance reproductive migration in different waters.

In this study, the observation of ovarian tissue sections of stage Ⅳ in CM, TZ and AQ sections revealed no significant difference in oocyte size. However, the proportion of yolk material composition of oocytes was different. Concretely, the ovarian tissue sections of the CM and AQ sections showed that oil droplets occupied about half of the cell area, and yolk granules were slightly smaller than the proportion of oil droplets, which was consistent with the results of stage Ⅳ ovarian tissue of the pond culture of *C. nasus*, *Parabramis pekinensis*, and *Hippocampus erectus* [18,35,36]. In contrast, ovarian tissue sections from the TZ section showed that yolk granules accounted for a larger proportion of oocytes and fewer oil droplets. This suggests that the maturity level of *C. nasus* collected in TZ section was lower than that in CM and AQ sections. Further development and maturation of *C. nasus* collected in TZ section were needed, probably because the target spawning grounds had not been reached yet, and it was necessary to continue migrating upstream to complete the reproduction of spawning [18,37].

4.2. Characterization of Changes in Serum Neutral Steroid Hormones

Sex steroid hormone levels can reflect the level of ovarian development in fish, among which E2 and 17α,20β-DHP are the main endogenous sex steroid hormones in fish, which play an important role in regulating the ovarian development process.

E2 was at a low level in stage II, consistent with the results of *Oreochromis niloticus* [38], *Epinephelus fuscoguttatus* [39], and *Schizothorax labrosus* [40]. Upon ovarian development to stage III, gonadotropins stimulate follicular and granulosa cells to co-synthesize E2, which acts through nuclear estrogen receptor signaling to induce hepatic synthesis of yolk proteins, and thus promotes ovarian vitellogenesis and accumulation [39], exhibiting a significant stage III elevation. Existing studies have shown that high concentrations of E2 inhibit oocyte development and ovulation [41], and decrease significantly in E2 concentration when the *C. nasus* ovary enters stage IV in order to ensure the normal process of oogenesis may be closely related to the high expression of maturation-inducing hormones (MIHs, e.g., 17α,20β-DHP), which induces the final maturation of the oocyte [3]. The findings in fish such as *Danio rerio* [42], *Epinephelus fasciatus* [43], and *Oncorhynchus keta* [44] were the same as those of *C. nasus* in the present study, in which 17α,20β-DHP was significantly elevated in stage IV. Also these factors have a roles in inducing ovarian cell maturation and is the dominant final maturation of the ovarian hormones. Sex steroid hormones are involved in the regulation of ovarian maturation and play a role in the completion of reproductive migration.

For different river sections, the E2 level in TZ section was significantly higher than that in CM and AQ sections, and the 17α,20β-DHP level was significantly lower inTZ section than in CM and AQ sections, which indicated that the level of maturation of *C. nasus* in TZ section was lower than that in CM and AQ sections. Some scholars have hypothesized that *C. nasus* migrates at the same speed and the developmental speed of *C. nasus* with similar body length is also similar, so the target spawning sites of *C. nasus* differ from each other due to the different starting points of *C. nasus* migration [30,45]. In this study, the stage IV *C. nasus* migrating to CM section were more mature, and it was hypothesized that they might have come from waters farther away from the entrance of the Yangtze River, and their target spawning grounds might have been located near CM section [28]. Similarly, the stage IV ovaries of *C. nasus* migrating to AQ section were also more mature, suggests that this population may have come from waters closer to the entrance of the Yangtze River, and that the target spawning grounds may be in AQ section and further waters after the same distance of migration [29]. The stage IV ovaries of *C. nasus* in TZ section were less mature, and it was hypothesized that there might not be any suitable target spawning grounds in TZ section and neighboring waters. As a result, the TZ section mainly functions as a migration corridor during migration [46].

4.3. Characterization of Gene Expression Related to Ovarian Development

Fshr, *lhr*, *kiss1*, and *foxl2* genes regulate the process of ovarian development and maturation by participating in physiological activities such as the synthesis of sex steroid hormones and accumulation of yolk material in oocytes. In female fish, *fshr* is mainly expressed in sphingocytes and granulosa cells, and *lhr* is mainly expressed in granulosa cells [47]. The traditional theory of animal reproduction suggests that the target organ of gonadotropin receptors (*fshr* and *lhr*) is the gonad, which indirectly affects the development and function of the reproductive organs through the regulation of gonadotropin secretion [48]. In *Oncorhynchus mykiss* [49] and *Ctenopharyngodon idella* [50] , the expression of *fshr* mRNA and *lhr* mRNA gradually increased with oocyte development. Similarly, in this study, *fshr* promoted yolk production and accumulation [50], and *lhr* was highly expressed in stage IV of *C. nasus*, which could promote the secretion of 17α,20β-DHP from the ovary. Thus, *lhr* and 17α,20β-DHP were the main factors regulating reproduction during oocyte maturation. *Kiss1* was significantly higher in stage III than in stage II and IV, and it mainly promoted yolk formation. The expression of *foxl2* was significantly higher in stage III and IV than in stage II. *Foxl2* indirectly regulates the synthesis of E2 through the regulation of the transcription gene *cyp19*, and may play a promotional role in yolk production and deposition [52].

Gene expression levels also varied in *C. nasus* in different river sections. *Fhsr* and *lhr* expression were both higher in TZ section. Migrating from the CM section, where fresh and sea water meet, to the TZ section, which is freshwater, the change in salinity significantly increased the synthesis of sex steroid hormone-related precursors [53], resulting in elevated expression of *fhsr* and *lhr*. It also suggests that these genes respond to the effects of osmotic pressure on the gonads by regulating the synthesis of regulatory steroid hormones.

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