Seasonal Changes in Plasma Hormones, Sex-Related Genes Transcription in Brain, Liver and Ovary during Gonadal Development in Female Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: The purpose of this study was to investigate the periodic seasonal changes in endocrine activity and gonadal development of female rainbow trout (*Oncorhynchus mykiss*) in a high-altitude cold-water environment. The fish were sampled monthly from January to November and the levels of plasma hormones (estradiol (E2), cortisol and thyroid hormones (THS)) and vitellogenin (VTG) were measured by ELISA. Moreover, the transcriptions of sex-related genes in the ovary, brain, and liver were detected by qRT-PCR. The results showed a seasonal fluctuation of plasma hormones and VTG together with the development of the ovary, which reached a peak from August to October. Similarly, the transcription of hypothalamic gonadotropin-releasing hormone-2 (cgnrh-2), hypothalamic gonadotropin-releasing hormone receptors (gnrhr) and follicle-stimulating hormone (fsh) in the brain varied from January to September, but the highest level was detected in September to November. In addition, the transcription of sex-related genes located in the ovary and liver increased significantly during August to October, accompanied by a continuous increase in the gonadosomatic index (GSI) and a decrease in the hepatosomatic index (HSI). Therefore, plasma hormones and sex-related genes regulate the development and maturation of *O. mykiss* oocytes with the change in seasons and peaked in November. The results of this study provide a reference for improving the efficiency of the artificial reproduction of *O. mykiss*.

Keywords: *Oncorhynchus mykiss*; gonadal development; seasonal changes; endocrine regulation; hypothalamic-pituitary-gonadal axis

1. Introduction

Reproduction is the basis of the whole ontogeny, which requires energy, ecology, anatomy, biochemistry and endocrine adaptations [1]. The obtainability of a high-quality fry and the capability to control fish breeding are the confining factors for fish culture [2]. Therefore, sufficient information related to reproductive constant, developmental biochemistry, and molecular regulation mechanism of gonadal development are very important in aquaculture.

Throughout the reproductive cycle, seasonal changes in tissue biochemical composition are related to gonadal weight, especially in females, in which the hepatic metabolism are stimulated during vitellogenesis [3]. Hence, monthly variations in the gonadosomatic index (GSI) helps to determine the breeding season of the fish, and study of the hepatosomatic index (HSI) is also important because the liver is a key organ in fish for production of vitellogenin which plays a significant role in the development of eggs [4,5]. Moreover, several studies have reported the seasonal endocrine and aromatase changes associated
with reproductive activity, such as in *O. mykiss* [6], salmonids [7], rainbow trout (*Salmo gairdneri*) [8], and frog (*Pelophylax esculentus*) [9–11].

Increasing evidence demonstrates that neurosteroids might regulate neurogenesis in the developing or adult central nervous system. In particular, it can lead to permanent sexual differentiation of certain structures involved in sexual behavior and the neuroendocrine control of reproduction [12,13]. For instance, a gonadotropin-releasing hormone (GnRH) is a neuropeptide that participates in the reproductive regulation of all vertebrates. The primary function of GnRH is to regulate the synthesis and release of pituitary gonadotropins (GtHs), follicle-stimulating hormone (FSH), and luteinizing hormone (LH), and then stimulate steroidogenesis and gonadal development [14]. At least two GnRH forms have been characterized in *O. mykiss*, salmon GnRH (*sgnrh-1*), and chicken II GnRH (*cgnrh-2*) [15]. The *sgnrh-1* appears to have a central role in eliciting the release of pituitary gonadotropins, whereas *cgnrh-2* appears to act as a neuromodulator [16]. Furthermore, cytochrome P450 aromatase is the key enzyme that converts androgens to estrogens, and participates in the gonadal development and differentiation of fish. In teleost fish, two separate genes, *cyp19a* and *cyp19b*, that encode distinct aromatase isoforms, have been identified. The activity of *cyp19a* and *cyp19b* are predominantly associated with the ovary and brain, respectively [17]. Therefore, examination of the transcription of the *gnrh*, *gnrhr*, and brain aromatase genes in the hypothalamus and pituitary might help to understand the reproductive process of *O. mykiss* during the seasonal changes. In addition, gonadal steroids are also involved in the regulation of the hypothalamus-pituitary-thyroid (HPT) axis [18,19], affecting the level of thyroxine (T4), free thyroxine (FT4), triiodothyronine (T3), and free triiodothyronine (FT3), thus affecting the development, growth and reproduction of fish [20–22].

Environmental and genetic factors affect sex determination through a complex process [23]. During sex determination, the onset of a cascade of transcriptional or mRNA splicing factors are activated by a primary signal, allowing the final differentiation of the gonads into testis or ovary [24]. *Foxl2*, which is a putative winged helix/fork head transcription factor gene and a sexually dimorphic marker of ovarian differentiation, is involved in the ovarian development [25]. Previous studies suggest that ovarian hormones regulate the transcription of *foxl2* thereby expanding the number of genes controlled by the hypothalamic-pituitary-gonadal (HPG) axis, e.g., *gnrhr* and *fsh*, that ultimately dictate reproductive fitness [26]. In addition, *foxl2* also upregulates the transcription of *cyp19a1a*, which leads to the increase in *cyp19a1a* transcription [27]. Moreover, four nuclear estrogen receptor genes, *era1*, *era2*, *erβ1*, and *erβ2*, have been detected in rainbow trout [28]. Studies showed the positively correlated relationship between the transcription of *ers* and *vtg* [29,30], as the oocyte matures, the transcription of the *vtg* gene gradually increases. Therefore, detecting the transcription of sex-related genes during gonadal development may help to understand the reproductive process of *O. mykiss* during seasonal changes.

*O. mykiss* is a cold-water economic fish of the genus *Oncorhynchus* of the family Salmonidae. Yunnan is rich in cold-water resources and suitable climatic conditions, which are necessary conditions to promote the sustainable development of the *O. mykiss* industry. However, at present, the low egg quality, low fertilization rate, and low hatching rate of cultured female *O. mykiss* still restricts the development of cold-water fish culture in Yunnan. Therefore, to explore the characteristics of seasonal changes on plasma hormones and sex-related genes transcription during gonadal development of female *O. mykiss*, and to understand its endocrine regulation mechanism, it is of great significance to provide the data reference for *O. mykiss* reproduction, so as to further promote the healthy development of *O. mykiss* farming in Yunnan.

2. Materials and Methods

2.1. Fishes and Sample Collection

The *O. mykiss* used in this study were bought from Kunming Tanghao Aquaculture Company of China. In December 2017, the fish were domesticated in the indoor water tank
of the circulatory system for a month in Yunnan Agricultural University and fed twice a day to satiety. Thirty-three female *O. mykiss* were sampled monthly from January to November 2018 (Table 1). After a fast for 2 d, three fish were anesthetized by 100 mg/L MS-222 (tricaine methane sulfonate, Sigma, St. Louis, MO, USA) before sampling every month. Plasma was separated from blood samples after centrifuging at 3000 rpms for 15 min, and then stored at −80 °C until assayed. The liver, brain and ovary were separated from the abdominal cavity and ventricle, then collected in RNAase-free tube and stored at −80 °C for further testing. The experimental animals used in this experiment are strictly in accordance with the requirements of the guidelines for the use of Experimental Animals of Yunnan Agricultural University, and were approved by the Experimental Ethics Committee of Yunnan Agricultural University (YNAU2017llwyh131).

| Month | Body Weight (g) | Liver Weight (g) | Ovary Weight (g) |
|-------|----------------|-----------------|-----------------|
| Jan.  | 82.37 ± 11.19  | 0.91 ± 0.24     | 0.03 ± 0.02     |
| Feb.  | 154.93 ± 1.46  | 1.30 ± 0.12     | 0.04 ± 0.002    |
| Mar.  | 181.40 ± 57.93 | 1.75 ± 0.57     | 0.18 ± 0.13     |
| Apr.  | 308.87 ± 62.43 | 2.84 ± 0.72     | 0.28 ± 0.06     |
| May   | 291.47 ± 62.76 | 2.58 ± 0.64     | 0.09 ± 0.04     |
| Jun.  | 550.73 ± 149.84| 5.77 ± 1.77     | 0.40 ± 0.13     |
| Jul.  | 661.73 ± 162.31| 5.17 ± 1.34     | 0.34 ± 0.24     |
| Aug.  | 909.17 ± 49.99 | 6.08 ± 0.86     | 7.90 ± 2.54     |
| Sept. | 1047.23 ± 212.67| 8.07 ± 1.26    | 11.76 ± 4.85    |
| Oct.  | 991.93 ± 60.76 | 6.70 ± 0.81     | 8.91 ± 11.29    |
| Nov.  | 1033.43 ± 286.97| 9.98 ± 5.62    | 47.86 ± 37.95   |

2.2. Determination of HSI and GSI

In order to calculate the values of HSI and GSI, the liver and ovary were weighed immediately after decapitation. HSI was calculated using the equation: HSI = [liver weight (g)/body weight (g)] × 100%. GSI was calculated as [ovary weight (g)/body weight (g)] × 100%.

2.3. Plasma Hormones and Vitellogenin (VTG) Analyses

Cortisol, E₂, and VTG were determined by the ELISA kit, which was produced by Shanghai Enzymatic Biotechnology Co., Ltd. (Shanghai, China), according to the instructions of the kit. The Intra-assay CV(%) is less than 10% and Inter-assay CV(%) is less than 15%. The minimum detectable dose of cortisol, E₂, and VTG are typically less than 10 pg/mL, 0.1 pmol/L and 1.0 ng/mL, respectively.

TH₅ were also determined by ELISA kit, which was produced by Beijing North Institute of Biotechnology Co., Ltd., Beijing, China, according to the instructions of the kit. The Intra-assay CV(%) and Inter-assay CV(%) is less than 15%. The minimum detection concentration is less than 10 ng/mL, 0.4 ng/mL, 4.0 pmol/L, and 2.0 pmol/L for T₄, T₃, FT₄, and FT₃, respectively. All samples were measured at 450 nm wavelength using a 96 Flat Bottom Transparent Polystyrol (Greiner BioOne, Kremsmünster, Austria). The standard curve was established to calculate the concentration of cortisol, E₂, VTG, and TH₅.

2.4. RNA Extraction and cDNA Synthesis

The total RNA of the brain, liver, and ovary of three female *O. mykiss* in each month were extracted using the TRIpure reagent (Aidlab Biotechnologies Co., Ltd., Beijing, China) following the manufacturer’s instruction. The quality and concentration of the total RNA were detected by NanoDrop 2000c apparatus (Thermo Fisher Scientific, Waltham, MA, USA), with an A260 nm/A280 nm ratio from 1.8 to 2.1. Then 1 µg total RNA was used for reverse transcription according to the manufacturer’s instruction of the TRUEscript 1st Strand cDNA Synthesis Kit user manual with gDNA Eraser (Aidlab Biotechnologies Co., Ltd., Beijing, China).
2.5. Gene Transcription

The transcription of sex-related genes during the oocyte development was detected using the quantitative real-time PCR. PCR reactions (20 µL) contained 1 µL of cDNA, 0.5 µL of each primer (10 µM), and 10 µL of 2X TSINGKE® Master qPCR Mix (SYBR Green I, Beijing TsingKe Biotech Co., Ltd., Beijing, China). The amplification procedure of these genes was: pre-heating at 95 °C for 3 min, followed by 40 cycles of 95 °C for 20 s, 55 °C for 20 s, and a final extension step at 72 °C for 20 s. The samples were analyzed in triplicate with a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The elongation factor 1-alpha (efa1α) was used as the internal control to calculate the relative transcription of target gene by the $2^{-\Delta\Delta Ct}$ method [31]. The primers of genes were shown in Table 2.

Table 2. Nucleotide primers used in real-time PCR.

| Gene   | Primer Name | Sequence (5′–3′) | GenBank No. or Article Source |
|--------|-------------|------------------|-------------------------------|
| era1   | era1-F      | CCCTGCTGGTGACAGAGAA | [28] |
|        | era1-R      | ATCCCTCCACCACCACATGAGACT |            |
| era2   | era2-F      | GTGGCAGTGACGACCAAC | [28] |
|        | era2-R      | ACCACCGAAGCTGCTTTTCTT |            |
| erβ1   | erβ1-F      | CCCAACGCGGTCCTAGCT | [28] |
|        | erβ1-R      | TCCCTCATGTCCTCTGAGGAA |            |
| erβ2   | erβ2-F      | TGGACCAGACAAGCTGATC | [28] |
|        | erβ2-R      | TCCTCAGTGTTGTAATG |            |
| vtg    | vtg-F       | TGGACTTGAGTGAGGAAC | AY049952.1 |
|        | vtg-R       | AGACGGGCTCAGTGTTGAAT |            |
| cyp19b | cyp19b-F    | GAGGAAGGCACGTGAAGATGAC | [32] |
|        | cyp19b-R    | GCTGGAAGAAACGACTGGG |            |
| fsh    | fsh-F       | GCCAACAAGGACTGAAACTAT | [33] |
|        | fsh-R       | GACAGCCCTCAGGTTGAAAT |            |
| cgnrh-II | cgnrh-II-F   | CTGTGAGGCGAGGAATG | [33] |
|        | cgnrh-II-R  | ACGGTTGATAGGTTGTCTAA |            |
| gnrhr  | gnrhr-F     | GCTTTTCTCACAACACATGTC | AJ272116.1 |
|        | gnrhr-R     | GGAACCTGGGACATTTTGAGAG |            |
| fshr   | fshr-F      | TCCAGTCGAGCTGAGTTGGA | [33] |
|        | fshr-R      | TCCTGACGTCGACAGAAAAAC |            |
| lhr    | lhr-F       | CTCTCAACCTTCAAATCGACTTC | [33] |
|        | lhr-R       | GGATATACCTGAGATACGCAA |            |
| cyp19a1a | cyp19a1a-F  | CTCCTCCTCATCCCTCCAGTT | [34] |
|        | cyp19a1a-R  | AGAGGAACTGCTGATATAAT |            |
| foxl2  | foxl2-F     | TGTGCTTGGATTGTTTTTTTTTGT | [34] |
|        | foxl2-R     | GTGTCGCTGGACCTCACGGCCA |            |
| efa1α  | efa1α-F     | AGGGCATCTGATCTACAACTGTCG | AF498320.1 |
|        | efa1α-R     | GGTGATACCAGGCTCCCTTCT |            |

2.6. Statistical Analysis

The Tukey method of a one-way analysis of variance (ANOVA) was used to evaluate the differences of value among the months through the SPSS 16.0 software (IBM Inc., Armonk, NY, USA). A value of $p < 0.05$ was considered statistically significant. All data were expressed as mean ± standard deviation (SD).
3. Results

3.1. Seasonal Changes in HSI and GSI

From January to July, no monthly significant differences in the HSI were observed. However, in August, HSI significantly reduced. After that, HSI returned to the normal levels from September to November (Figure 1A). Moreover, from January to October, no significant differences of GSI values were observed. The highest value of GSI of 4.11% was observed in November (Figure 1B).

![Figure 1](image)

*Figure 1.* Monthly changes in HSI (A) and GSI (B) values of female *O. mykiss* during the oocyte development. *: Significant differences between the Jan and other month (Tukey method of the one-way ANOVA test, *p* < 0.05).

3.2. Plasma Cortisol, E\textsubscript{2} and VTG Levels of Female O. mykiss during Oocyte Development

Plasma cortisol levels of females gradually increased from January to July (354.02 ± 19.60~485.85 ± 10.65 pg/mL). Then, cortisol levels decreased rapidly in August (423.58 ± 22.31 pg/mL) and increased again in September (594.65 ± 7.05 pg/mL) and remained at a high level until November (Figure 2A). Plasma E\textsubscript{2} levels of females fluctuated from January to July (5.80 ± 0.07~12.00 ± 0.45 pmol/L) and peaked in May. E\textsubscript{2} levels then increased rapidly in September (13.05 ± 0.77 pmol/L) and decreased again from October and November (Figure 2B). Plasma VTG levels of females fluctuated and were low in January to July (65.56 ± 3.95~97.96 ± 5.70 ng/mL), increased in September and reached the peak (126.99 ± 1.66 ng/mL). VTG levels then decreased rapidly in October and remained at a low level until November (Figure 2C).
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Different letters denote statistically significant differences among the groups (Tukey method of the one-way ANOVA test, \( p < 0.05 \)).

Monthly changes in cortisol (Figure 2A), \( E_2 \) (Figure 2B) and VTG (Figure 2C) values of female \( O. \) mykiss during the oocyte development. Different letters denote statistically significant differences among the groups (Tukey method of the one-way ANOVA test, \( p < 0.05 \)).

3.3. Plasma TH\(_3\) Levels of Female \( O. \) mykiss during Oocyte Development

Plasma concentrations of both \( T_4 \), FT\(_4\), \( T_3 \), and FT\(_3\) were determined in female \( O. \) mykiss during the oocyte development (Figure 3). Plasma \( T_4 \) levels remained at around 30 ng/mL from January to May, and then significantly increased in June. The highest plasma \( T_4 \) levels were observed in August (122.59 ± 15.08 ng/mL). After that, \( T_4 \) levels gradually declined from September to November (Figure 3A). Plasma \( T_3 \) levels of females fluctuated throughout the year. From January to April, \( T_3 \) levels increased first and then decreased (1.01 ± 0.05−1.74 ± 0.23 ng/mL). After that, plasma \( T_3 \) remained elevated from May and reached the peak in June (2.24 ± 0.10 ng/mL). However, \( T_3 \) concentrations continued to decrease from July to November (Figure 3B). The changes in FT\(_4\) levels in the plasma were similar to \( T_4 \). Plasma FT\(_4\) increased in June and reached peak levels by the beginning of July (8.69 ± 0.21 pmol/L), and then maintained a high level until October (10.80 ± 0.22−10.97 ± 1.00 pmol/L) (Figure 3C). Then, a significant decrease was observed in November. Similar to \( T_3 \), FT\(_3\) levels fluctuated irregularly throughout the year. The highest level of FT\(_3\) appeared in October (9.48 ± 0.48 pmol/L) (Figure 3D).

3.4. Seasonal Changes in the Levels of Female \( O. \) mykiss Brain Genes during Oocyte Development

The transcription levels of genes in the brain were examined through an areal-time PCR assay (Figure 4). The transcription of \( cyp19b \) did not fluctuate significantly from January to July. However, a significant decrease was detected in August and September. From October to November, \( cyp19b \) levels gradually raised and then peaked in November (Figure 4A). The levels of \( cgnrh-2 \) were low in January to August, and increased significantly in September and October in association with active vitellogenesis. In November, the \( cgnrh-2 \) level decreased to the similar levels of January to August (Figure 4B). Moreover, similar to \( cgnrh-2 \), the levels of \( gnrh \) were low from January to August. After that, the levels of \( gnrh \) substantially increased in September and October, and the level in October was significantly higher than those in other months. The transcription of \( gnrh \) significantly decreased in November (Figure 4C). The transcription levels of \( fsh \) were low from January to August. However, the levels of \( fsh \) fluctuated irregularly from September to November, showing a transcription trend that decreased first and then increased. The transcription peaks of \( fsh \) were observed in September and November (Figure 4D).
3.4. Seasonal Changes in the Levels of Female *O. mykiss* Brain Genes during Oocyte Development

From October to November, the transcription of `erβ2` showed transcription peaks in September (Figure 4G). Similar to `cgnrh-2`, `foxl2` transcription remained at the same levels from January to August, and then significantly increased from August to November. The transcription peak of `cyp19a1a` gradually increased and reached its peak in November (Figure 5B). Transcripts of `cyp19b` did not fluctuate significantly from June to September, which reached the highest levels in September. In October and November, the transcription of `fshr` was observed from January to August, which significantly increased from August and reached the maximum in September. After that, the transcription of `erβ2` gradually decreased to the similar levels of January to August (Figure 5H). However, irregular changes were observed in `fshβ2` during the period from January to May, followed by a gradually increase from August and reached the maximum in September. After that, the transcription of `lhr` transcription remained at the same levels from January to August, which significantly decreased in November (Figure 4C). The transcription levels of genes in the brain were examined through an areal-time PCR assay (Figure 4). The transcription of `gnrhr` level in October was significantly higher than those in other months. The transcription of `fshα2` gradually decreased from October to November (Figure 5C). The levels of `fshβ2` increased in September. After that, the transcription of `erβ2` gradually decreased to the same level in August (Figure 5H). However, irregular changes were observed in `fshβ2` during the period from January to May, followed by a gradually increase from August and reached the maximum in September. After that, the transcription of `lhr` transcription remained at the same levels from January to August, which significantly decreased in November (Figure 4C). The transcription levels of genes in the brain were examined through an areal-time PCR assay (Figure 4). The transcription of `gnrhr` level in October was significantly higher than those in other months. The transcription of `fshα2` gradually decreased from October to November (Figure 5C). The levels of `fshβ2` increased in September. After that, the transcription of `erβ2` gradually decreased to the same level in August (Figure 5H).

**Figure 3.** Monthly changes in THS of female *O. mykiss* during the oocyte development. (A), T4; (B), T3; (C), FT4; (D), FT3. Different letters denote statistically significant differences among the groups (Tukey method of the one-way ANOVA test, p < 0.05).

**Figure 4.** Monthly changes in genes transcription of female *O. mykiss* brain during the oocyte development. (A), `cyp19b`; (B), `cgnrh-2`; (C), `gnrhr`; (D), `fsh`. Different letters denote statistically significant differences among the groups (Tukey method of the one-way ANOVA test, p < 0.05).
3.5. Seasonal Changes in the Levels of Female O. mykiss Gonadal Genes during Oocyte Development

The real-time PCR was further used to validate the differentially expressed gonadal genes during seasonal changes (Figure 5). The transcription levels of *fshr* did not change significantly during the period from January to May, followed by a gradually increase from June to September, which reached the highest levels in September. In October and November, the transcription of *fshr* also remained at a high level (Figure 5A). Different from *fshr*, the levels of *lhr* transcription were low from January to September, and then gradually increased and reached its peak in November (Figure 5B). Transcripts of *cyp19a1a* remained at the same levels from January to July, and then significantly increased from August to November. The transcription peak of *cyp19a1a* was observed in November (Figure 5C). The levels of *foxl2* transcription were low from January to July, and significantly increased from August and reached the maximum in September. After that, the transcription of *foxl2* gradually decreased to the same level in August (Figure 5D).

According to the real-time PCR data, *era1* and *era2* were expressed at low levels from January to August and then became significantly high in September, followed by a gradually decrease from October to November (Figure 5E,F). However, irregular fluctuations of the *erβ1* transcription were observed from January to August, which showed transcription peaks in September (Figure 5G). Similar to *era1* and *era2*, the *erβ2* transcription remained at the same levels from January to August, and then significantly increased in September. After that, the transcription of *erβ2* gradually decreased to the same level in January to August (Figure 5H).

3.6. Seasonal Changes in the Levels of Female O. mykiss Liver Genes during Oocyte Development

Both the *era1* and *era2* gene transcription profile showed the lowest transcription levels from January to July, but a significant elevation of *era1* transcription was observed in November, while *era2* transcription increased sharply between October and November (Figure 6A,B). There was no significant change in *erβ1* transcription during the annual cycle. The *erβ2* transcription was detected in the liver during the annual cycle with low levels of transcription, except for the October, in which the transcription level was significantly higher compared with January to July (Figure 6C,D).

![Figure 5. Cont.](image-url)
3.6. Seasonal Changes in the Levels of Female $O. \text{mykiss}$ Liver Genes during Oocyte Development

Both the $er\alpha_1$ and $er\alpha_2$ gene transcription profile showed the lowest transcription levels from January to July, then a significant elevation of $er\alpha_1$ transcription was observed...
Figure 6. Monthly changes in genes transcription of female O. mykiss liver during the oocyte development. (A), era1; (B), era2; (C), erβ1; (D), erβ2. Different letters denote statistically significant differences among the groups (Tukey method of the one-way ANOVA test, p < 0.05).

Figure 7. Monthly changes in vtg transcriptions of female O. mykiss during the oocyte development. (A), vtg gene transcription in ovary; (B), vtg gene transcription in liver. Different letters denote statistically significant differences among the groups (Tukey method of the one-way ANOVA test, p < 0.05).

4. Discussion

O. mykiss is an iterative, oviparous fish with a synchronized set of annual reproductive cycles. In the whole reproductive cycle, GSI and HSI may help to identify the breeding period or reproductive peak of fish, and further reflect the ovarian maturation time and spawning time of O. mykiss. In S. Gairdneri, GSI elevated gradually from June to August, increased significantly from September to December and reached a peak in December, and then decreased again from January to May. Similarly, HSI values increased gradually from March to December, and then decreased again in January and February [35]. Therefore, it showed that the ovarian maturation and spawning period of S. Gairdneri is in December. In this study, GSI increased gradually from August to November and peaked in November, while HSI was the lowest in August. When HSI decreases and GSI increases, it indicates that the liver loses weight during reproduction, and the VTG synthesized by the liver is transported to the gonads to promote gonadal maturation [36]. Therefore, the period of rapid gonadal development of O. mykiss is from August to November, and November is the most suitable breeding season for this species.
Steroid hormones such as testosterone, estradiol, progesterone, and corticosteroids play a key role in the reproductive process of fish. These hormones act on the reproductive process of fish directly or through feedback mechanisms. In female fish, E2 is necessary for ovarian development and controls egg maturation, ovulation, and egg laying. Meanwhile, circulating E2 regulates the transcription of vtg in hepatocytes, leading to the synthesis of several closely related VTG proteins [37]. In this study, the plasma levels of cortisol, E2 and VTG in female O. mykiss changed significantly during oocyte development. Especially in the pre-oviposition stage, the level of E2 in plasma increased, the yolk generation was active, and then the level of VTG in plasma increased subsequently. In the study of catfish (Silurus asotus), the levels of E2 and plasma cortisol increased during the period of yolk generation and spawning [38]. Female O. mykiss had a low gonad E2 synthesis January to August. However, the plasma E2 content increased significantly in September, indicating that the ovarian development of female O. mykiss was the fastest in September in this study. Similarly, the plasma VTG content of female O. mykiss also has a similar trend with E2. The content of VTG has a gradually increasing trend from January to June. In September, the plasma VTG content increased significantly, indicating that the synthesis of VTG is the largest in September, followed by the size of the oocyte, which increases rapidly. In addition, the trend of female O. mykiss plasma cortisol and VTG is consistent, reaching a peak in September, and then decreasing. Therefore, the trend of plasma cortisol is consistent with E2 and VTG, indicating that cortisol is also involved in the development of female O. mykiss oocytes in this study. During the reproductive cycle, plasma cortisol levels also increase from spawning or spawning periods, such as in the plaice (Pleuronectes platessa) [39], two species of trout, Salmo trutta L. and S. gairdneri [40], O. mykiss [41]. Therefore, in O. mykiss reproduction, the increase in the plasma cortisol level during reproductive periods may play a key role in ovarian growth and vitellogenesis in addition to stress [42,43].

In fish species, gonadal steroids can also affect circulating levels of thyroid hormone and/or thyroid activity [18,19]. THs include T3, T4, FT3, and FT4 [44]. Thyroids participate in the physiologic stress response envisaged chiefly because of the involvement in THs in almost all aspects of the physiologic processes [45–47]. The capability of the thyroid axis is to respond to the stimuli which arise from the other factors that engage in its cross talk [48]. Therefore, thyroid hormones have a wide range of effects on the development, growth, and reproduction of fish [20,21]. These effects are also passed on from generation, because THs play a pivotal role in the early development of offspring [21,49]. The plasma THs levels of many fishes show periodic changes. These hormones are related to sexual maturity [50,51] and reproductive cycles [52,53]. In this study, plasma concentrations of THs were determined in female O. mykiss during the year of oocyte development. Plasma TH4 levels increased in August and decreased in November before spawning. This is similar to the study of two strains of rainbow trout (O. mykiss Shasta and Kamloops), the levels of FT3 and FT4, T3 and T4 were the lowest during the spawning period, and the circulating THs showed similar seasonal changes [54]. These results indicated that THs participated in the regulation of yolk accumulation in rainbow trout.

With the change in seasons, the HPG axis plays an important role in oocyte development of O. mykiss. GnRH, synthesized and released by the hypothalamus, is a key hormone that regulates reproduction [55]. In salmonid fishes, two forms of GnRH, sgnrh-1 and cgnrh-2, have been detected [15]. GnRH is conveyed to the pituitary via the hypothalamo-hypophysial portal vessels, and regulates synthesis and release of GtH [56]. In the process of signal transduction, ligands (cgnrh-2, fsh, lh) bind and interact with their receptors (gnrhr, fshr, lhr) distributed in target cells to achieve their physiological functions [57]. In this study, the transcription level of cgnrh-2 and gnrhr gradually increased from August to October. Moreover, a previous study showed that the transcription of fshr is associated predominantly with vitellogenesis, while the lhr is prevalent during oocyte maturation and ovulation [57]. In female greater amberjack (Seriola dumerili) [58], ovarian fshr and plasma E2 gradually increased during ovarian development, which suggested that fsh plays a role in ovarian development and during the post-spawning period [58]. In this study,
the ovarian \textit{fsh}, \textit{fshr} of \textit{O. mykiss} increased continuously from August to September and decreased from September to October, but \textit{fsh} increased significantly again in November. Similarly, the ovarian \textit{lhr} increased continuously from August to November, and the peak was in November. Therefore, the transcription of \textit{fsh} and \textit{lhr} plays a crucial role in the oocyte development of \textit{O. mykiss} and has seasonal correlation. In addition, GnRH can act as a neuromodulator, and administration of exogenous GnRH facilitates sexual behavior in many species [59]. Therefore, \textit{cgmrh-2} can affect the transcription of \textit{fsh} and \textit{lhr} through \textit{gnrhr}, and then participate in the regulation of \textit{E2} synthesis in \textit{O. mykiss}.

Aromatase is a key enzyme in the synthesis of estrogen, also known as estrogen synthetase. It catalyzes the conversion of testosterone and androstendione to estrone and estradiol in animals, and converts androgen to estrogen. Therefore, aromatase shows its regulatory role in the early differentiation of female gonadal gland in nonmammalian vertebrates [60]. The \textit{cyp19} encodes cytochrome \textit{P450} aromatase. Two \textit{cyp19} genes, \textit{cyp19a} and \textit{cyp19b}, which belong to two independent \textit{CYP19} subfamilies, were identified. The \textit{cyp19a} is transcribed in the ovary, while the \textit{cyp19b} gene is transcribed in the brain [61]. The transcription of the \textit{O. mykiss cyp19b} is consistent with the gonadal development period. From January to July, the transcription of the \textit{O. mykiss cyp19b} is at a high level. The \textit{cyp19b} promotes the release of estrogen and gonadotropin. Then, the transcription of the \textit{O. mykiss cyp19b} decreased from August, and significantly increased until the gonad maturation process. These results indicate that \textit{cyp19b} is based on the transcription of the brain, which regulates the final maturation and ovulation of \textit{O. mykiss} in November.

The \textit{foxl2} is the fork transcription factor in the process of the ovarian development [62]. As shown in previous studies, the \textit{foxl2} transcription is a genetic factor that activates the transcription of aromatase [63]. In the \textit{O. mykiss} gonadal development of this study, the transcription of \textit{foxl2} reached its peak in September, decreased gradually from October to November, and decreased the lowest in November. Therefore, \textit{foxl2} may have a regulatory relationship with \textit{cyp19a1a}, and the transcription of \textit{cyp19a1a} gradually increases from August to November and peaked in November. A previous study also revealed that \textit{cyp19a1a} and \textit{cyp19a1b} were co-expressed with \textit{foxl2}, except in the early yolk stage of the ovary [24]. In summary, during fish gonadal development, the highest transcription level of \textit{foxl2} was earlier than \textit{cyp19a1a}, suggesting that \textit{foxl2} is involved in fish gonadal differentiation and the maintenance of ovarian function [64].

In addition, estrogen regulates ovarian development, differentiation, and maintenance, as well as oogenesis, and stimulates liver synthesis of \textit{vtg} and choriogenin [29,30]. These effects are principally mediated through \textit{ers} which belong to the nuclear hormone receptor superfamily. After binding of a ligand to the ligand-binding domain (LBD) of ERS, this complex binds as homo dimer to estrogen response elements (EREs) in the promoter regions of estrogen responsive target genes and regulates their transcription [65]. Four nuclear estrogen receptor genes were identified in \textit{O. mykiss}: \textit{era1}, \textit{era2}, \textit{erβ1}, and \textit{erβ2} [28]. Recent knockout studies in goldfish (\textit{Carassius auratus Linnaeus}) and zebrafish (\textit{Danio rerio}) have shown that \textit{erβ1} and \textit{erβ2} are necessary for the estrogen-mediated up-regulation of \textit{era} and \textit{vtg} transcription [66,67]. The \textit{vtg} was also positively correlated with the \textit{ers} subtype [29,30]. In this study, under the effect of estrogen in female \textit{O. mykiss}, the transcription of \textit{ers} in the ovary increased significantly in September, and decreased gradually from September to November. Similarly, the transcription of \textit{vtg} in the ovary increased gradually in August to November and peaked in the November. As an important exogenous organ, the transcription of \textit{ers} and \textit{vtg} in the liver also increased with the change in season. These results showed that the increasing of estrogen level and its receptor genes transcription, as well as \textit{vtg}, participated in the accumulation of vitelloprotein during female \textit{O. mykiss} ovarian development with seasonal changes. Therefore, cortisol, \textit{E2}, \textit{THS} can interfere with the transcription of sex-related genes on the HPG axis, thereby promoting the transcription of \textit{vtg} genes in the ovary and liver, increasing the plasma \textit{VTG} levels, and finally leading to the reproduction of \textit{O. mykiss} in November.
5. Conclusions

In conclusion, this experiment studied the periodic seasonal changes in plasma hormones, and sex-related genes transcription in the brain, liver, and ovary during the gonadal development of female *O. mykiss* under the regulation of HPG axis. The plasma hormones gradually increased from January to August, and peaked from August to October, which promotes the synthesis of VTG, and then accelerated the development and maturation of oocytes in *O. mykiss*. Similarly, the transcription level of sex-related genes in the HPG axis increased significantly since August and maintained a high level until November, which affected the synthesis of estrogen, and then participated in the regulation of gonadal development. In addition, estrogen can affect the transcription of vtg by increasing the ers transcription, thereby regulating the synthesis and accumulation of yolk in *O. mykiss* oocytes, and ultimately affects the value of GSI in November. Therefore, these sex-related hormones and genes can accelerate the synthesis and accumulation of yolk by affecting the HPG axis from August, and reach a peak in November to promote the spawning and reproduction of *O. mykiss*. These results strongly proved that November is the most suitable season for *O. mykiss* breeding in Yunnan.

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Institutional Review Board Statement: The experimental animals used in this experiment are strictly in accordance with the requirements of the guidelines for the use of Experimental Animals of Yunnan Agricultural University, and have been approved by the Experimental Ethics Committee of Yunnan Agricultural University (YNAU201711wyh131).

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References

1. Rocha, M.J. *Fish Reproduction*; CRC Press: Boca Raton, FL, USA, 2008.
2. Karimi, S.; Kochanian, P.; Salati, A.P.; Gooraninejad, S. Plasma sex steroids and gonadosomatic index variations during ovarian development of female wild yellowfin seabream (*Acanthopagrus latus*). *Ichthyol. Res.* 2013, 61, 68–75. [CrossRef]
3. Tolussi, C.E.; Gomes, A.D.; Ribeiro, C.D.S.; Caneppele, D.; Moreira, R.G.; Honj, R.M. Mobilization of energetic substrates in the endangered catfish *Steindachneridion parahybae* (Siluriformes: Pimelodidae): Changes in annual reproductive cycle in captivity. *Neotrop. Ichthyol.* 2018, 16. [CrossRef]
4. Jan, M.; Ahmed, I. Assessment of fecundity, gonadosomatic index and hepatosomatic index of snow trout, *Schizothorax plagiosotomus* in river Lidder, in Kashmir Himalaya, India. *Int. J. Fish. Aquat. Stud. Delhi* 2016, 4, 370–375.
5. Jan, M.; Jan, N. Studies on the fecundity (F), gonadosomatic index (GSI) and hepatosomatic index (HSI) of *Salmo trutta fario* (Brown trout) at Kokernag trout fish farm, Anantnag, Jammu and Kashmir. *Int. J. Fish. Aquat. Stud. Delhi* 2017, 5, 170–173.
6. Estay, F.; Colihueque, N.; Araneda, C. Comparison of Oogenesis and Sex Steroid Profiles between Twice and Once Annually Spawning of Rainbow Trout Females (*Oncorhynchus mykiss*). *Sci. World J.* 2012, 2012, 1–7. [CrossRef]
7. Teitsma, C.; Desdoits-Lethimonier, C.; Tujague, M.; Anglade, I.; Saligaut, D.; Bailhache, T.; Pakdel, F.; Kah, O.; Ducouret, B. Identification of potential sites of cortisol actions on the reproductive axis in rainbow trout. *Comp. Biochem. Physiol. Part C Pharmacol. Toxicol. Endocrinol.* 1998, 119, 243–249. [CrossRef]
8. Cyr, D.G.; Eales, J.G. Influence of thyroidal status on ovarian function in rainbow trout, *Salmo gairdneri*. *J. Exp. Zoöl.* 1988, 248, 81–87. [CrossRef]
9. Di Fiore, M.M.; Assisi, L.; Botte, V. Aromatase and testosterone receptor in the liver of the female green frog, *Rana esculenta*. *Life Sci.* 1998, 62, 1949–1958. [CrossRef]
10. Burrone, L.; Santillo, A.; Pinelli, C.; Baccari, G.C.; Di Fiore, M.M. Induced synthesis of P450 Aromatase and 17β-estradiol by D-aspartate in frog brain. *J. Exp. Biol.* 2012, 215, 3559–3565. [CrossRef]
11. Paolucci, M.; Di Fiore, M.M. Sex Steroid Binding Proteins in the Plasma of the Green Frog, Rana esculenta: Changes during the Reproductive Cycle and Dependence on Pituitary Gland and Gonads. Gen. Comp. Endocrinol. 1994, 96, 401–411. [CrossRef]

12. Morris, J.A.; Jordan, C.L.; Breedlove, S.M. Sexual differentiation of the vertebrate nervous system. Nat. Neurosci. 2004, 7, 1034–1039. [CrossRef]

13. Vizziano-Cantonnent, D.; Anglade, I.; Pellegrini, E.; Gueguen, M.-M.; Fostier, A.; Guiguen, Y.; Kah, O. Sexual dimorphism in the brain aromatase expression and activity, and in the central expression of other steroidogenic enzymes during the period of sex differentiation in monosex rainbow trout populations. Gen. Comp. Endocrinol. 2011, 170, 346–355. [CrossRef]

14. Dickey, J.T.; Swanson, P. Effects of Salmon Gonadotropin-Releasing Hormone on Follicle Stimulating Hormone Secretion and Subunit Gene Expression in Coho Salmon (Oncorhynchus kisutch). Gen. Comp. Endocrinol. 2000, 118, 436–449. [CrossRef] [PubMed]

15. Von Schalburg, K.R.; Warby, C.M.; Sherwood, N.M. Evidence for Gonadotropin-Releasing Hormone Peptides in the Ovary and Testis of Rainbow Trout. Biol. Reprod. 1999, 60, 1338–1344. [CrossRef] [PubMed]

16. Okuzawa, Z.; Amano, M.; Kobayashi, M.; Aida, K.; Hanyu, I.; Hasegawa, Y.; Miyamoto, K. Differences in salmon GnRH and chicken GnRH-II contents in discrete brain areas of male and female rainbow trout according to age and stage of maturity. Gen. Comp. Endocrinol. 1990, 80, 116–126. [CrossRef]

17. Leatherland, J.F. Reflections on the thyroidology of fishes: From molecules to humankind. Gen. Comp. Endocrinol. 2005, 141, 101–115. [CrossRef]

18. Blanton, M.L.; Specker, J.L. The Hypothalamic-Pituitary-Thyroid (HPT) Axis in Fish and Its Role in Fish Development and Reproduction. Crit. Rev. Toxicol. 2007, 37, 97–115. [CrossRef]

19. Power, D.M.; Llewellyn, L.; Faustino, M.; Nowell, M.A.; Björnsson, B.T.; Einarsdóttir, I.E.; Canario, A.V.; Sweeney, G.E. Thyroid hormones in growth and development of fish. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 2001, 130, 447–459. [CrossRef]

20. Maclatchy, D.L.; Eales, J. Short-term treatment with testosterone increases plasma 3,5,3′-triiodo-l-thyronine and hepatic l-thyroxine 3′-monodeiodinase levels in arctic char, Salvelinus alpinus. Gen. Comp. Endocrinol. 1988, 71, 10–16. [CrossRef]

21. Cyr, D.; Bromage, N.; Duston, J.; Eales, J. Seasonal patterns in serum levels of thyroid hormones and sex steroids in relation to photoperiod-induced changes in spawning time in rainbow trout, Salmo gairdneri. Fish Physiol. Biochem. 2001, 25, 191–364. [CrossRef]

22. Leatherland, J.F. Reflections on the thyroidology of fishes: From molecules to humankind. Guelph Ichthyol. Rev. 1994, 2, 1–67.

23. Power, D.M.; Llewellyn, L.; Faustino, M.; Nowell, M.A.; Björnsson, B.T.; Einarsdóttir, I.E.; Canario, A.V.; Sweeney, G.E. Thyroid hormones in growth and development of fish. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 2001, 130, 447–459. [CrossRef]

24. Devlin, R.H.; Nagahama, Y. Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. Aquaculture. 2002, 208, 191–364. [CrossRef]

25. Wang, D.-D.; Zhang, G.-R.; Wei, K.-J.; Ji, W.; Gardner, J.P.A.; Yang, R.-B.; Chen, K.-C. Molecular identification and expression of chicken GnRH-II contents in discrete brain areas of male and female rainbow trout according to age and stage of maturity. Gen. Comp. Endocrinol. 1990, 80, 116–126. [CrossRef]

26. Herndon, M.K.; Nilson, J.H. Maximal Expression of Foxl2 in Pituitary Gonadotropes Requires Ovarian Hormones. PLoS ONE 2015, 10, e0212652. [CrossRef] [PubMed]

27. Wang, D.S.; Kobayashi, T.; Zhou, L.Y.; Nagahama, Y. Molecular cloning and gene expression of Foxl2 in the Nile tilapia, Oreochromis niloticus. Biochem. Biophys. Res. Commun. 2004, 320, 83–89. [CrossRef]

28. Nagler, J.J.; Cavileer, T.; Sullivan, J.; Cyr, D.G.; Rexroad, C., III. The complete nuclear estrogen receptor family in the rainbow trout: Discovery of the novel ERα2 and both ERβ isoforms. Genes. 2007, 392, 164–173. [CrossRef]

29. Guiguen, Y.; Fostier, A.; Piferrer, F.; Chang, C.-F. Ovarian aromatase and estrogens: A pivotal role for gonadal sex differentiation in northern snakehead Channa argus. Fish Physiol. Biochem. 2015, 41, 1419–1433. [CrossRef]

30. Mu, W.J.; Wen, H.S.; Li, J.F.; He, F. Cloning and expression analysis of Foxl2 during the reproductive cycle in Korean rockfish, Sebastes schlegelii. Fish Physiol. Biochem. 2013, 39, 1419–1430. [CrossRef] [PubMed]

31. Wang, D.-D.; Zhang, G.-R.; Wei, K.-J.; Ji, W.; Gardner, J.P.A.; Yang, R.-B.; Chen, K.-C. Molecular identification and expression of the Foxl2 gene during gonadal sex differentiation in northern snakehead Channa argus. Fish Physiol. Biochem. 2015, 41, 1419–1433. [CrossRef] [PubMed]

32. Wang, D.S.; Kobayashi, T.; Zhou, L.Y.; Nagahama, Y. Molecular cloning and gene expression of Foxl2 in the Nile tilapia, Oreochromis niloticus. Biochem. Biophys. Res. Commun. 2004, 320, 83–89. [CrossRef]

33. Nagler, J.J.; Cavileer, T.; Sullivan, J.; Cyr, D.G.; Rexroad, C., III. The complete nuclear estrogen receptor family in the rainbow trout: Discovery of the novel ERα2 and both ERβ isoforms. Genes. 2007, 392, 164–173. [CrossRef]

34. Guiguen, Y.; Fostier, A.; Piferrer, F.; Chang, C.-F. Ovarian aromatase and estrogens: A pivotal role for gonadal sex differentiation in northern snakehead Channa argus. Fish Physiol. Biochem. 2015, 41, 1419–1433. [CrossRef] [PubMed]

35. Wang, D.S.; Kobayashi, T.; Zhou, L.Y.; Nagahama, Y. Molecular cloning and gene expression of Foxl2 in the Nile tilapia, Oreochromis niloticus. Biochem. Biophys. Res. Commun. 2004, 320, 83–89. [CrossRef]

36. Nagler, J.J.; Cavileer, T.; Sullivan, J.; Cyr, D.G.; Rexroad, C., III. The complete nuclear estrogen receptor family in the rainbow trout: Discovery of the novel ERα2 and both ERβ isoforms. Genes. 2007, 392, 164–173. [CrossRef]

37. Guiguen, Y.; Fostier, A.; Piferrer, F.; Chang, C.-F. Ovarian aromatase and estrogens: A pivotal role for gonadal sex differentiation in northern snakehead Channa argus. Fish Physiol. Biochem. 2015, 41, 1419–1433. [CrossRef] [PubMed]
38. Lamba, V.J.; Goswami, S.V.; Sundararaj, B.I. Circannual and circadian variations in plasma levels of steroids (cortisol, estradiol-17β estrone, and testosterone) correlated with the annual gonadal cycle in the catfish, Heteropneustes fossilis (Bloch). Gen. Comp. Endocrinol. 1983, 50, 205–225. [CrossRef]
39. Wingfield, J.; Grimm, A. Seasonal changes in plasma cortisol, testosterone and oestradiol-17β in the plaice, Pleuronectes platessa L. Gen. Comp. Endocrinol. 1997, 31, 1–11. [CrossRef]
40. Pickering, A.D.; Christie, P. Changes in the concentrations of plasma cortisol and thyroxine during sexual maturation of the hatchery-reared brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 1981, 44, 487–496. [CrossRef]
41. Kusakabe, M.; Nakamura, I.; Young, G. 11β-Hydroxysteroid Dehydrogenase Complementary Deoxyribonucleic Acid in Rainbow Trout: Cloning, Sites of Expression, and Seasonal Changes in Gonads. Endocrinology 2003, 144, 2534–2545. [CrossRef]
42. Shankar, D.S.; Kulkarni, R.S. Tissue cholesterol and serum cortisol level during different reproductive phases of the female freshwater fish Notopterus notopterus (Pallas). J. Environ. Biol. 2007, 28, 137–139. [PubMed]
43. Hou, Y.Y.; Han, X.D.; Suzuki, Y. Annual changes in plasma levels of cortisol and sex steroid hormones in male rainbow trout, Oncorhynchus mykiss. Chin. J. Oceanol. Limnol. 2001, 19, 217–221.
44. Abdollahpour, H.; Falahatkar, B.; Efatpanah, I.; Meknakhah, B.; Van Der Kraak, G. Hormonal and physiological changes in Sterlet sturgeon Acipenser ruthenus treated with thyroxine. Aquaculture 2019, 507, 293–300. [CrossRef]
45. Gorbman, A. Thyroid function and its control in fishes—ScienceDirect. Fish Physiol. 1969, 2, 241–274.
46. Grau, E.G. Environmental Influences on Thyroid Function in Teleost Fish. Am. Zool. 1988, 28, 329–335. [CrossRef]
47. Leatherland, J.F. Endocrine Factors Affecting Thyroid Economy of Teleost Fish. Am. Zool. 1988, 28, 319–328. [CrossRef]
48. Peter, M.S. The role of thyroid hormones in stress response of fish. Gen. Comp. Endocrinol. 2011, 172, 198–210. [CrossRef]
49. Kang, D.-Y.; Chang, Y.J. Effects of maternal injection of 3,5,3'-triiodo-l-thyronine (T3) on growth of newborn offspring of rockfish, Sebastes schlegeli. Aquaculture 2004, 234, 641–655. [CrossRef]
50. Cyr, D.G.; Idler, D.R.; Audet, C.; McLeese, J.M.; Eales, J.G. Effects of Long-Term Temperature Acclimation on Thyroid Hormone Deiodinase Function, Plasma Thyroid Hormone Levels, Growth, and Reproductive Status of Male Atlantic Cod, Gadus morhua. Gen. Comp. Endocrinol. 1998, 109, 24–36. [CrossRef]
51. Pavlidis, M.; Greenwood, L.; Mourot, B.; Kokkari, C.; Le Menn, F.; Divanach, P.; Scott, A. Seasonal Variations and Maturity Stages in Relation to Differences in Serum Levels of Gonadal Steroids, Vitellogenin, and Thyroid Hormones in the Common Dentex (Dentex dentex). Gen. Comp. Endocrinol. 2000, 119, 14–25. [CrossRef]
52. Eales, J. Modes of action and physiological effects of thyroid hormones in fish. Fish Endocrinol. 2006, 2, 767–808.
53. Supriya, A.; Raghuveer, K.; Swapna, I.; Rasheeda, M.K.; Kobayashi, T.; Nagahama, Y.; Gupta, A.D.; Majumdar, K.C.; Senthilkumar, M. Thyroid hormone modulation of ovarian recrudescence of air-breathing catfish Clarias gariepinus. Fish Physiol. Biochem. 2005, 31, 267–270. [CrossRef] [PubMed]
54. Pavlidis, M.; Dessypris, A.; Christofidis, I. Seasonal fluctuations in plasma thyroid hormones, in two strains of rainbow trout (Oncorhynchus mykiss), during the first and second reproductive cycle: Relation with their photoperiodically altered spawning time. Aquaculture 1991, 99, 365–385. [CrossRef]
55. Miller, R.P. GnRHs and GnRH receptors. Anim. Reprod. Sci. 2005, 88, 5–28. [CrossRef]
56. Sherwood, N.M.; Lovejoy, D.A.; Coe, I.R. Origin of mammalian gonadotropin-releasing hormones. Endocr. Rev. 1993, 14, 241–254. [CrossRef] [PubMed]
57. Yaron, Z.; Levavi-Sivan, B. Endocrine regulation of fish reproduction. Environ. Physiol. Gen. Environ. 2011, 2, 1500–1508.
58. Nyuji, M.; Kazeto, Y.; Izumida, D.; Tani, K.; Suzuki, H.; Hamada, K.; Mekuchi, M.; Gen, K.; Soyano, K.; Okuzawa, K. Greater amberjack Fish, Lh, and their receptors: Plasma and mRNA profiles during ovarian development. Gen. Comp. Endocrinol. 2016, 225, 224–234. [CrossRef] [PubMed]
59. Pavlidis, M.; Dessypris, A.; Christofidis, I. Seasonal fluctuations in plasma thyroid hormones, in two strains of rainbow trout (Oncorhynchus mykiss), during the first and second reproductive cycle: Relation with their photoperiodically altered spawning time. Aquaculture 1991, 99, 365–385. [CrossRef] [PubMed]
60. Tchoudakova, A.; Kishida, M.; Wood, E.; Callard, G.V. Promoter characteristics of two cyp19 genes differentially expressed in the brain and ovary of teleost fish. J. Steroid Biochem. Mol. Biol. 1998, 78, 427–439. [CrossRef]
61. Chiang, E.F.L.; Yan, Y.L.; Tong, S.K.; Hsiao, P.H.; Guiguen, Y.; Postlethwait, J.; Chung, B.C. Characterization of duplicated zebrafish cyp19 genes. J. Exp. Zool. 2001, 290, 709–714. [CrossRef]
62. Baron, D.; Cocquet, J.; Xia, X.; Fellous, M.; Guiguen, Y.; Veitia, R.A. An evolutionary and functional analysis of FoxL2 in rainbow trout gonad differentiation. J. Mol. Endocrinol. 2004, 33, 705–715. [CrossRef] [PubMed]
63. Wang, D.-S.; Kobayashi, T.; Zhou, L.-Y.; Paul-Prasanth, B.; Iijiri, S.; Sakai, F.; Okubo, K.; Morohashi, K.-I.; Nagahama, Y. FoxL2 Up-Regulates Aromatase Gene Transcription in a Female-Specific Manner by Binding to the Promoter as Well as Interacting with Ad4 Binding Protein/ Steroidogenic Factor 1. Mol. Endocrinol. 2007, 21, 712–725. [CrossRef] [PubMed]
64. Liu, H.; Mu, X.; Gui, L.; Su, M.; Li, H.; Zhang, G.; Liu, Z.; Zhang, J. Characterization and gonadal expression of FOXL2 relative to Cyp19a genes in spotted scat Scatophagus argus. Genes 2015, 561, 6–14. [CrossRef] [PubMed]
65. Tohyama, S.; Miyagawa, S.; Lange, A.; Ogino, Y.; Mizutani, T.; Tatarazako, N.; Katsu, Y.; Ibara, M.; Tanaka, H.; Ishibashi, H.; et al. Understanding the Molecular Basis for Differences in Responses of Fish Estrogen Receptor Subtypes to Environmental Estrogens. Environ. Sci. Technol. 2015, 49, 7439–7447. [CrossRef]
66. Griffin, L.B.; January, K.E.; Ho, K.W.; Cotter, K.; Callard, G.V. Morpholino-Mediated Knockdown of ERα, ERβa, and ERβb mRNAs in Zebrafish (Danio rerio) Embryos Reveals Differential Regulation of Estrogen-Inducible Genes. *Endocrinology* 2013, 154, 4158–4169. [CrossRef]

67. Nelson, E.; Habibi, H.R. Functional Significance of Nuclear Estrogen Receptor Subtypes in the Liver of Goldfish. *Endocrinology* 2010, 151, 1668–1676. [CrossRef]