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Research Article

MicroRNA Dynamics in Gonadal Development and Reproductive Regulation of Striped Murrel, *Channa striata* (Bloch, 1793)

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MicroRNAs (miRNAs) are essential regulators of gene expression, influencing a wide range of biological processes, including reproduction and gonadal development. This study investigated the expression patterns of various miRNAs in the gonads of *Channa striata* across different reproductive stages: prespawning, spawning, and postspawning. The Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI) showed significant seasonal variations, with GSI peaking during the spawning season and HSI reaching its highest value in the prespawning period, reflecting reproductive preparation. Our findings highlight the distinct roles of several miRNAs in regulating reproductive processes. The miR-21 was upregulated during spawning in both sexes, indicating its key role in gametogenesis, with persistent expression in males through postspawning, suggesting a role in sperm quality maintenance. MiR-22 also showed upregulation during spawning, supporting its involvement in gamete maturation and steroidogenesis, with reduced expression postspawning. MiR-34a was notably upregulated in male gonads postspawning, highlighting its role in spermatogenic recovery and sperm motility regulation. The miR-133 displayed complex patterns: In females, it was downregulated during spawning and upregulated postspawning, while in males, it was upregulated during spawning but downregulated afterward, highlighting its role in reproductive and metabolic processes. In addition, miR-200 was upregulated during spawning and postspawning in both sexes, underscoring its role in gamete maturation. Lastly, the miR-202-5p was consistent during prespawning and spawning in females but downregulated postspawning, with increased expression during spawning and postspawning in males, indicating its role in sperm maturation. These findings underscore a complex network of miRNAs that regulate gonadal development and reproductive success in *C. striata*.

Keywords: aquaculture; *C. striata*; fish reproduction; microRNA

1. Introduction

MicroRNAs (miRNAs) are small noncoding RNA molecules, typically comprising 18–26 nucleotides, which play a crucial role in regulating gene expression [1]. These molecules are found across a wide range of organisms, from unicellular entities and viruses to complex plants and animals [2]. MiRNAs modulate messenger RNAs (mRNAs) through complementary base pairing, leading to either degradation or translational repression. Their discovery and functional characterizations have significantly advanced our understanding of regulatory mechanisms in

both plants and animals. MiRNAs regulate approximately 30–70% of genes [3] and have the ability to target hundreds of mRNAs, with multiple binding sites for different or identical miRNAs [4]. Research has demonstrated that certain miRNAs are involved in regulating gametogenesis, gonadal development, organ development, fat metabolism, cell proliferation, apoptosis, cell differentiation, signal transduction, adaptive immune responses, and various diseases [5, 6]. The expression of miRNAs is often tissue-specific and highly conserved across different organisms, making comparative genomics a logical approach for identifying novel orthologues [7].

Recent advancements have expanded the identification of miRNAs, facilitating the analysis of miRNA profiles in both model and nonmodel fish species. MiRNAs are cataloged in databases such as https://www.miRBase.org, which provides sequences, targets, and nomenclature for these molecules [8]. In fish, miRNAs have been linked to physiological processes such as growth and reproduction. For example, in the common carp (*Cyprinus carpio*), small RNA deep sequencing revealed 150 unique and differentially expressed miRNAs during critical stages of ovarian development, including primordial gonads, juvenile stages, and adult ovaries [9]. Similarly, in the gonads of the spotted knifejaw (*Oplegnathus punctatus*), 247 conserved and 41 novel miRNAs were identified, with 36 differentially expressed miRNAs regulating sex determination and gonadal development in males [10].

A recent study using small RNA-seq in the Chinese giant salamander (*Andrias davidianus*) identified 374 miRNAs, including nine sex-biased miRNAs involved in early gonadal differentiation, offering valuable insight into sex-specific regulatory networks [11]. These findings underscore the significance of miRNA profiling during critical reproductive phases. Similarly, in fish, miRNAs have been associated with gametogenesis and gonadal development, as demonstrated in common carp and spotted knifejaw [9, 10]. However, no studies on miRNAs in *Channa striata* are currently available in miRBase.

C. striata (Bloch, 1793), known as snakehead murrel, is one of the most important food fish in India, Thailand, Vietnam, Malaysia, and the Philippines [12, 13]. It has great consumer preference due to its taste, high nutritional value, and medicinal qualities. Stripped murrel is considered a high-value food fish and is marketed in live condition [14, 15]. This fish survives in adverse water conditions. Thus, it could be a suitable species for freshwater aquaculture [13]. Nowadays, the natural breeding grounds of this commercially important species have been damaged due to increasing anthropogenic activities. The unavailability of quality seed is a major constraint in the murrel farming. Thus, earlier attempts were made to synchronize the murrel brooders for better maturation using HCG pellets and implanted intramuscular (500–1000 IU/kg body weight of fish) on dorsal side of fish [16]. However, asynchronous maturation of those species during breeding season creates a serious problem in seed production. Therefore, researchers are putting efforts to understand the mechanism of reproduction of this commercially important species. Molecular approaches, such as identifying and validating reproductive-related miRNAs, could provide valuable insights into the molecular mechanisms underlying maturation and spawning in nonmodel food fish like *C. striata*.

Thus, this study will analyze the growth biology during reproductive period, as well as the expression of selected miRNAs in the gonad, liver, and brain tissues of both male and female *C. striata*.

2. Materials and Methods

2.1. Mining of Conserved miRNAs. miRBase (https://microrna.sanger.ac.uk) is a publicly accessible database containing miRNA sequences and annotations from published studies, including precursor and mature miRNAs for 16 fish species (Version 22.1, October 2020). To identify conserved miRNAs, sequences, transcriptome, and genome of *Channa* spp. available in the NCBI database were compared against the miRBase. Reproduction-related miRNAs, previously reported, were analyzed for complementary sites in key genes. The identification process involved two steps: First, mature miRNA sequences were used to search for hits in the transcriptome using NCBI BLAST (2.7.1+), selecting mRNA sequences with over 75% query coverage. These potential targets were then confirmed using the RNAhybrid tool [17].

2.2. Experiment and Sampling. Brooders of snakehead murrel (*Channa striata*), weighing between 450 and 530 g, were obtained from the murrel hatchery of ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar (20.1839° N, 85.8512° E). The brooders administered intramuscular HCG pellets (1500–2000 IU/kg of fish body weight) based on previous induced breeding methods by [17]. Gonads from 3 male and 3 female fish were dissected during prespawning (April), spawning (August), and postspawning (October) seasons and preserved in RNAlater (Qiagen, USA). Measurements of length, weight, and gonad weight were recorded, and the Gonadosomatic Index (GSI) was calculated after anesthetizing the fish using MS-222 (100 mg/L, Sigma, USA).

2.3. Analysis of Growth Parameters and Reproductive Indices. Length, weight, gonad, and liver metrics, including the GSI and Hepatosomatic Index (HSI), were analyzed for *C. striata*. These indices are essential for assessing reproductive performance [18] and were calculated across prespawning, spawning, and postspawning stages using the following formulas:

$$\text{GSI} = \frac{\text{Weight of ovary}}{\text{Weight of fish}} \times 100, \quad (1)$$

$$\text{HSI} = \frac{\text{Weight of liver}}{\text{Weight of fish}} \times 100. \quad (2)$$

2.4. Isolation of RNA and cDNA Synthesis. Total RNA was extracted using the miRNeasy Kit (QIAGEN, Germany) according to the manufacturer's instructions. RNA quality and quantity were assessed using a Nanodrop spectrophotometer (Thermo Scientific, USA), and integrity was verified through agarose gel electrophoresis. cDNA synthesis was performed using the miScript II RT kit (QIAGEN, Hilden, Germany), and cDNA quality was validated using housekeeping genes.

2.5. Quantitative Real-Time PCR. Primers for mature miRNA sequences were designed (Table 1). The cDNA libraries were generated by reverse transcription (RT), and real-time PCR amplification was conducted for the quantification of target miRNAs using the Applied Biosystems ABI 7500 PCR machine (USA). The PCR protocol included initial denaturation at 95°C for 10 min, followed by denaturation at 95°C for 10 s, annealing at 57°C for 20 s, and extension at 72°C for 15 s. The U6 gene was used as an internal control for normalizing miRNA expression levels. Each sample was tested in triplicate to ensure reliability. Gel electrophoresis and melt curve analyses confirmed the specificity of the amplifications.

2.6. Statistical Analysis. Gene expression levels were evaluated using the $2^{-\Delta\Delta Ct}$ method. Statistical analysis was performed in R Studio, employing one-way ANOVA followed by Duncan's multiple range test. A significance level of $p < 0.05$ was considered statistically significant, with results reported as means ± standard error of the mean.

Table 1: Primers used for qPCR of miRNAs.

S. no.	Primer	Sequence
1	U6	CTCGCTTCGGCAGCACA
2	miR-200b-5p	TAATACTGCCTGGTAATGAT
3	miR-22	AAGCTGCCAGCTGAAGAACT
4	miR-34a	TGGCAGTGTCTTAGCTGG TTG
5	miR-202-5p	TTCCTATGCATATACCTCTTTG
6	miR-21	TAGCTTATCAGACTGGTGTTGGC
7	miR-133b-5p	GCTGGTCAAATGGAACCAAGTC

3. Result

3.1. Analysis of Growth Parameters and Reproductive Indices. The mature male and female brooders were selected and the gonad weight peaked during the spawning season, with subsequent decreases in the prespawning and postspawning seasons for both sexes. This trend was consistent with variations observed in both the GSI and HSI values (Figure 1). Specifically, gonad weight reached its maximum during the spawning season, whereas liver weight was also highest during the prespawning season when compared to the spawning and postspawning periods (Figure 2).

3.2. Expression Analysis of Selected miRNAs. The expression profile of selected miRNAs was carried out, which includes miR-34a, miR-202-5p, miR-21, miR-133b, miR-22, and miR-200. U6 was used as the internal reference gene. All the selected miRNAs showed differential expression in the brain, liver, and gonadal tissues of murrel during prespawning, spawning, and postspawning stages (Figures 3, 4, 5, 6, 7, 8). The amplification of respective miRNAs was checked using the melt curve and amplification profile during RT-PCR.

4. Discussion

In this study, we selected striped murrel (*C. striata*) individuals with body weights of 450 g in males and 530 g in females during the prespawning season. These weights correspond to the mature size range of 300–600 g for females and 400–800 g for males [19]. During the breeding season, the GSI for females ranged from 3.91 to 4.31, while for males, it ranged from 0.15 to 0.20. Our findings indicate the highest GSI values during the spawning season, consistent with the body weights observed in males (450 g) and females (530 g) as reported by Kumar et al. [19], thereby aligning with the results of our study.

This trend is further consistent with variations in the GSI and HSI values (Figure 1). Specifically, peak gonad weight was recorded during the spawning season, while the highest liver weight was noted during the prespawning period (Figure 2). These observations highlight a significant correlation between reproductive stages and physiological characteristics in *C. striata*. The increase in GSI during spawning reflects enhanced reproductive activity, whereas the elevated HSI during the prespawning period suggests a preparatory phase for reproductive investment.

Furthermore, the GSI values recorded in our study are consistent with those reported for the peak breeding season [19], suggesting that the fish were sampled at an optimal time to examine miRNA expression. This alignment supports the reliability of our findings regarding the reproductive physiology of *C. striata* and its correlation with miRNA expressions.

Most of the research on miRNAs has concentrated on their involvement in regulating a wide array of physiological processes [20]. The availability of high-throughput sequencing data has enabled mining of conserved miRNAs, and efforts are on to make the bioinformatics analysis of the predicted precursor miRNAs more robust and reliable [16]. In this work, earlier reported conserved miRNAs were taken up for analysis using the RNAhybrid tool and dG value of 5′ termini to make the prediction more robust. By leveraging Watson–Crick complementarity in the seed region between the miRNA and the target region of the gene [1], six miRNAs—miR-21, miR-22, miR-34a, miR-133b, miR-200, and miR-202-5p—were selected based on their strong binding affinity to target genes involved in reproduction, as confirmed by RNAhybrid analysis. These miRNAs have also been previously reported in teleosts to regulate key reproductive processes such as gametogenesis, steroidogenesis, and gonadal maturation. Their evolutionary conservation and functional roles in fish biology justified their selection for expression profiling in *C. striata*.

The identified miRNAs were utilized to analyze their expression patterns during different gonadal stages (prespawning,

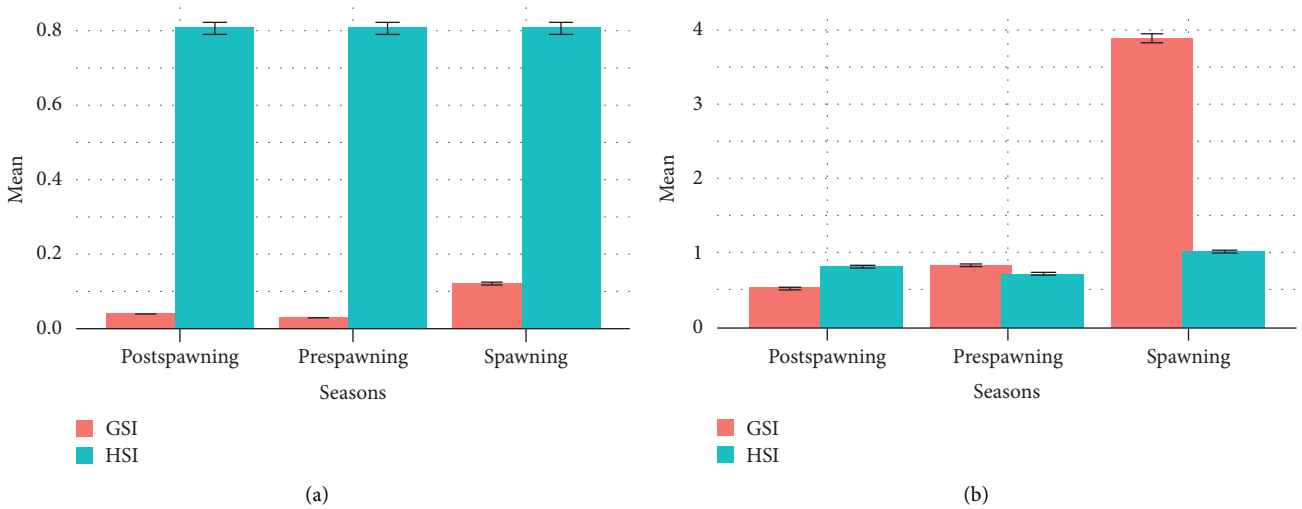

Figure 1: Mean values of Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI) in *C. striata* during different reproductive stages (postspawning, prespawning, and spawning), with standard error bars indicating variability: (a) male; (b) female.

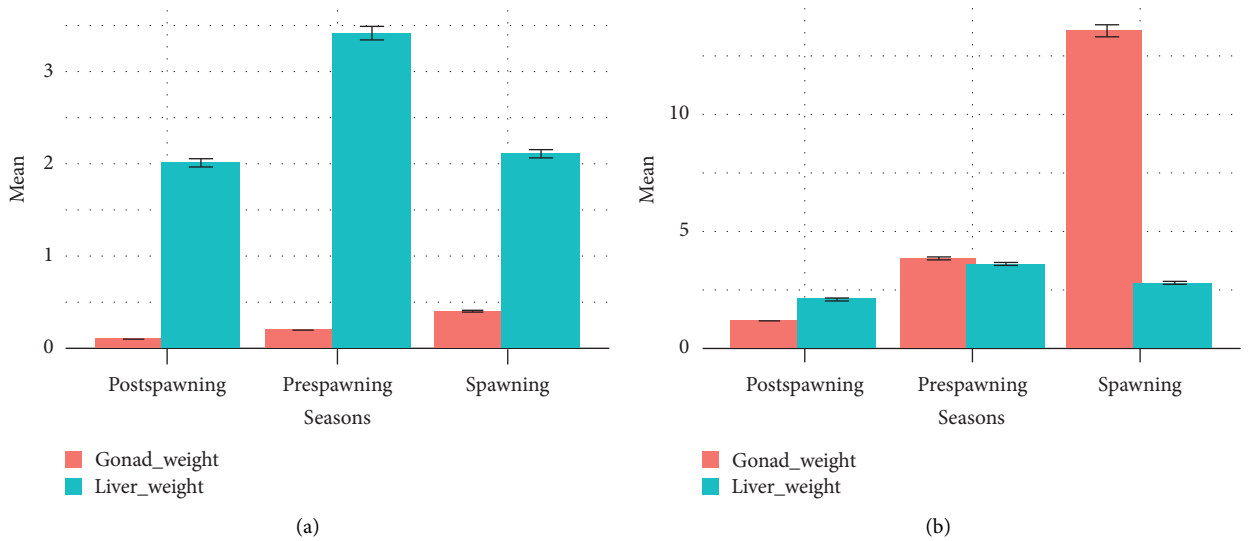

Figure 2: Mean values of gonad weight and liver weight in *C. striata* during different reproductive stages (postspawning, prespawning, and spawning), with standard error bars indicating variability: (a) male; (b) female.

spawning, and postspawning) in the gonadal tissues of both male and female *C. striata*. The miR-21 is widely expressed in the ovary tissues of numerous fish species, such as common carp (*C. carpio*) [9], zebrafish (*Danio rerio*) [21], Yellowfin Seabream (*Acanthopagrus latus*) [22], Medaka (*Oryzias latipes*) [23], *Oryzias melastigma* [24], and Olive flounder (*Paralichthys olivaceus*) [25]. Its expression is particularly high in the semen of *Salmo salar*, indicating a significant role in reproductive processes [26].

In our study (Figure 3), miR-21 expression in *C. striata* varied across different tissues and reproductive stages. In male gonads, miR-21 was highly expressed during the spawning and postspawning stages compared to the prespawning stage, suggesting a crucial role in spermatogenesis. This miRNA might regulate genes involved in cell proliferation, apoptosis, and differentiation of spermatogonial cells—processes essential for producing viable sperm. The abundant expression of miR-21 in the semen of *S. salar* [26] further supports its involvement in ensuring sperm quality and fertility.

In female gonads, miR-21 expression was upregulated during the spawning stage but downregulated in the postspawning stage. In other species, high levels of miR-21 in ovarian tissues are associated with follicular development, oocyte maturation, and overall ovarian function [27]. For *C. striata*, the upregulation of miR-21 during spawning suggests it may be a key regulator in these processes, promoting successful reproduction.

In liver tissues, miR-21 showed downregulation during both the spawning and postspawning stages in both sexes. This pattern suggests that miR-21 might be involved in preparing the liver for the reproductive activities during

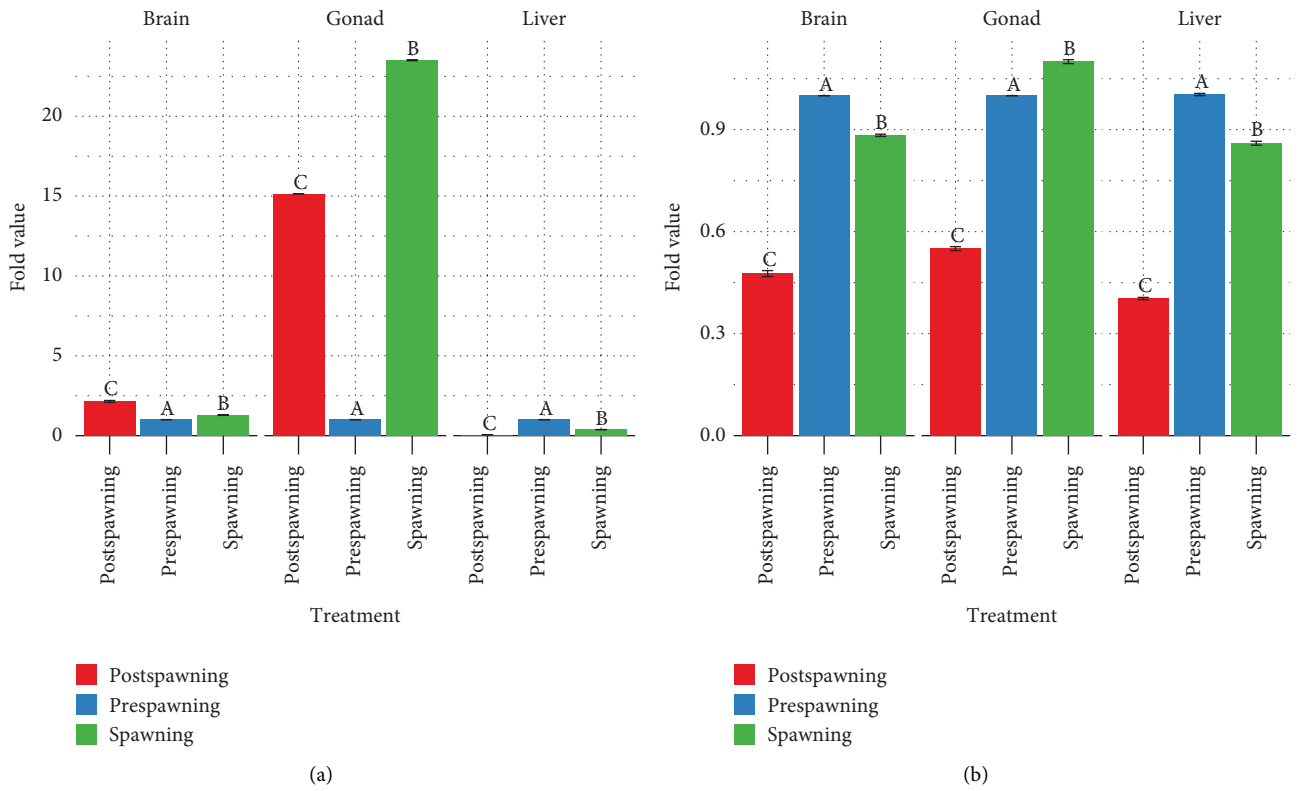

FIGURE 3: Relative expression of miR-21 in *C. striata* in prespawning, spawning, and postspawning ((a) male and (b) female). Values are presented as mean ± standard error ($n = 3$). Statistical significance ($p < 0.05$) is indicated by different letters, determined using ANOVA followed by Tukey's post hoc test.

spawning and its subsequent recovery after the reproductive phase. In brain tissues, miR-21 was downregulated during the spawning and postspawning stages compared to the prespawning stage. This expression pattern indicates a potential role for miR-21 in regulating neuroendocrine signals that control reproductive processes in *C. striata*.

MiR-22 has been recognized as a key regulator in gonadal development across various teleost species, including *C. carpio* [9], *Trachinotus ovatus* [28], *Oryzias latipes* [23], and *Danio rerio* [29]. It has been demonstrated to regulate estrogen receptor 6 (*esr6*), a crucial transcription factor for reproductive system maturation in mammals [30]. This regulation is essential for proper gonadal development and function, affecting processes such as oocyte maturation and spermatogenesis.

In this study, miR-22 expression patterns were analyzed across different tissues and reproductive stages in *C. striata*. In the gonads, miR-22 was upregulated during the spawning stage in both male and female fish, indicating its involvement in active reproductive processes. This upregulation suggests that miR-22 may play a role in the maturation of gametes, potentially by regulating genes associated with steroidogenesis and germ cell development. In the postspawning stage, miR-22 expression was downregulated in both sexes, reflecting a decreased need for active gonadal function as the reproductive season ends.

Additionally, miR-22 expression in liver and brain tissues was assessed (Figure 4). In the liver, miR-22 showed upregulation during the prespawning stage, which could be linked to preparatory processes for reproduction, such as vitellogenin synthesis. During the spawning stage, miR-22 exhibited intermediate expression levels, while in the postspawning stage, it was downregulated. This pattern suggests a role in managing the liver's metabolic and synthetic functions in response to reproductive demands.

In brain tissues, miR-22 was upregulated during the spawning stage and downregulated in the postspawning stage in both males and females. This expression profile implies that miR-22 may be involved in neuroendocrine regulation during reproduction, potentially influencing behaviors and physiological processes related to spawning. The upregulation during spawning could be associated with the coordination of reproductive activities, while the downregulation in the postspawning stage may indicate a return to nonreproductive physiological states.

Recently, noncoding RNAs such as miRNAs are gaining attention for their role in spermatogenesis [31]. The miR-34 family is known to regulate various aspects of spermatogenesis in fish [32]. In silico target gene prediction using TargetScan Fish (release 6.2) identified *gsk3a* (glycogen synthase kinase-3a) as a target of miR-34a. *Gsk3a* is implicated in regulating sperm motility, a crucial factor in successful fertilization [33].

The function of miR-34a in spermatogenesis has been explored using miR-34a-knockout zebrafish, generated through the CRISPR/Cas9 system [32]. Contrary to

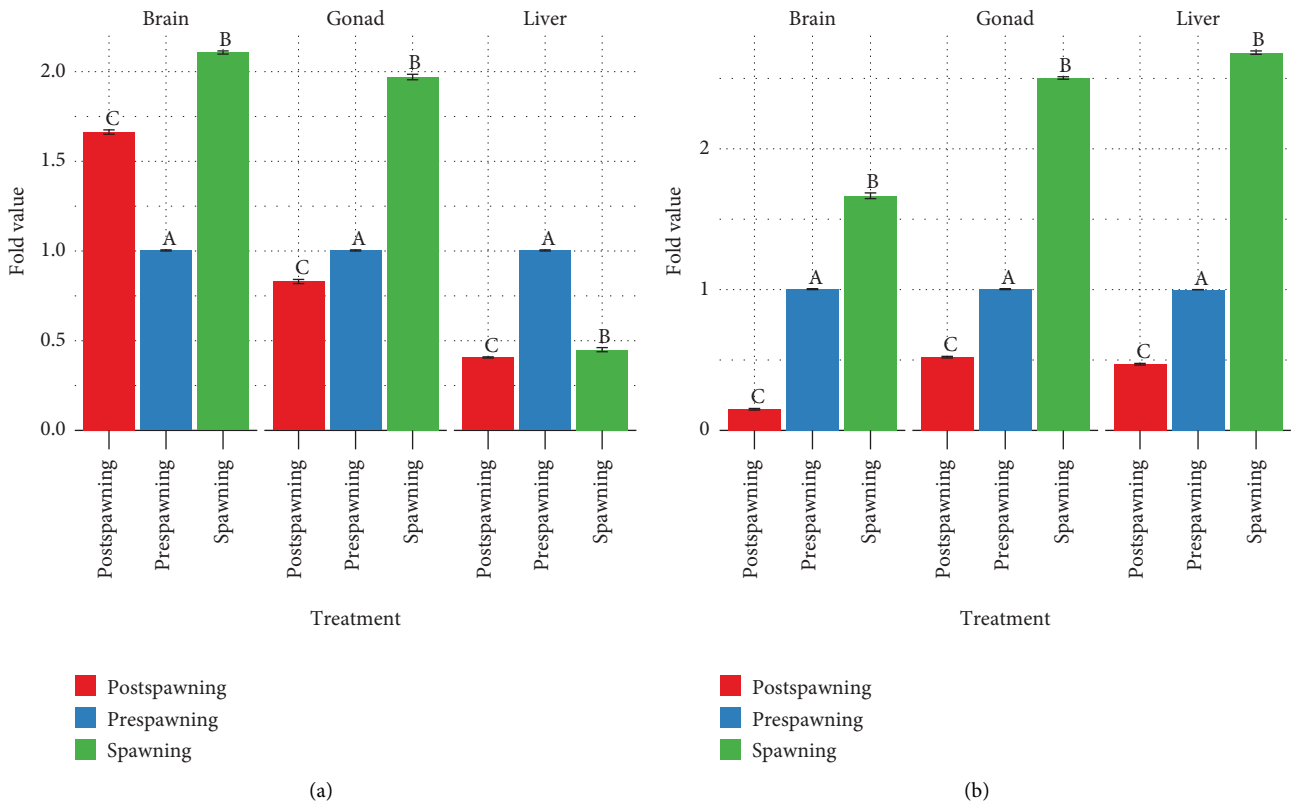

FIGURE 4: Relative expression of miR-22 in *C. striata* in prespawning, spawning, and postspawning ((a) male and (b) female). Values are presented as mean ± standard error ($n = 3$). Statistical significance ($p < 0.05$) is indicated by different letters, determined using ANOVA followed by Tukey's post hoc test.

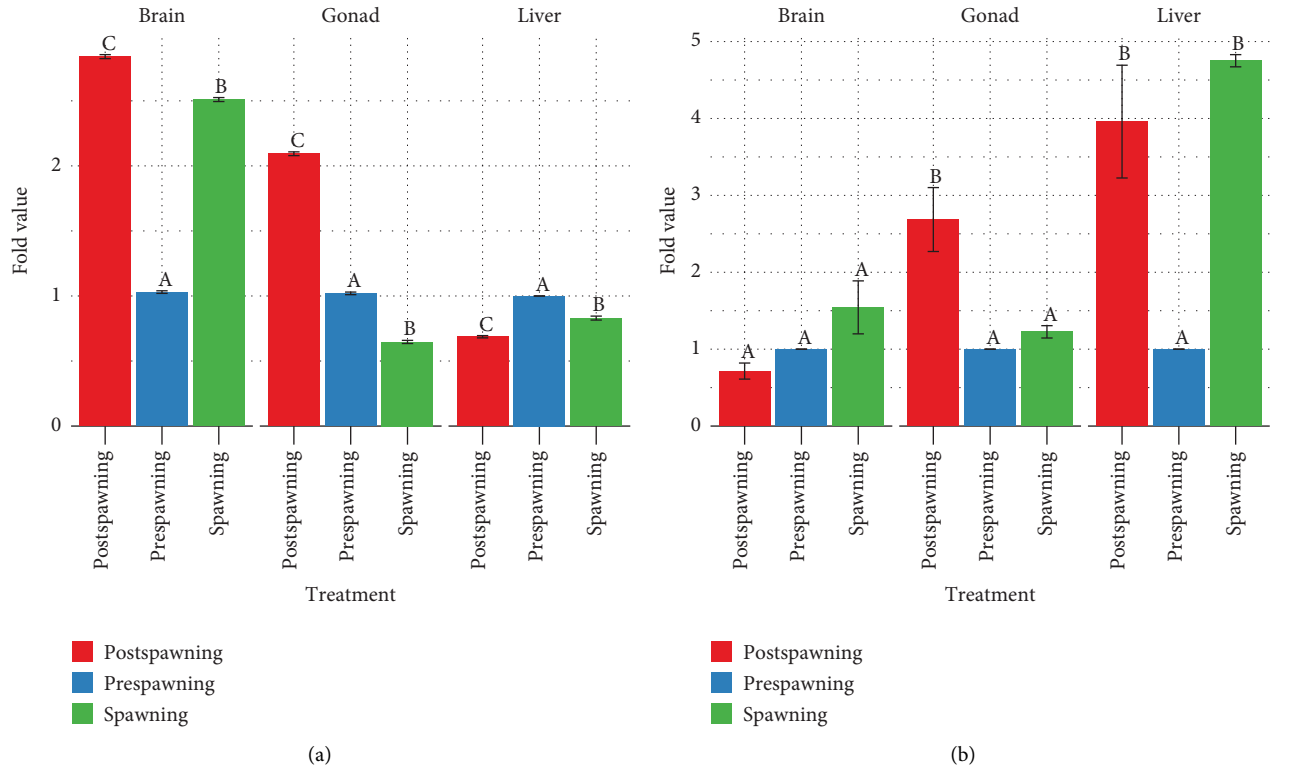

FIGURE 5: Relative expression of miR-34a in *C. striata* in prespawning, spawning, and postspawning ((a) male and (b) female). Values are presented as mean ± standard error ($n = 3$). Statistical significance ($p < 0.05$) is indicated by different letters, determined using ANOVA followed by Tukey's post hoc test.

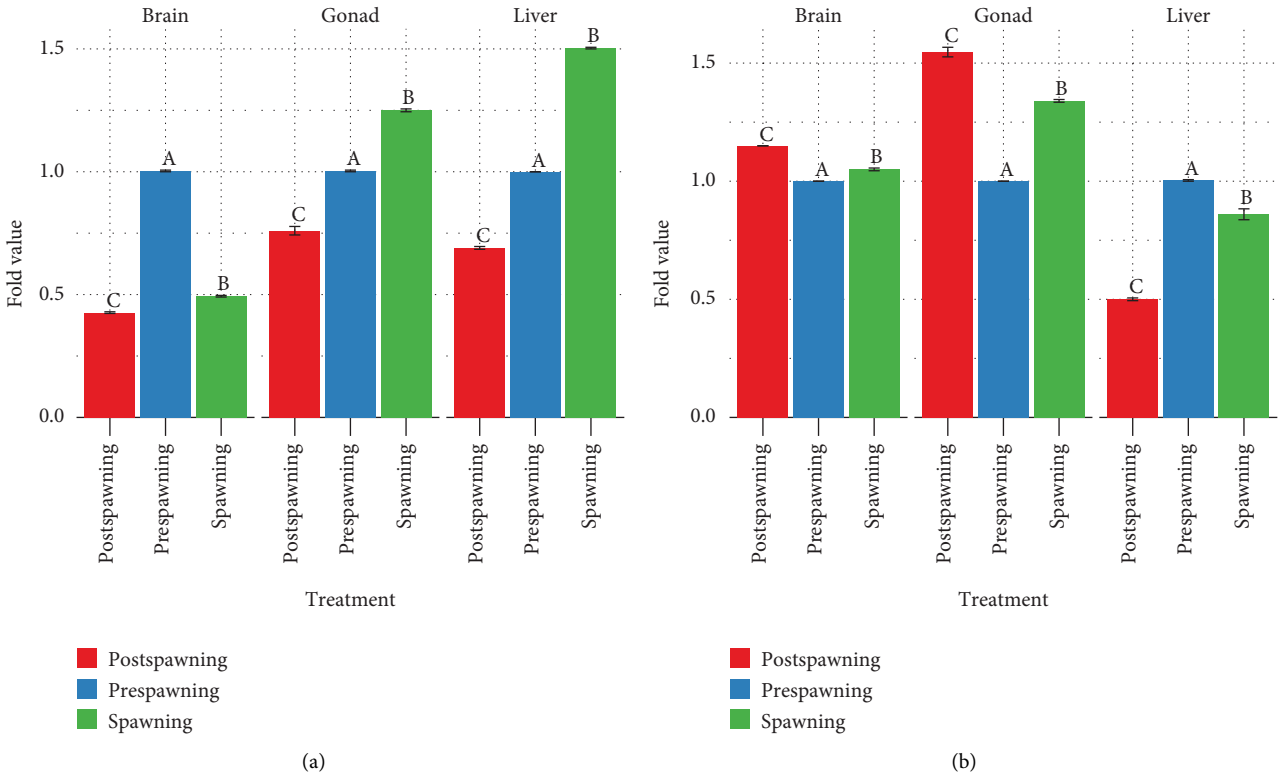

FIGURE 6: Relative expression of miR-133 in *C. striata* in prespawning, spawning, and postspawning ((a) male and (b) female). Values are presented as mean ± standard error ($n = 3$). Statistical significance ($p < 0.05$) is indicated by different letters, determined using ANOVA followed by Tukey's post hoc test.

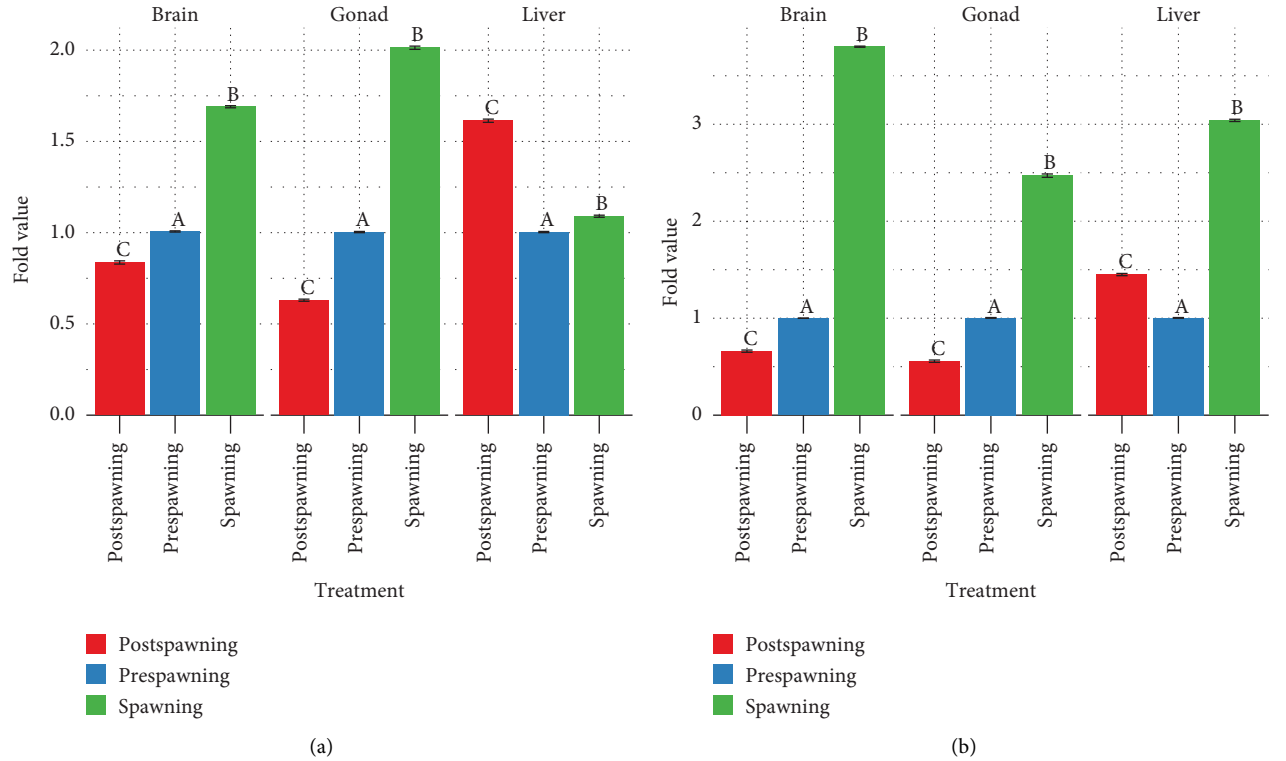

FIGURE 7: Relative expression of miR-200 in *C. striata* in prespawning, spawning, and postspawning ((a) male and (b) female). Values are presented as mean ± standard error ($n = 3$). Statistical significance ($p < 0.05$) is indicated by different letters, determined using ANOVA followed by Tukey's post hoc test.

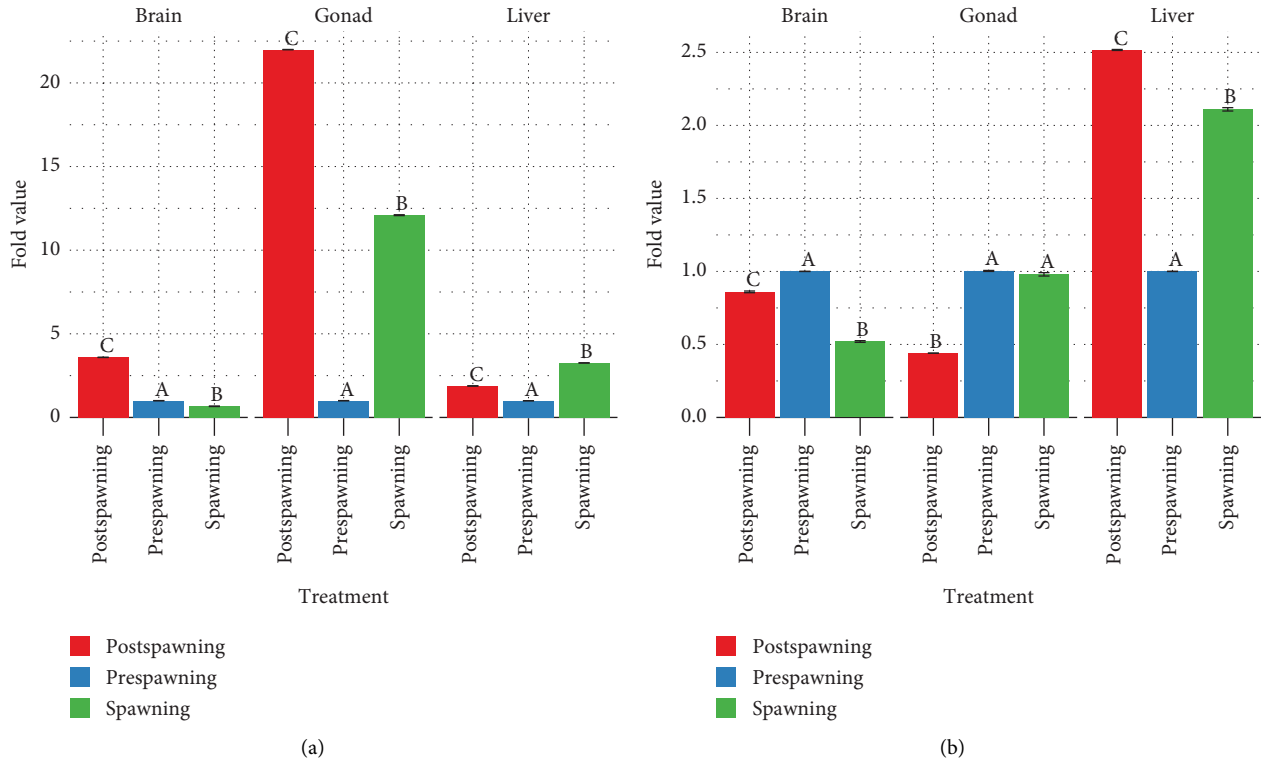

FIGURE 8: Relative expression of miR-202 in *C. striata* in prespawning, spawning, and postspawning ((a) male and (b) female). Values are presented as mean ± standard error ($n = 3$). Statistical significance ($p < 0.05$) is indicated by different letters, determined using ANOVA followed by Tukey's post hoc test.

expectations, miR-34a-knockout zebrafish exhibited no significant defects in testis morphology or sperm quantity. However, a notable increase in progressive sperm motility was observed, which is a critical factor influencing in vitro fertilization rates. Breeding experiments further revealed that miR-34a-knockout male zebrafish had a higher fertilization rate compared to wild-type males when mated with wild-type females. These findings suggest that miR-34a plays a regulatory role in sperm motility.

One of the key targets of miR-34a identified in these studies is glycogen synthase kinase-3a (*gsk3a*), a gene implicated in the regulation of sperm motility. Luciferase reporter assays demonstrated that miR-34a downregulates gsk3a by targeting its 3′ untranslated region, thereby modulating sperm motility. This interaction between miR-34a and *gsk3a* suggests a regulatory mechanism through which miR-34a influences reproductive outcomes in zebrafish.

These findings are consistent with our study's observation of miR-34a upregulation during the postspawning season in male *C. striata* gonads (Figure 5). The increased expression of miR-34a in the postspawning phase may indicate a role in the recovery and maintenance of spermatogenic processes after peaking reproductive activity. By targeting genes such as gsk3a, miR-34a likely contributes to the modulation of sperm motility and maturation, crucial for successful fertilization.

Moreover, the broader implications of miR-34a′s regulatory functions extend beyond gsk3a. Other genes essential for spermatogenesis, such as *crem* and *spag6*, are also potential targets of miR-34a [32]. Crem is involved in the transcriptional regulation of spermatogenesis, while *spag6* is associated with sperm flagellum formation, indicating a comprehensive role of miR-34a in fine-tuning spermatogenic processes.

In addition to its role in the gonads, miR-34a was downregulated during both the spawning and postspawning stages in liver tissues of both males and females. This downregulation might reflect a decrease in metabolic activities associated with reproductive effort. Conversely, in the brain, miR-34a was upregulated during both the spawning and postspawning stages in both males and females. This upregulation could indicate a role in neuroendocrine regulation, possibly influencing reproductive behaviors or stress responses related to reproductive events.

MiR-133 has been identified as an important regulator in various teleost species, including *S. salar* [7], and has been shown to influence estrogen synthesis in mice [34]. In tilapia, miR-133b targets *tagln2*, a crucial factor in the early stages of oogenesis [34]. Its role in gonadal development has also been reported in species such as *C. carpio* and *Trachinotus ovatus* [9, 28].

In our study, miR-133 exhibited distinct expression patterns in the gonadal and nongonadal tissues of *C. striata* (Figure 6). In the ovary, miR-133 was upregulated during the spawning and postspawning stages, suggesting a role in supporting oogenesis and estrogen synthesis in females. In contrast, in the male gonad, miR-133 was upregulated

during spawning but downregulated in the postspawning stage, indicating a potentially different regulatory mechanism or function in spermatogenesis or testicular maintenance.

In liver tissues, the expression of miR-133 displayed a sex-specific pattern. It was downregulated during the spawning stage in females, possibly reflecting the liver's role in reducing the synthesis of vitellogenin and other reproductive proteins after peaking reproductive activity. In males, miR-133 was upregulated during spawning, potentially indicating a role in managing energy and metabolic processes related to spermatogenesis. Both sexes showed downregulation in the postspawning stage, which might be associated with a shift in metabolic focus after the reproductive period.

In brain tissues, miR-133 was downregulated during both the spawning and postspawning stages in males, while it was upregulated in females during these stages. This differential expression suggests that miR-133 could be involved in modulating neuroendocrine functions related to reproductive behavior or stress response, which may vary between sexes.

The role of miR-200 in regulating reproductive processes has been highlighted in various studies, including its confirmed function in controlling sperm motility in zebrafish using CRISPR/Cas9 technology [35]. Moreover, knockout studies on miR-200 located on chromosome 23 in zebrafish revealed impaired spawning, further underscoring its critical role in reproduction [36].

In our study (Figure 7), miR-200 expression was upregulated in the testes during the spawning and postspawning stages in *C. striata*, indicating its involvement in spermatogenesis and possibly in the regulation of sperm motility. The similar upregulation observed in the ovaries during the spawning stage suggests a role in oocyte maturation or other ovarian functions critical for reproduction. This is consistent with the role of miR-200 in regulating reproductive processes, potentially influencing the expression of genes involved in gamete maturation and fertilization.

In liver tissues, miR-200 was upregulated during both the spawning and postspawning stages, suggesting that it may play a role in preparing the organism for the metabolic demands of reproduction and the recovery phase that follows. The liver's role in synthesizing essential proteins, such as vitellogenin, which are critical for egg development, may be influenced by miR-200. In brain tissues, miR-200 exhibited upregulation during the spawning stage and downregulation postspawning. This pattern indicates a possible role in the neuroendocrine regulation of reproductive behaviors and physiological responses.

The miR-202-5p has emerged as a significant regulator in the reproductive processes of fish, influencing critical stages such as late vitellogenesis [2] and overall fecundity [37]. Additionally, miR-202 has been widely observed in the testes and ovaries of various fish species, including Olive flounder (*Paralichthys olivaceus*), Rainbow trout, Nile Tilapia, medaka, and zebrafish [21, 25, 29, 37]. For instance, miR-202-5p has been shown to modulate ovarian tissues during late vitellogenesis in fish [2]. In female medaka (*Oryzias latipes*), miR-202-5p controls fecundity, a critical aspect of reproductive success.

This was evidenced by studies involving miR-202 knockout medaka generated using CRISPR/Cas9 technology. The knockout medaka exhibited a significant reduction or complete absence of egg production, and the remaining eggs were nonfertilizable, thereby preventing successful reproduction [37]. This profound effect was attributed to the downregulation of several key genes essential for folliculogenesis, including *cyp19a1a*, *star*, *cyp17*, *inha*, *foxl3*, *foxl2b*, and *gsdf*, that play crucial roles in steroidogenesis and the development of ovarian follicles, highlighting the central role of miR-202-5p in regulating these pathways [37, 38]. The downregulation of such key genes in miR-202 knockout models underscores the miRNA's importance in ensuring the proper maturation and function of gonadal tissues.

In our study (Figure 8), the expression of miR-202-5p in female *C. striata* was nearly equal during the spawning stage compared to the prespawning stage, with a noticeable downregulation observed during the postspawning stage. This expression pattern aligns with previous findings that highlight the role of miR-202-5p in modulating ovarian tissues during late vitellogenesis. For example, Juanchich et al. [2] reported that miR-202-5p plays a crucial role in regulating this stage in fish. Additionally, miR-202-5p has been shown to control fecundity in female medaka [37] which correlates with the increased ovary weight during spawning compared to the post- and prespawning seasons.

In the male gonad, the upregulation of miR-202-5p during the spawning and postspawning stage of *C. striata* suggests its significant role in sperm maturation. The miR-202-5p, detected in spermatogonial cells in male *S. salar*, is abundantly expressed in the gonads of both male and female fish species [39]. The primary transcript, pri-miR-202, is enriched in the testis and acts as a transcriptional target of SOX9/SDF1, playing a regulatory role in promoting testis differentiation [40]. This suggests that miR-202-5p is crucial in regulating spermatogenesis by modulating gene expression involved in sperm development and maturation.

MiR-202-5p expression in liver tissues showed upregulation during both spawning and postspawning stages in both males and females. This pattern may be associated with the liver's role in supporting reproductive activities through metabolic processes, such as steroidogenesis and nutrient provision. In brain tissues, miR-202-5p was upregulated during the postspawning stage in males, while downregulation occurred during the spawning stage in males and both spawning and postspawning stages in females. These differences indicate a potential role for miR-202-5p in regulating neuroendocrine functions related to reproductive behavior and physiological adjustments.

The seasonal changes in GSI and HSI closely align with the differential expression of key miRNAs, suggesting their involvement in regulating reproductive and metabolic processes. The peak GSI during spawning corresponds with the upregulation of miR-21 and miR-22, which are linked to gametogenesis and steroidogenesis. Increased HSI during

prespawning reflects metabolic preparation, coinciding with the regulation of miR-133 and miR-202-5p. During postspawning, the upregulation of miR-34a in males aligns with reduced GSI and indicates a role in spermatogenic recovery. These patterns highlight a coordinated relationship between physiological indices and miRNA expression across reproductive stages in *C. striata*.

Overall, the differential expression of selected miRNAs across various tissues during the reproductive cycle highlights its multifaceted role in regulating not only gamete maturation but also broader physiological processes necessary for successful reproduction. These findings provide insights into the complex molecular mechanisms governing reproductive physiology in *C. striata*, with potential implications for understanding reproductive strategies in other fish species as well.

5. Conclusion

In conclusion, the differential expression of miRNAs in the gonads of *C. striata throughou*t various reproductive stages highlights their crucial roles in regulating gametogenesis and reproductive physiology. The observed patterns of upregulation and downregulation across different miRNAs underscore their involvement in key processes such as gamete maturation, oocyte development, and sperm production. These miRNAs appear to coordinate the physiological adjustments required for successful reproduction, including both the preparation for and recovery from spawning. Overall, this study enhances our understanding of the molecular mechanisms governing fish reproduction and provides a foundation for further research into reproductive strategies and miRNA functions in other aquatic species.

Data Availability Statement

The data that support the conclusions of this study can be obtained from the corresponding author upon reasonable request.

Ethics Statement

All ethical principles of ICAR-CIFA, Bhubaneshwar, India, were followed for the handling of animals in this experiment.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Gitanjali Behera and Kiran D. Rasal participated in conceptualization; Gitanjali Behera conducted the experiment and drafted the manuscript; Paramita Banerjee Sawant and Rajesh Kumar did critical revision of the manuscript; and Lakshman Sahoo and Narinder K. Chadha participated in data analysis, technical, and material support. Gowrimanohari Rakkannan participated in data visualization, proofreading, and structuring the manuscript; Jitendra Kumar Sundaray supervised the experiment and approved the final manuscript. Gitanjali Behera and Gowrimanohari Rakkannan contributed equally to the manuscript.

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