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Analysis of Relative Gene Expression of some Brain-Pituitary-Gonad Axis Genes of the Ripe Wild and Captive Female *Liza ramada*

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ABSTRACT

The thin-lipped grey mullet, *Liza ramada*, a commercially significant fish in Egypt, was studied for its expression patterns along the brain-pituitary-gonad axis in female *L. ramada* during the spawning season of 2022 in both captive and wild habitats. The inspected genes included *Kiss2*, *Gpr54*, *Gnrh1*, and *Drd2* genes in the brain and ovary, as well as *Lhβ*, *Fshβ*, and *Slα* genes in the pituitary and ovary. Relative expressions of all studied genes either in the brain, or pituitary, and ovary were higher in ripe females than in immature ones. Captive females had higher *Drd2* gene expressions; nevertheless, mature wild females had higher expression values for the other investigated genes that were measured in the brain, or pituitary and ovary. The study found correlations between gene expression patterns in wild and captive females ($P \leq 0.05$), suggesting that confinement may affect reproductive physiology. The findings suggest focusing on hormones for artificial fertilization therapies in confined females.

INTRODUCTION

The thinlip grey mullet, *Liza ramada* (family: Mugilidae, order: Perciformes), is a pelagic species that inhabits a range of habitats **(Tancioni *et al.*, 2015)**. Fry move in schools from the ocean to estuaries, coastal lagoons, rivers, and lakes to find better trophic conditions before returning to the sea to spawn. Migratory juveniles, like other mullets, spend their growing, sexual development, and adult phases in inland waters (fresh and brackish water) **(Ortiz-Zarragoitia *et al.*, 2014)**.

In freshwater, brackish water, and the ocean, *L. ramada* is an essential target species for farming. It is seen to be the best option for aquaculture due to its rapid development, ability to use a variety of artificial and natural meals effectively, tolerance to a varied variety of environmental circumstances, and resilience to stressors and illness. Egypt has acknowledged this fish species as commercially valuable **(El-Sayed & El-Ghobashy, 2011)**.

Due to the lack of ovulation, spawning, or final oocyte maturation of captive females, their supply is mainly reliant on the wild. The current natural fry collection is

insufficient to satisfy the increasing demand for juvenile *L. ramada*; the number of wild thin-lipped mullets has been steadily falling. Since Egypt uses fry for all agriculture, there is a growing concern about this resource's continued depletion and sudden price spikes. As a result, the construction and development of practical artificial propagation procedures for thinlip mullet are required to sustain supplies **(Mousa, 2010)**.

Comprehending the biological state of the reproductive cycle and artificial spawning of the fish is crucial to identifying potential causes of fish preoviposition mortality and creating effective hatchery technologies. However, scarce data are provided about the physiology of mullets during the reproductive cycle and induced spawning.

The BPG axis regulates reproductive activity in fish and other vertebrates by being a member of a sophisticated neuroendocrine network. The integration of internal and external inputs is mostly carried out by the neuroendocrine neurons located in the hypothalamus of the brain **(Zohar *et al.*, 2010)**.

Kisspeptins are thought to have a significant role in controlling the release of gonadotropin-releasing hormone (*Gnrh*) from the hypothalamus in several vertebrates including fish **(Pinilla *et al.*, 2012)**. Furthermore, mature peptides identified as kisspeptins are generated by kiss genes and are believed to act through their receptors (*kissr*) **(Akazome *et al.*, 2010)** on a variety of neuronal systems in the fish brain. The two paralogous *kiss* genes (*kiss1* and *kiss2*) and their receptors (*Gpr54*) genes (*kissr1, kissr2*) were discovered by **Mechaly *et al.* (2013)**.

Gnrh is the principal BPG axis modulator. This neuroendocrine hormone, which is a decapeptide, is thought to be crucial for controlling teleost reproduction, mostly through stimulating the pituitary gland to release gonadotropin **(Sower, 2015)**. When puberty begins in vertebrates, the hypothalamus releases growth hormone (*Gnrh*). Three different variants of *Gnrh* exist: *Gnrh1, Gnrh2,* and *Gnrh3*. The majority of fish have been shown to have *Gnrh1* **(Lethimonier *et al.*, 2004)**. It binds selectively to the receptors of follicle-stimulating hormone (*Fsh*) and luteinizing hormone (*Lh*) to control their synthesis and secretion from the pituitary gland **(Mechaly *et al.*, 2012)**.

Levavi-Sivan *et al.* (2010) showed that dopamine (*DA*) is a significant inhibitor of *Lh* release in a range of teleosts, particularly cyprinid fish. It inhibits both basal and *Gnrh*-induced *Lh* release, both *in vivo* and *in vitro*. The significance of the *DA-D2* receptor (*Drd2*) in teleosts has garnered considerable attention since it has been shown that *DA* acts directly on gonadotrophs in a variety of fish species through *Drd2* **(Zohar *et al.*, 2010)**.

The pituitary hormone somatolactin (*Sl*), which is exclusive to fish, is closely connected to the growth hormone series. According to **Degani (2015)**, it is involved in the regulation of several physiological processes including reproduction. There are two *Sl* subtypes known to exist in fish: Slα and *Slβ.* Meanwhile, *Slα* is present in all fish species, whereas *Slβ* appears mainly in basal teleost species **(Shepherd, 2007)**.

Based on the study of **Kawauchi *et al.* (2006)**, these isoforms are classified as paralogs, which means that they are homologous genes that developed by gene duplication during evolution, especially in bony fish. They were important in the development of functional specialization and gene diversity.

This study compares the relative gene expression of several genes in females raised in captivity versus those captured in the wild to investigate the potential molecular biology function of the BPG axis during the spawning season of female *L. ramada* that may be influenced by environmental cues. We selected significant reproductive genes from the brain and pituitary gland that are involved in stimulating and releasing hormones during reproduction, particularly during the spawning phase as Kisspeptins; *Kiss2* and *Gpr54* (as a *Gnrh* stimulator), *Gnrh1* (as a stimulator to *Fsh* and *Lh*), and (*DA* receptor) *Drd2*; (as an inhibitor of *Gnrh*) were investigated in the origin (the brain). Additionally, the genes of the pituitary gland *Lhβ*, *Fshβ*; (stimulators for steroids) and *Slα* (has an unclear function in the reproduction) were studied. All investigated genes were examined in the ovary as a target organ. This method is probably going to provide insightful information about how environmental influences and captivity affect this species' reproductive biology. This study represented the first investigation of *L. ramada* during the spawning season that compared the genetic expression of different reproductive genes of wild and captive populations.

MATERIALS AND METHODS

1. Study area and sampling

Females of ripe *L. ramada* were taken from the natural water of Bogaz El-Gamil (Inlet of El-Gamil area in the northwest of Portsaid with El-Manzala wetland) while migrating to their spawning grounds in the Mediterranean Sea from November (2022) to January (2023). The study was conducted in the genetics and genetic engineering laboratory at Al-Mataryiah Station for Aquatic Resources (NIOF) in Egypt, where they were brought in alive. Ten well-ripe female specimens were meticulously chosen. The females ranged in length from 40.8 to 53cm and weight from 519.8 to 1031.3g overall. The grown *L. ramada* was taken from an earthen aquaculture farm at Ras El-Bar, Damietta Governorate. Ten captive-bred, ripe females were measured, ranging from 277 to 488g for the total weight and measuring between 30 and 36cm in total length. Two additional control groups of fish were taken from the aforementioned habitats. Dissections were directly executed *in situ*. All fish-handling techniques were sanctioned by the regional authorities and the UPV/EHU Ethics Committee on Animal Experimentation. Organs under investigation were promptly preserved in liquid nitrogen up to processing, including the entirety of the brain or pituitary and a section of the gonad.

2. Extraction of total RNA and cDNA synthesis

Total RNA was extracted from the homogenized tissue samples using sonication in Trizol (Transzol, China), depending on the manufacturer's instructions. Approximately, 100mg of the ovary was taken, as opposed to the entire tissue used in the brain or pituitary. The purity and quantity of the extracted RNA were also evaluated using a Nanodrop (ND-1000UV). Following the extraction process, samples were treated with DNase (Invitrogen, Thermo Fisher, USA) for 5 minutes at 37°C to eliminate any potential DNA contamination. The integrity of RNAs was examined on the gel, as displayed in Fig. (1).

First-strand cDNA was synthesized from total RNA (2µg) using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Thermo Fisher, USA) and oligo-dt primers. The following temperature conditions were used to conduct the reaction: 60 minutes of incubation at 42°C and extension at 70°C for 5min.

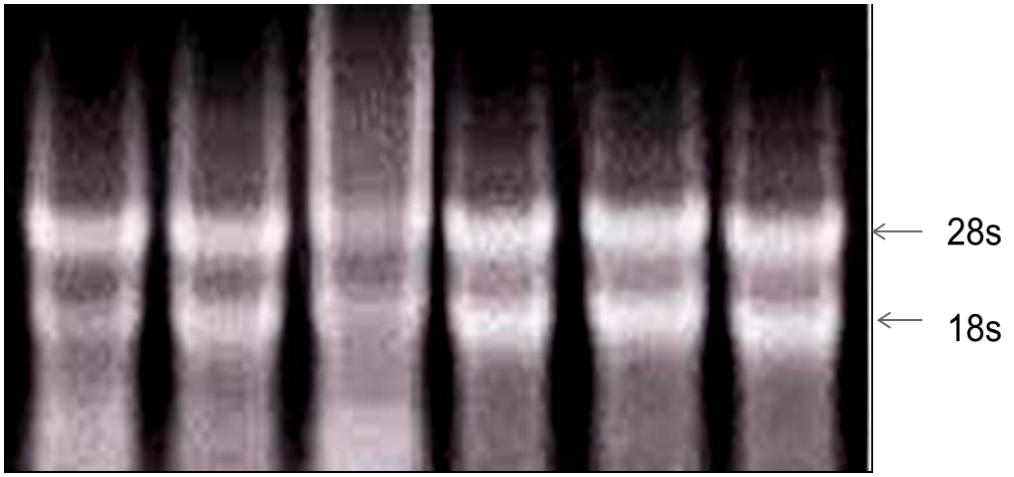

Fig.1 The examined integrity of RNA

3. Quantitative real-time PCR (QRT-PCR)

For the RT-QPCR assays, mullet *β-actin* served as the reference gene (Accession No. XM047572269). Little differences in actin mRNA levels exist between the different organs **(Nocillado *et al.*, 2007)**. *Gnrh1*, *Drd2*, *Kiss2*, and *Gpr54* are studied in the brain and ovary, while *Lhß*, *Fshß*, and *Slα* are studied in the pituitary and ovaries. The genes-specified primers are examined and scanned for the target amplifications, as displayed in Fig. (2) and listed in Table (1). For our experiment, NCBI was used for specially constructed primers using partial or complete cDNA sequences. The experiment was set up using a Thermoscientific 7300 Real-Time PCR System thermocycler. Three independent reactions were performed on the positively transcribed cDNA and corresponding controls. The 25µl reaction volume consisted of 12.5µL of SYBR Green fluorescent dye master mix (Maxima, Thermo Fisher), 0.6pmol of the specific primer pair, 5µL diluted cDNA template, and the remaining volume was RNase water. Cycling parameters were as follows: 50°C for

an initial step for 2 and 10min at 95°C, a denaturing step at 95°C for 15s for 40 cycles, and 60°C for an annealing step at 30s, and finally an extension step at 72°C for 30s. As a calibrator, the cDNAs of the immature female mullets were utilized. One reference gene was used to normalize the data, making it easier to evaluate the target levels of gene expression levels across a range of tissues. The QRT-PCR experiments were examined using the $2^{-\Delta\Delta Ct}$ method as described by **Livak and Schmittgen (2001)** and **Pfaffl (2001)** for normalization.

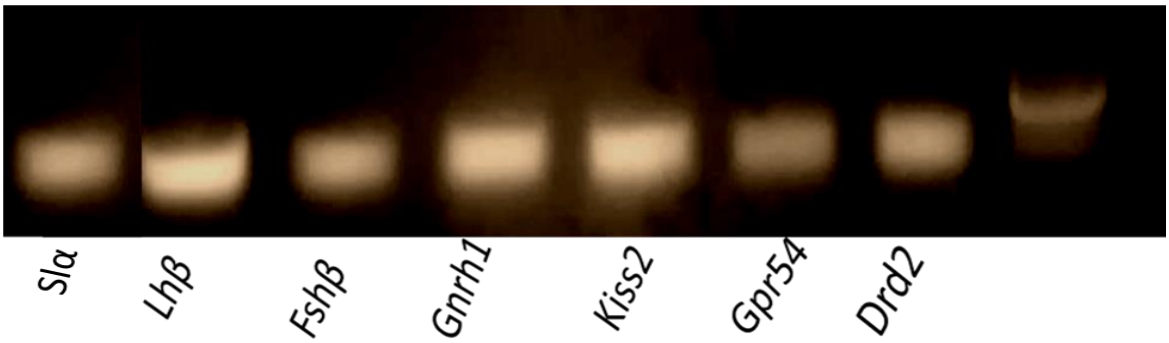

Fig. 2 The specific gene sequences with the primers

Table 1. Gene-specific primers used in this study for QRT-PCR analysis

Gene	Forward (5′–3′)	Reverse (5′–3′)	Length	Tm °c (annealing)	Accession number
Lhβ	ATCTGGGCCTTTAGTCCAGC	TCTTGACAGGGTCCTTGGTG	160	60	MF574169
Fshβ	ATTAAAGGATGCCCGGTGGG	GCCATGCACTAGCAGGATGA	163	60	NC061772.1
Gnrh1	GGAAGAGGGAACTGGACAGC	GATTTTGGCGAAAGGCGTGT	116	60	KT248847.1
Slα	GGCGCATGACAAGAAAGCAAG	GCATGATGGATGACCCGATCT	212	60	XM047594351
Gpr54	TGTTGTCAACGAGGGGGAAG	TTGGAAACGTAGTCCGCCC	196	63	DQ683737
Drd2	TGTTGTCAACGAGGGGGAAG	GGATCCCCGATTGGCTCTTT	234	63	XM047595197
Kiss2	TGGTCCTCCATCCGGTACAT	TCCAGGGGCAAGTGTTTGTT	188	63	XM047575306
β-actin	TCAAGATCATTGCCCCACCA	TCTGCGCCTGAGTGTGTAAT	250	63	XM047572269

4. Statistical analysis

The statistical software GraphPad Prism 5 was used to calculate the relative gene expressions of the target genes. The standard error (SEM) of values is represented as means ±. The actin gene was used to standardize the expression levels among the experimental groups. One-way ANOVA was utilized to evaluate the data, and a nonparametric T-test with Pearson correlation was employed to conclude the significance of the group differences. $P \leq 0.05$ was designated as the level of statistical significance.

RESULTS

1. Analysis of *Kiss2, Gpr54, Gnrh1,* and *Drd2* genes transcription patterns in the brain

As shown in Fig. (3), the brains of mature wild and captive female *L. ramada* showed varying expression levels of the genes under investigation. The brains of the mature wild females displayed a considerable rise in the relative expression of *Kiss2* (6.67-fold; $P \leq 0.01$), but the brains of captive females showed less increase in mRNA expression (5.76-fold; $P \leq 0.01$). Additionally, *Gpr54* (*Kissr2*) expression was found to be 4.25-fold ($P \leq 0.05$) in the mature wild female and somewhat (2.6-fold; $P \leq 0.05$) in the captive ones. Additionally, the brains of captive females had considerably lower relative expression levels of *Gnrh1* (2.1-fold; $P \leq 0.05$) than the brains of wild females (6.97-fold; $P \leq 0.01$). Finally, the expression of *Drd2* in the brains of wild females was 1.94-fold; $P \leq 0.01$, while the level of 4.12-fold; $P \leq 0.01$ in the captive ones was detected.

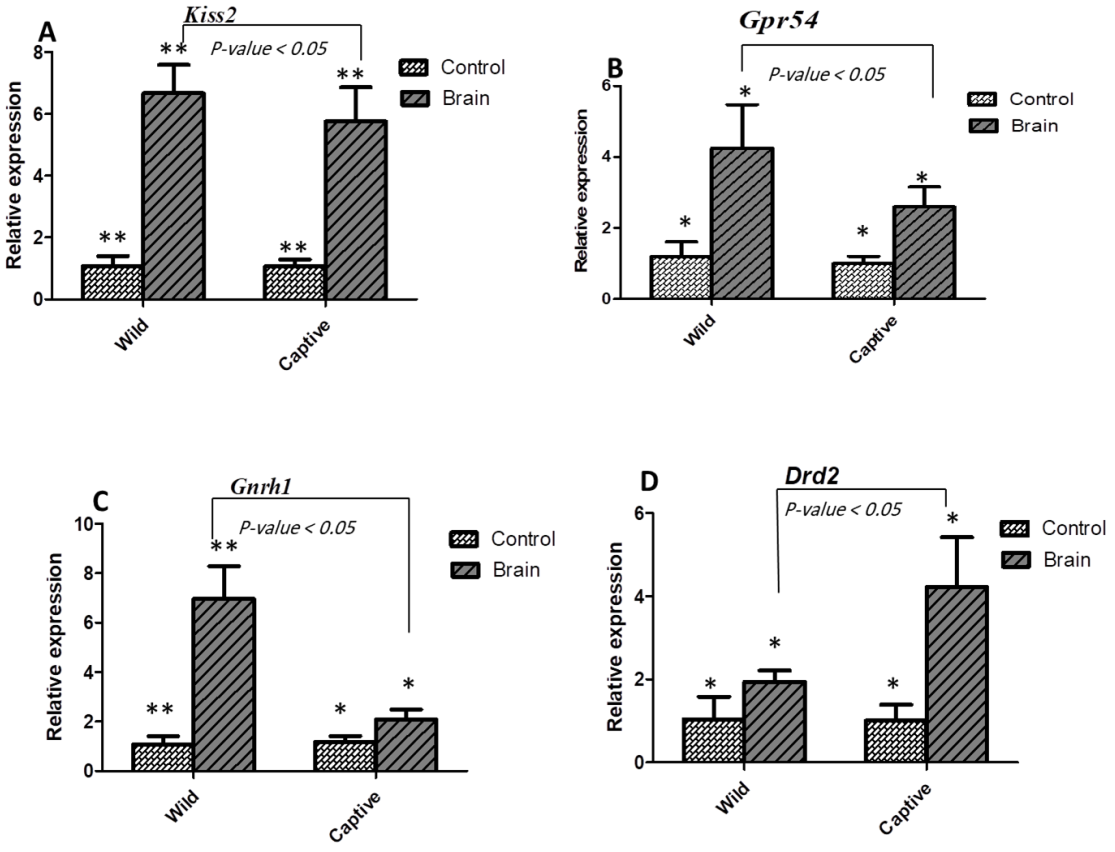

Fig. 3 Relative gene expression levels of **(A)** *Kiss2,* **(B)** *Gpr54,* **(C)** *Gnrh1,* and **(D)** *Drd2* were normalized to *β-actin* in the brain of wild and captive females of *L. ramada* during the spawning period. For each group, the data are displayed as mean ± SEM (n = 3). Significant differences are indicated by (*) *($P \leq 0.05$)* or ** for *($P \leq 0.01$)*

2. Analysis of *Kiss2, Gpr54, Gnrh1* and *Drd2* genes transcription patterns in the ovary

When compared to their expression in immature females, the ovaries of mature wild and captive females revealed higher relative expressions of *Gnrh1*, *kiss2*, *Gpr54*, and *Drd2*, as illustrated in Fig. (4). The *kiss2* analysis showed a significant increase in the wild females (5.33-fold; $P \leq 0.01$) compared to the confined females (2.37-fold; $P \leq 0.01$). In wild females, the relative expression of *Gpr54* was 3.89-fold ($P \leq 0.05$), whereas in captive females, it was 1.8-fold ($P \leq 0.05$). Furthermore, *Gnrh1* showed a considerable rise (3.33-fold; $P \leq 0.05$) in the ovaries of wild females while the reduced expression (1.5-fold; $P \leq 0.05$) in captive females was noticed. Finally, *Drd2* was expressed as well (1.46-fold; $P \leq 0.05$) in the ovaries of wild females, while the increased level (3.07-fold; $P \leq 0.05$) was observed in the captive ones.

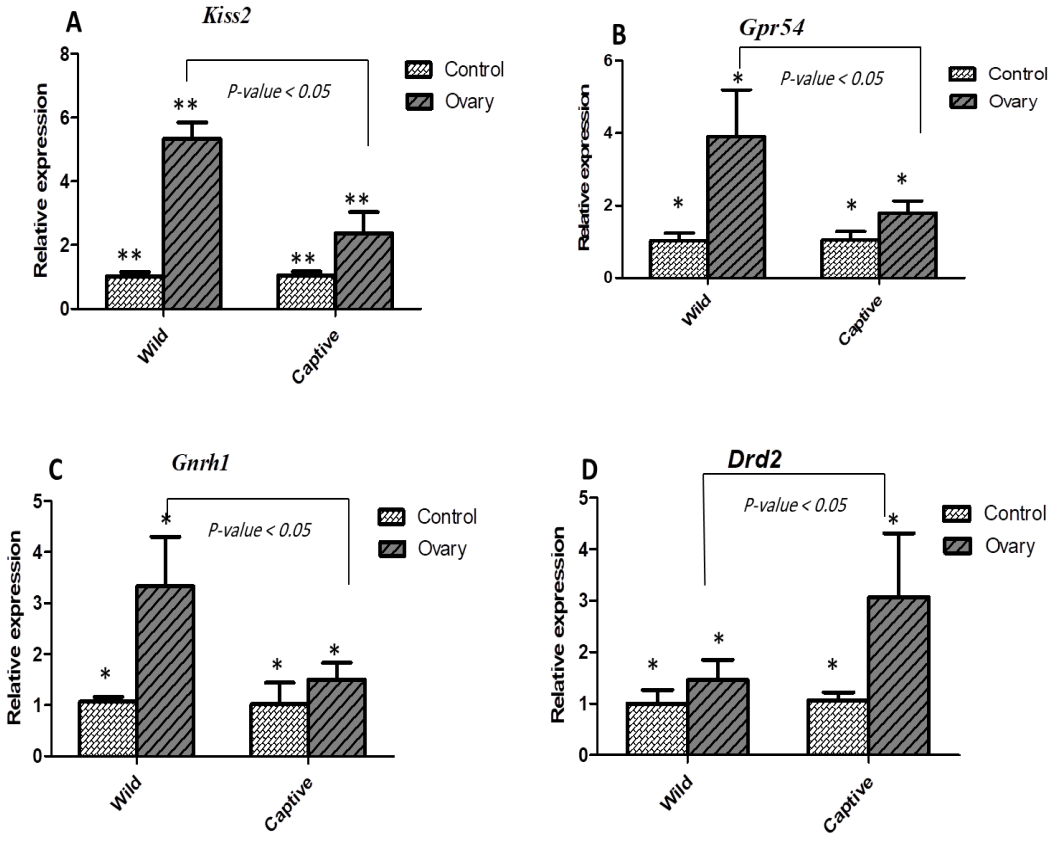

Fig. 4. Relative gene expression levels of **(A)** *Kiss2*, **(B)** *Gpr54*, **(C)** *Gnrh1*, and **(D)** *Drd2* were normalized to *β-actin* in the ovary of wild and captive females of *L. ramada* during the spawning period. For each group, the data are displayed as mean ± SEM (n = 3). Significant differences are indicated by an asterisk (*) ($P \leq 0.05$) or a ** ($P \leq 0.01$).

3. Correlation between *Kiss2*, *Gpr54*, *Gnrh1* and *Drd2* in the wild and captive females

Table (2) displays the varying patterns of correlation between the analyzed gene expression profiles in the brain and ovary of wild and captive females. The *Drd2* and *Kiss2* genes showed a reverse correlation in the ovaries of wild and captive females, while a direct association in the brains during the spawning season was detected. Whereas, a direct correlation was shown with significant values for *Gpr54* and *Gnrh1* genes in the brains and ovaries of wild females compared to the values of captive ones.

Table 2. Pearson correlation coefficients for the genes *Kiss2*, *Gpr54*, *Gnrh1*, and *Drd2* in the brain and ovary between wild and captive females

	Gene	Brain	Ovary
Pearson correlation	***Gpr54***	0.25*	-0.06*
	Drd2	0.99*	0.1*
	Kiss2	0.94*	0.99*
	Gnrh1	0.88*	-0.38*

* indicates significance ($P \leq 0.05$).

4. Analysis of *Fshß*, *Lhß*, and *Slα* transcription patterns in the pituitary gland

The genes that were analyzed in the pituitaries of ripe females demonstrated an increase in their relative expression in comparison to the immature ones, as shown in Fig. (5). In the wild female pituitaries, *Fshß* was higher (9.3-fold; $P \leq 0.01$) than in the captives (4.5-fold; $P < 0.05$). Furthermore, compared to captive females (5.94-fold; $P < 0.01$), wild females reported *Lhß* values (6.26-fold; $P < 0.01$). The expression of *Slα* in the pituitaries of wild females was 3.5-fold ($P < 0.05$), while the pituitaries of captive females showed a slight variation (2.85-fold; $P < 0.05$).

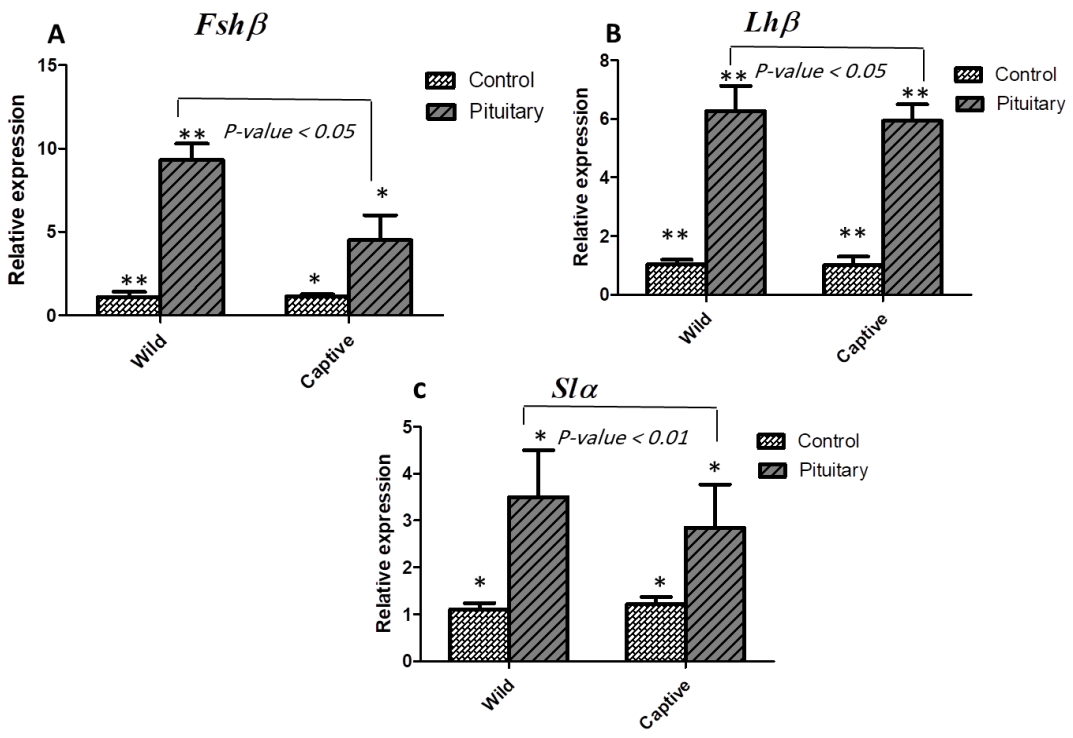

Fig. 5. Relative gene expression levels of **(A)** *Fshβ*, **(B)** *Lhβ*, and **(C)** *Slα* were normalized to *β-actin* in the pituitary of wild and captive females of *L. ramada* during the spawning period. For each group, the data are displayed as mean ± SEM (n = 3). Significant differences are indicated by an asterisk (*) ($P \leq 0.05$) or a ** ($P \leq 0.01$)

5. Analysis of *Lhß, Fshß,* and *Slα* transcription patterns in the ovary

As illustrated in Fig. (6), there was a notable increase in the expression of these genes in the ovaries of ripe females when compared to the immature ones. The ovaries of wild females showed a relative expression of 7.16-fold ($P < 0.01$) for *Fshß*, whereas in captives, it was 3.62-fold *(*$P < 0.05$). Compared to captive females (3-fold; $P < 0.05$), ovaries of wild females displayed 5.63-fold; $P < 0.05$ for *Lhß*. Lastly, *Slα* has an approximate expression of 2.17-folds *(*$P < 0.05$) in the wild, while the expression of 1.7-folds *(*$P < 0.05$) was detected in captivity.

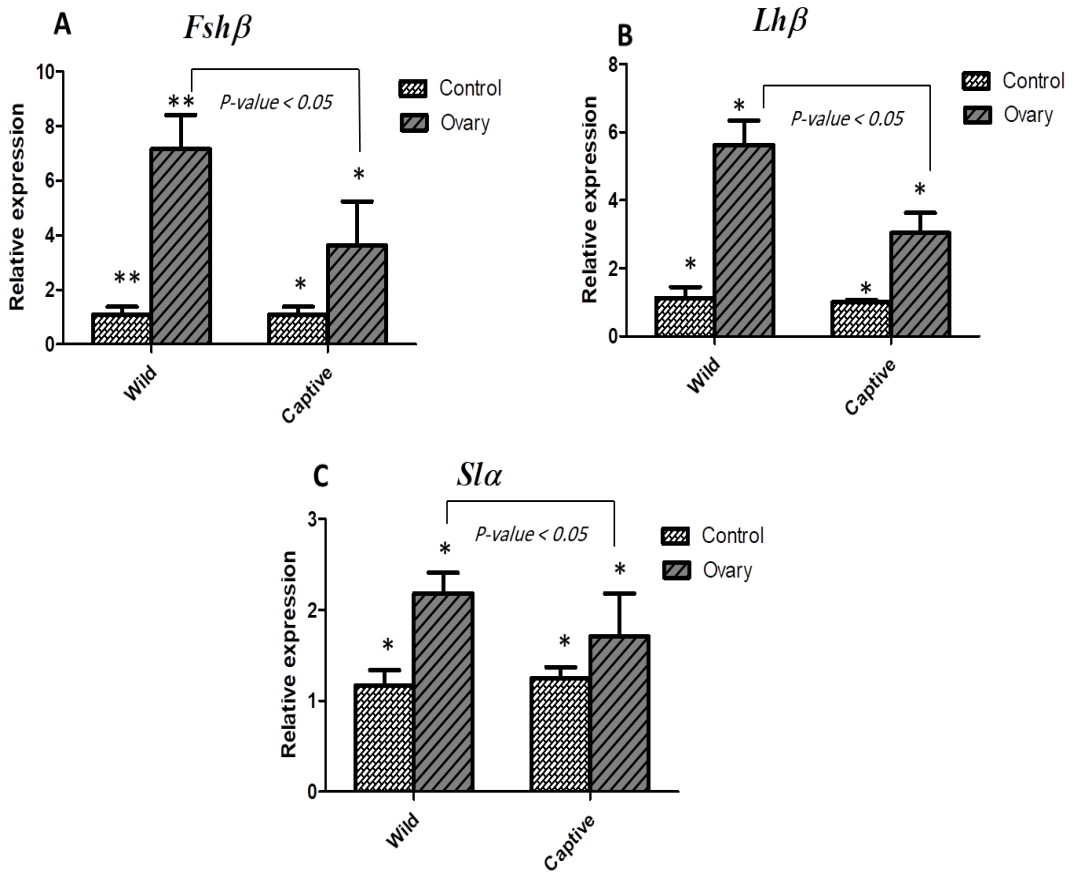

Fig. 6. Relative gene transcription levels **(A)** *Fshβ*, **(B)** *Lhβ*, and **(C)** *Slα* were normalized to *β-actin* in the ovary from wild and captive females of *L. ramada* during the advanced pubertal stage. For each group, the data are displayed as mean ± SEM (n = 3). Significant differences are indicated by an asterisk (*) ($P \leq 0.05$) or a ** ($P < 0.01$)

6. Correlation between *Fshβ, Lhβ,* and *Slα* in the wild and captive environments

The expression patterns of three genes; *Fshβ, Lhβ,* and *Slα* in the pituitary and ovary of wild females seem to be significantly correlated with those of their counterparts in captive females. *Fshβ* and *Slα* in the pituitaries and ovaries displayed a direct association between wild and captive females. However, *Lhβ* represented an inverse correlation in the pituitaries and ovaries between the two habitats, as shown in Table (3).

Table 3. Pearson correlation for *Fshβ*, *Lhβ*, and *Slα* in the pituitary and ovary between wild and captive females

	Gene	Pituitary	Ovary
Pearson correlation	***Lhβ***	-0.33*	-0.96*
	Fshβ	0.99*	0.96*
	Slα	0.99*	-0.99*

*represents significance ($P \leq 0.05$).

DISCUSSION

By contrasting wild and cultured broodstocks during the spawning season, this research stated for the first time the gene expression profiles by QRT-PCR in three separate tissues (brain, pituitary, and ovary) in female *L. ramada*. Our paper shed light on the potential effects of confinement on patterns of reproductive gene expression that differ from those found in the natural environment. Comprehending these patterns is crucial to understanding the reproductive physiology of *L. ramada*, which has consequences for artificial fertilization methods of fish species as well as their natural populations.

Our results support the hypothesis that the unique brain gene expression patterns correspond with the spawning season, with significant variations observed in females in the wild compared to those kept in captivity. Compared to captive settings, these genes have higher relative expressions in the wild. It seems that the brains and ovaries of wild female *L. ramada* express less of the gene than do captive ones, only for *Drd2* expression. The disparities between females in the wild and captivity may be due to environmental influences, conditions in captivity, or other factors affecting gene regulation.

According to **Zmora *et al.* (2012)**, adult female striped bass had significantly higher amounts of *kiss1* and *kiss2* mRNAs, as well as their receptors, *kissr1* and *kissr2* (*Gpr54*), in their brains than juvenile and prepubertal fish. Moreover, higher expression of *kiss1* and *kiss2* was found in Indian major carp during the pre-spawning and spawning periods **(Saha *et al.*, 2016)**.

According to **Kitahashi *et al.* (2009)** observations, zebrafish *Kiss1* and *Kiss2* mRNA levels were significantly elevated at the beginning of the pubertal phase and remained high throughout maturity. This suggests that *kiss-Gnrh* systems play a role in the regulation of pituitary gonadotropins. Additional data supporting the importance of the kiss-Gnrh system in fish for the integration of metabolic signals with environmental cues and the transport of this information to the reproductive axis was shown in the studies of **Selvaraj *et al.* (2012)**, **Thakuria *et al.* (2017)** and **Faheem *et al.* (2019)**.

The findings of **Guzmán *et al.* (2009**) were documented in captive-reared female Senegalese soles (*Solea senegalensis*), revealing a decrease in *Gnrh1* expression in comparison to wild counterparts. Furthermore, during the spawning season, **El-kady *et al.* (2024)** observed that the expression pattern of *Gnrh1* in female *M. cephalus* animals was the same in both wild and captive environments. These investigations support our findings regarding female *L. ramada* and also imply that stress associated with captive-rearing may block *Gnrh1* transcription, which would then lead to the failure of *L. ramada* vitellogenesis.

According to **Monbrison *et al.* (2003)**, there could be two plausible reasons for the claimed absence of gonadotropin in captive fish's circulation: either the pituitary produces inadequate gonadotropin; the hypothalamus secretes insufficient *Gnrh*, or a combination of both causes. By targeting the endocrine axis that regulates gonad function, this strategy may help prevent possible failure.

Estrogen not only stimulates the Gnrh system in juvenile fish but also activates the brain-pituitary dopaminergic pathway **(Linard *et al.*, 1995)**, increasing the expression of *Drd2* **(Levavi-Sivan *et al.*, 2005)**. *Drd2* transcripts have been found in the ovaries of tilapia **(Levavi-Sivan *et al.*, 2005)** and grey mullet **(Nocillado *et al.*, 2007)**, suggesting a direct *DA* impact on the fish gonad. It has been shown that gonadal steroids change the expression of the *Drd2* gene in Anguilla Anguilla **(Pasqualini *et al.*, 2009)**.

Representative species of other teleosts likewise exhibit dopaminergic suppression of reproduction, such as rainbow trout (*Oncorhynchus mykiss*) **(Vacher *et al.*, 2003**) and tilapia (*Oreochromis mossambicus*) **(Yaron *et al.*, 2003)**. Aquaculture depends on these species. **Aizen *et al.* (2005)** found that a key problem along the reproductive neuroendocrine axis that prevents spontaneous spawning in captive grey mullet (*Mugil cephalus*) is dopaminergic suppression.

In regards to *Fshβ, Lhβ,* and *Slα* expression, the present investigation found that the mRNA levels of these genes were higher in the pituitaries and ovaries of wild female broodstocks than in those of their cultured counterparts.

Lh and *Fsh* are present on the gonads to produce more gonadal factors and sex steroids, which are necessary for gamete development and maturation, as demonstrated by **Kusakabe *et al.* (2006)** and **Zmora *et al.* (2007)**. They are produced in distinct cells inside the pituitary and display distinct expression patterns at different stages of the reproductive cycle.

However, at the spawning time, both sexes of goldfish showed the same amounts of *Lhβ* mRNA, and females showed higher amounts of *Fshβ* mRNA than males **(Sohn *et al.*, 1999)**. Previous research **(Zohar & Mylonas, 2001)** revealed that the levels of pituitary *Lhβ* mRNA in female striped bass raised in culture and those in the wild were identical. According to the study of **Nyuji *et al.* (2012)**, farmed fish have lower brain *Gnrh1*, pituitary *Fsh*, and *Lh* expression levels than wild fish at similar reproductive stages.

According to findings reported by **Bhandari *et al.* (2006)** and **El-kady *et al.* (2024)**, the pituitary of *M. cephalus* exhibits higher levels of *Slα* mRNA during the maturation stage. Furthermore, before and during spawning, both *Slα* and *Slβ* underwent significant improvements, increasing by two-folds for *Slα* and seven-folds for *Slβ* **(Benedet *et al.*, 2008)**. **Degani *et al.* (2015)** found that *Sl* may be involved in vitellogenesis rather than maturation in female blue gourami.

Selvaraj *et al.* (2021) stated that kisspeptins, *Gnrh*, and sex steroids are among the minor actions of the BPG axis that have been predominantly connected to the dysfunction of the reproductive system in captivity. According to **Nyuji *et al.* (2020)**, feeding schedule, dose, and water temperature are examples of external factors that affect the expression of the BPG axis gene, while internal factors like fish development and age also have an impact. The reproductive process will be harmed if the BPG system is predicted to be deteriorating in captivity according to **Guzmán *et al.* (2009)**. BPG axis obstruction may affect reproductive function in jack mackerel kept in captivity, as elucidated in the study of **Imanaga *et al.* (2014)**.

Based on our research, there might be a genetic basis for the reproductive variations between wild and confined breeders, which could be caused by genetic inbreeding within captive generations. The findings of this study may indicate that suppression of the BPG axis, namely the brain transcription of *Kiss2* and *Gnrh1* and the pituitary's manufacture of *GTH* subunits, is the cause of the captive female *L. ramada*'s inability to reproduce during the spawning season. To understand how stress from captivity increases *Gnrh1* transcription inhibition and exacerbates vitellogenesis, more research is required on the gene expression profiles of *Gnrh1* in wild female *L. ramada* during ovarian development, specifically before and during the early stages of vitellogenesis.

In summary, it was demonstrated that fish kept in captivity exhibited the lowest levels of *Kiss2, Gpr54, Grnh1, Fshβ,* and *Lhβ* contrasted with the wild female *L. ramada*. *Gnrh1* transcription levels in the brains were inversely linked with the expression of *Lhβ* and *Fshβ* in the pituitaries of captive fish, however *Gnrh1* expressions in the brains were greater and tended to correlate with the expression of *Fshβ, Lhβ,* and *Slα* in the pituitaries of wild females. In contrast, fish kept in captivity revealed more *Drd2* mRNA levels than wild fish that have stumpy expression levels.

Because of the detected variances in its gene expression in the two habitats and the relationship between *kiss2* and *Lhβ* secretion, we propose that this study elucidated the function of the kisspeptin system (*Kiss2* and *Gpr54*) and how it contributes to spawning. To ascertain how much of an impact it has on the spawning process, it is crucial to study it in a lab setting by injecting it into captive mullet fish, *L. ramada*. The study found that *Gnrh1* and *Drd2* significantly affect reproduction. In the wild, their inverse relationship facilitates the completion of spawning, but in captivity, their direct correlation inhibits it. The study offers more contexts for the involvement of *Slα* because statistically significant connections were established.

REFERENCES

Aizen, J.; Meiri, I.; Tzchori, I.; Levavi-Sivan, B. and **Rosenfeld, H.** (2005). Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. Gen. Comp. Endocrinol., *142*(1-2): 212-221.

Akazome, Y.; Kanda, S.; Okubo, K. and **Oka, Y.** (2010). Functional and evolutionary insights into vertebrate kisspeptin systems from studies of fish brain. J. Fish Biol., *76*(1): 161-182.

Benedet, S.; Björnsson, B. T.; Taranger, G. L. and **Andersson, E.** (2008). Cloning of somatolactin alpha, beta forms and the somatolactin receptor in Atlantic salmon: seasonal expression profile in pituitary and ovary of maturing female broodstock. Reprod. Biol. Endocrinol., *6*: 1-17.

Bhandari, R. K.; Nakamura, M.; Kobayashi, T. and Nagahama, Y. (2006). Suppression of steroidogenic enzyme expression during androgen-induced sex reversal in Nile tilapia (*Oreochromis niloticus*). Gen. Comp. Endocrinol., *145*(1), 20-24.

Chi, M. L.; Ni, M.; Li, J. F.; He, F.; Qian, K.; Zhang, P.; Chai, S. H. and **Wen, H. S.** (2015). Molecular cloning and characterization of gonadotropin subunits (GTHα, FSHβ and LHβ) and their regulation by hCG and Gnrha in Japanese sea bass *(Lateolabrax japonicas)* in vivo. Fish Physiol Biochem., *41*: 587–601

Degani, G. (2015). Somatolactin transcription during oogenesis in female blue gourami *(Trichogaster trichopterus)*. ABC., 5(7): 279-285.

El-kady, M. A.; Mansour, H. A.; Elmor, M. E. and Ali, A. A. (2024). Comparative Gene Expression Level of Reproductive Genes Along the Brain-Pituitary-Gonad Axis in the Ripe Female of the Wild and Captive Grey Mullets; *Mugil cephalus*. EJABF., *28*(1): 20-36.

El-Sayed, A. F. M. and **El-Ghobashy, A. E.** (2011). Effects of tank colour and feed colour on growth and feed utilization of thinlip mullet (*Liza ramada*) larvae. Aquac. Res., *42*(8): 1163-1169.

Faheem, M.; Jahan, N.; Khaliq, S. and **Lone, K. P.** (2019). Modulation of brain kisspeptin expression after bisphenol-A exposure in a teleost fish, *Catla catla*. Fish Physiol. Biochem., *45*: 33-42.

Guzmán, J. M.; Rubio, M.; Ortiz-Delgado, J. B.; Klenke, U.; Kight, K.; Cross, I.; Sánchez-Ramos, I.; Riaza, A.; Rebordinos, L.; Sarasquete, C. and **Zohar, Y.** (2009). Comparative gene expression of gonadotropins (FSH and LH) and peptide levels of gonadotropin-releasing hormones (GnRHs) in the pituitary of wild and cultured Senegalese sole (*Solea senegalensis*) broodstocks. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. Comp Biochem Phys., *153*(3): 266-277.

Imanaga, Y.; Nyuji, M.; Amano, M.; Takahashi, A.; Kitano, H.; Yamaguchi, A. and **Matsuyama, M.** (2014). Characterization of gonadotropin-releasing hormone and gonadotropin in jack mackerel (*Trachurus japonicus*): comparative gene expression analysis with respect to reproductive dysfunction in captive and wild fish. Aquac., *428*: 226-235.

Kawauchi, H. and **Sower, S. A.** (2006). The dawn and evolution of hormones in the adenohypophysis. Gen. Comp. Endocrinol.,*148*(1): 3-14.

Kusakabe, M.; Nakamura, I.; Evans, J.; Swanson, P. and **Young, G.** (2006). Changes in mRNAs encoding steroidogenic acute regulatory protein, steroidogenic

enzymes and receptors for gonadotropins during spermatogenesis in rainbow trout testes. J. Endocrinol., *189*(3): 541-554.

Lethimonier, C.; Madigou, T.; Muñoz-Cueto, J. A.; Lareyre, J. J. and **Kah, O.** (2004). Evolutionary aspects of GnRHs, GnRH neuronal systems and GnRH receptors in teleost fish. Gen. Comp. Endocrinol., *135*(1): 1-16.

Levavi-Sivan, B.; Bloch, C. L.; Gutnick, M. J. and **Fleidervish, I. A.** (2005). Electrotonic coupling in the anterior pituitary of a teleost fish. Endocr. J., *146*(3): 1048-1052.

Levavi-Sivan, B.; Bogerd, J., Mañanós, E. L., Gómez, A. and Lareyre, J. J. (2010). Perspectives on fish gonadotropins and their receptors. Gen. Comp. Endocrinol., *165*(3): 412-437.

Linard, B.; Bennani, S. and **Saligaut, C.** (1995). Involvement of estradiol in a catecholamine inhibitory tone of gonadotropin release in the rainbow trout *(Oncorhynchus mykiss)*. Gen. Comp. Endocrinol., *99*(2): 192-196.

Livak, K. J. and **Schmittgen, T. D.** (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-} ΔΔCT method. Methods., *25*(4): 402-408.

Mechaly, A. S.; Vin, S. J. and **Piferrer, F.** (2012). Sex-specific changes in the expression of kisspeptin, kisspeptin receptor, gonadotropins and gonadotropin receptors in the Senegalese sole (*Solea senegalensis*) during a full reproductive cycle. Comp. Biochem. Physiol. A., *162*: 364–371

Mechaly, A. S.; Viñas, J. and **Piferrer, F.** (2013). The kisspeptin system genes in teleost fish, their structure and regulation, with particular attention to the situation in Pleuronectiformes. Gen. Comp. Endocrinol., *188*: 258-268.

Monbrison, D.; Tzchori, I.; Holland, M. C.; Zohar, Y.; Yaron, Z. and **Elizuri, A.** (2003). Acceleration of gonadal development and spawning induction in the Mediterranean grey mullet, *Mugil cephalus*: preliminary studies. Aquac., *220*(1-4): 725–735.

Mousa, M. A. (2010). Induced spawning and embryonic development of *Liza ramada* reared in freshwater ponds. Anim. Reprod. Sci., *119*: 115-122.

Nocillado, J. N.; Levavi-Sivan, B.; Carrick, F. and **Elizur, A.** (2007). Temporal expression of G-protein-coupled receptor 54 (GPR54), gonadotropin-releasing hormones (Gnrh), and dopamine receptor D2 (Drd2) in pubertal female grey mullet, *M. cephalus*. Gen. Comp. Int. J. Endocrinol., *150*(2): 278-287.

Nyuji, M.; Hongo, Y.; Yoneda, M. and **Nakamura, M.** (2020). Transcriptome characterization of BPG axis and expression profiles of ovarian steroidogenesis-related genes in the Japanese sardine. BMC Genomics., *21*: 1-18.

Nyuji, M.; Selvaraj, S.; Kitano, H.; Ohga, H.; Yoneda, M.; Shimizu, A.; Kaneko, K.; Yamaguchi, A. and **Matsuyama, M.** (2012). Changes in the expression of pituitary gonadotropin subunits during reproductive cycle of multiple spawning female chub mackerel *(Scomber japonicas*). Fish Physiol. Biochem., *38*: 883-897.

Ortiz-Zarragoitia, M.; Bizarro, C.; Rojo-Bartolomé, I.; De Cerio, O. D.; Cajaraville, M. P. and **Cancio, I.** (2014). Mugilid fish are sentinels of exposure to endocrine disrupting compounds in coastal and estuarine environments. Mar. Drugs., *12*(9): 4756-4782.

Pasqualini, C.; Weltzien, F. A.; Vidal, B.; Baloche, S.; Rouget, C.; Gilles, N.; Servent, D.; Vernier, P. and **Dufour, S.** (2009). Two distinct dopamine D2 receptor

genes in the European eel: molecular characterization, tissue-specific transcription, and regulation by sex steroids. Endocr. J., *150*(3): 1377-1392.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Res., *29*(9): e45-e45.

Pinilla, L.; Aguilar, E.; Dieguez, C.; Millar, R. P. and **Tena-Sempere, M.** (2012). Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol. Rev., *92*(3): 1235-1316.

Saha, A.; Pradhan, A.; Sengupta, S.; Nayak, M.; Samanta, M.; Sahoo, L. and **Giri, S. S.** (2016). Molecular characterization of two kiss genes and their expression in rohu *(Labeo rohita)* during annual reproductive cycle. CBP., *191*: 135-145.

Selvaraj, S.; Chidambaram, P.; Ezhilarasi, V.; Kumar, P. P.; Samuel Moses, T. L. S.; Antony, C. and **Ahilan, B.** (2021). A review on the reproductive dysfunction in farmed finfish. Annu. Res. Rev. Biol., *36*(10): 65-81.

Selvaraj, S.; Kitano, H.; Amano, M.; Nyuji, M.; Kaneko, K.; Yamaguchi, A. and **Matsuyama, M.** (2012). Molecular characterization and expression profiles of three GnRH forms in the brain and pituitary of adult chub mackerel *(Scomber japonicus)* maintained in captivity. Aquac., *356*: 200-210.

Shepherd, S. A. and **Brook, J. B.** (2007). Distribution and ontogenetic shifts in habitat and abundance of the temperate western blue groper, *Achoerodus gouldii* (Richardson). J. Fish Biol., *71*(5): 1457-1478.

Sohn, Y. C.; Yoshiura, Y.; Kobayashi, M. and **Aida, K.** (1999). Seasonal changes in mRNA levels of gonadotropin and thyrotropin subunits in the goldfish, *Carassius auratus*. Gen. Comp. Endocrinol., *113*(3): 436-444.

Song, H., Wang, M., Wang, Z., Yu, H., Wang, Z. and **Zhang, Q.** (2016). Identification and characterization of kiss2 and kissr2 homologs in Paralichthys olivaceus. Fish Physiol. Biochem., *42*: 1073-1092.

Sower, S. A. (2015). The reproductive hypothalamic-pituitary axis in lampreys. *Lampreys:* Biol. Conserv., *1*: 305-373.

Tancioni, L.; Caprioli, R.; Dawood Al-Khafaji, A. H.; Mancini, L.; Boglione, C.; Ciccotti, E. and **Cataudella, S.** (2015). Gonadal disorder in the Thinlip grey mullet (Liza Ramada, Risso 1827) as a biomarker of environmental stress in surface waters. Int. J. Environ. Res. Public Health., *12*(2): 1817-1833.

Thakuria, D.; Shahi, N.; Singh, A. K.; Khangembam, V. C.; Singh, A. K. and **Kumar, S.** (2017). Conformational analysis of a synthetic fish kisspeptin 1 peptide in membrane mimicking environments. Plos one., *12*(10): e0185892.

Vacher, C.; Pellegrini, E.; Anglade, I.; Ferriére, F.; Saligaut, C. and **Kah, O.** (2003). Distribution of dopamine D2 receptor mRNAs in the brain and the pituitary of female rainbow trout: an in situ hybridization study. J. Comp. Neurol., *458*(1): 32-45.

Wong, A. O.; Chang, J. P. and **Peter, R. E.** (1993). Characterization of D1 receptors mediating dopamine-stimulated growth hormone release from pituitary cells of the goldfish, *Carassius auratus*. Endocr. J. *133*(2): 577-584.

Yaron, Z.; Gur, G.; Melamed, P.; Rosenfeld, H.; Elizur, A. and **Levavi-Sivan, B.** (2003). Regulation of fish gonadotropins. Int. Rev. Cytol., *225*: 131-185.

Zmora, N.; Stubblefield, J.; Zulperi, Z.; Biran, J.; Levavi-Sivan, B.; Muñoz-Cueto, J. A. and **Zohar, Y.** (2012). Differential and gonad stage-dependent roles of

kisspeptin1 and kisspeptin2 in reproduction in the modern teleosts, morone species. Biol. Reprod., *86*(6): 177-1.

Zmora, N.; Trant, J.; Chan, S. M. and **Chung, J. S.** (2007). Vitellogenin and its messenger RNA during ovarian development in the female blue crab, *Callinectes sapidus*: gene expression, synthesis, transport, and cleavage. Biol. Reprod., *77*(1): 138-146.

Zohar, Y. and **Mylonas, C.C.** (2001). Endocrine manipulations of spawning in cultured fish: from hormones to genes. Aquac., 197: 99-136.

Zohar, Y.; Muñoz-Cueto, J. A.; Elizur, A. and **Kah, O.** (2010). Neuroendocrinology of reproduction in teleost fish. Gen. Comp. Endocrinol., *165*(3): 438-455.