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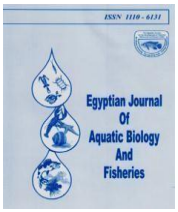

Comparative Gene Expression Level of Reproductive Genes Along the Brain-Pituitary-Gonad Axis in the Ripe Female of the Wild and Captive Grey Mullets; *Mugil cephalus*

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ABSTRACT

The present study highlights distinctions between the wild and captivity environments during the spawning season by providing light on the expression patterns of crucial genes in the BPG axis of female *Mugil cephalus*. Genes from the brain and ovaries (*Kiss2*, *Gpr54*, *Gnrh1*, and *Drd2*), as well as the pituitary and ovary (*Lhβ, Fshβ,* and *Slα*) were examined. According to the research, ripe females have higher relative expressions of all the genes analyzed in all the organs (brain, pituitary, and ovary) than do immature females. While *Drd2* expression in captive females' brains and ovaries was higher than in wild females, the other genes under investigation that were evaluated in the brain, pituitary, or ovary showed higher expression values in mature wild females than in captive ones. A correlation between gene expression patterns in the studied organs of the wild females was recorded either directly or inversely, with their counterparts in captive ones. Results showed significant variance ($p < 0.05$) between captive and wild females. Overall, the noted connections and noteworthy variations in gene expression suggest possible effects of confinement on the reproductive physiology of these fish, with a particular emphasis on various hormones for experimental treatments for the captive females for artificial fertilization.

INTRODUCTION

Mugil cephalus is a euryhaline and eurythermal species belonging to the family Mugilidae. It is a member of the order Mugiliformes, which comprises Actinopterygian teleosts **(Whitfield *et al.* 2012; Abo-Taleb *et al.* 2021)**. **Whitfield *et al.* (2012)** mentioned in their study that juvenile and sub-adult stages of *M. cephalus* migrate towards fresh and brackish waters in estuaries and lagoons during the post-spawning period. In autumn and winter, an inverse migration to the open sea occurs, likely for spawning or seeking refuge. The migration patterns of *M. cephalus* for spawning have been extensively studied and documented worldwide, as mentioned in **Katselis *et al.* (2005, 2007)**. It was documented by a study by **Ramos-Júdez *et al.* (2021)** regarding grey mullet farming in Egypt that *M. cephalus* relies on inbred fry collected from the wild. Nevertheless, the challenges associated with seasonal reproduction and reproductive dysfunctions in captivity, ultimately lead to more dependable hatchery-based seed production.

A specific study conducted by **AAEl-Darawany *et al.* (2016)** stated that the stress related to exhaustive culture conditions and the deficiency of natural environmental signals may impact the reproductive capabilities of grey mullets in captivity. Mullets, like many other fish species, have a specific period or season during which they engage in spawning activities. Nevertheless, in captivity, often experience reproductive dysfunctions. The main cause of these reproductive dysfunctions is attributed to the stress associated with intensive culture conditions and the absence of appropriate environmental cues. This is chiefly important due to the commercial consequence of grey mullets, which likely implies a high demand for them in the market and a need for sustainable aquaculture practices.

The brain-pituitary-gonad (BPG) axis is a crucial endocrine system that exhibits a significant role in the regulation of reproductive functions in vertebrates. This axis involves a complex interaction of hormones and feedback mechanisms that control the development and functioning of the gonads **(Yan, 2016)**.

Kisspeptins are a group of peptides that are critical regulators of the BPG axis, influencing the release of *Gnrh* and, consequently, the entire cascade of events leading to puberty and the maintenance of regular reproductive function **(Pinilla *et al.* 2012)**.

Somoza *et al.* (2020) mentioned that in teleosts, two kisspeptin genes, *Kiss1* and *Kiss2*, have been identified. These genes encode for two types of receptors; *Gpr54-1* and *Gpr54-2* and they play roles in regulating reproductive processes. In addition, **Colledge, (2009)** reported that the *Kiss-Gpr54* signaling system is one of the tracks controlling *Gnrh* secretion from the hypothalamus. The study by **Selvaraj *et al.* (2022)** likely contributes to the understanding of *Gnrh* isoforms in teleosts. One of the well-known isoforms is *Gnrh1*, which is engaged in the control of reproductive development, such as the release of gonadotropins and subsequent regulation of gonadal functions. **Valencia *et al.* (2020)** documented that the synthesis and release of *Lh* and *Fsh* from the pituitary to the bloodstream has been stimulated by the discharge of *Gnrh* from the hypothalamus of the brain.

Somatolactin (*Sl*) is indeed a hormone that belongs to the growth hormone/prolactin (GH/PRL) family, and it has been studied in the context of fish endocrinology **(Zhu *et al.* 2004)**. In fish, it often exists as two isoforms known as *Slα* and *Slβ.* These isoforms are considered paralogs, indicating that they are homologous genes that arose through gene duplication during evolution, particularly in bony fish, and they played a significant role in the generation of gene diversity and functional specialization **(Kawauchi *et al.* 2006)**. It has been concerned with numerous physiological processes, for instance, stress response, smoltification, gonadal maturation, and gonadal steroid biosynthesis **(Bertolesi and McFarlane, 2021)**.

Dufour *et al.* (2010) reported the well-established significant role of dopamine (*DA*) in the regulation of reproduction, particularly in the context of the hypothalamic-pituitary-gonadal (BPG) axis in teleost fish.

Our study aims to investigate the molecular basis for differences in the expression of certain genes related to the BPG axis in various tissues (brain, pituitary, and ovary) of female *M. cephalus*. The inspected genes are *Kiss2*, *Gpr54*, *Drd2*, and *Gnrh1* in the brain, as well as *Lhβ, Fshβ,* and *Slα* in the pituitary. All of these genes are also examined in the ovary. This study involves comparing individuals raised in captivity with those captured from the wild. This approach is likely to contribute valuable insights into the impact of captivity and environmental factors on the reproductive biology of this species.

MATERIALS AND METHODS

1. Study area and sampling

Females of mature *M. cephalus* of wild fish have been taken from their natural habitat of El-Manzala Lake in the area of Bogaz El-Gamil of the Mediterranean Sea at the time of their migration for spawning during September and October 2022. They were directly transported alive to the laboratory of genetics and genetic engineering at Al-Mataryiah Station for Aquatic Resources, NIOF, Egypt where the study was carried out. Ten ripe female samples were nominated prudently. The full length of the females ranged from 42 to 47 cm, and the whole weight ranged from 784.4 to 1141.1 g. The cultured *M. cephalus* was collected from an outdoor earthen pond of an aquaculture farm at Ras El-Bar, Damietta governorate. Ten individuals of captive ripe females were taken with total lengths ranging from 44 to 50.5 cm, and total weights ranging from 919 to 1544 g. Another two groups of control fish were collected from the previously mentioned habitats. The sample management and processes were permitted according to the UPV/EHU Ethics. Dissections were directly executed in situ. The whole brain including the pituitary gland and a portion of the gonad of all fish were collected and immediately stored in liquid nitrogen until processing.

2. Extraction of total RNA and cDNA synthesis

Total RNA was extracted from the pituitary, brain, and ovary samples of *M. cephalus* females using TRI reagent solution (Transzol, China). Almost 100 mg of the ovary was taken, whereas in the case of the brain or the pituitary, the entire tissue was used. The integrity of RNA is examined on the gel as in **Fig. 1**. Additionally, the purity and quantity of the extracted RNA were assessed using a Nanodrop (ND-1000 UV). To eliminate potential DNA contamination, the RNA samples were treated with DNase (Invitrogen, Thermo Fisher, USA) at 37 ˚C for 5 minutes.

To synthesize a first strand of cDNA, 2 µg of total RNA were used with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Thermo Fisher, USA) and oligo-dt primers. The reaction was accomplished consistent with the thermal conditions of incubation at 42 ˚C for 60 min and 70 ˚C for 5 min.

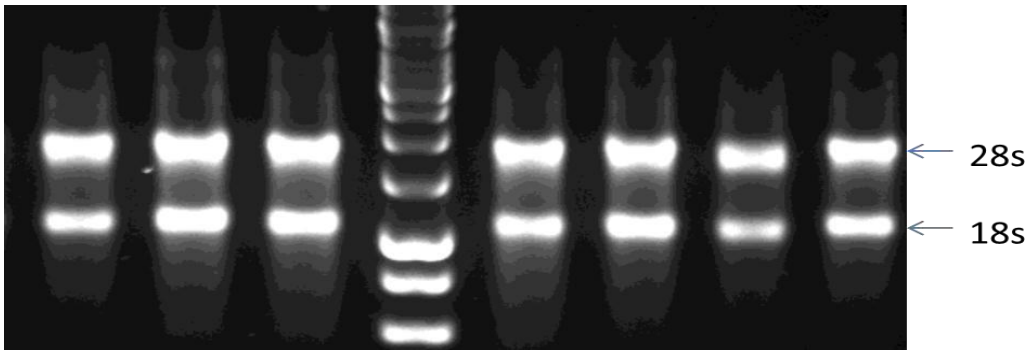

Fig. 1. The examined integrity of RNA

3. Real-time quantitative PCR (RT-QPCR)

The mullet *β-actin* was utilized as a reference gene for the QPCR assays (GenBank Accession No. XM047572269). The levels of mRNA of actin are relatively constant in the different tissues **(Nocillado *et al.* 2007)**. Mullet *Kiss2, Gpr54, Gnrh1*, and *Drd2* are studied in the brain and ovary while *Fshß*, *Lhß*, and *Slα* are examined in the pituitary and ovary. The gene-specific primers used for the target and reference gene amplification are shown in **Table 1** and it is scanned as in **Fig. 2**. Primers were specifically designed via (NCBI) for our study based on partial or complete cDNA sequences. The reaction was established by a Real-Time PCR system thermocycler (7300 Thermoscientific). Triplicate reactions were performed for the positively transcribed cDNA and their corresponding controls, respectively.

The 25 µl reaction volume consisted of 12.5 µL of SYBR Green fluorescent dye master mix (Maxima, Thermo Fisher), 0.6 pmol of the specific primer pair, 5 µL diluted cDNA template and the remaining volume was RNase water. Cycling parameters were as follows: 50 °C for an initial step for 2 min and 10 min at 95 °C, a denaturing step at 95°C for 15 s for 40 cycles, and 60 °C for an annealing step at 30 s, and finally an extension step at 72 °C for 30 s.

The cDNA from the immature female mullets was used as a calibrator. The data was normalized with a single internal reference gene to simplify the assessment of expression levels of the target genes in various tissues. The investigation of RT-QPCR assays was performed by the $2^{-\Delta\Delta Ct}$ method according to **Livak and Schmittgen, (2001)** and the normalization according to the method of **Pfaffl, (2001)**.

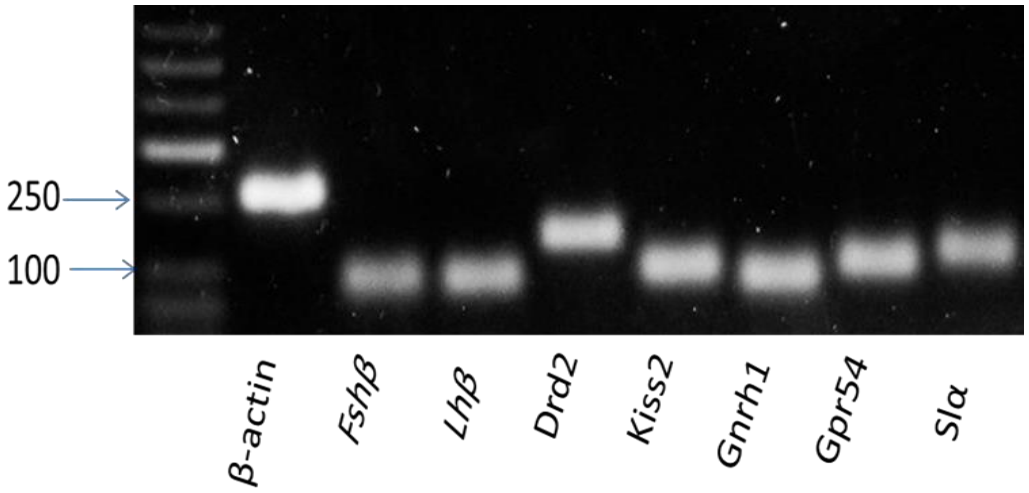

Fig. 2 The specific gene sequences with the primers

Table. 1 Gene-specific primers used for RT-QPCR analysis

Gene	Forward (5′–3′)	Reverse (5′–3′)	Length	Tm ˚C	Accession number
Lhβ	ATCTGGGCCTTTAGTCCAGC	TCTTGACAGGGTCCTTGGTG	160	60	MF574169
Fshβ	ATTAAAGGATGCCCGGTGGG	GCCATGCACTAGCAGGATGA	163	60	NC061772
Gnrh1	GGAAGAGGGAACTGGACAGC	GATTTTGGCGAAAGGCGTGT	116	60	KT248847
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

*Primers were designed by NCBI.

4. Statistical analysis

The transcription profile of the target genes was analyzed using SPSS statistical software (version 25). Values are expressed as means ± standard error (SEM). Expression levels in the experimental groups were normalized with the actin gene. The data were analyzed using one-way ANOVA and a nonparametric T-test with Pearson correlation (Graph-Pad prism 5) was used to assess the significance of the differences between groups. The level of statistical significance was set at $P < 0.05$.

RESULTS

1. Expression levels in the brain for *Kiss2, Gpr54, Gnrh1*, and *Drd2*

Figure 3 illustrates the temporal variations in the expression levels of the studied genes with maturation in the brains of captive and wild females. The brains of wild females showed significantly higher levels of *Kiss2* (10.47-fold; $P \leq 0.001$), while the expression of it in captive females was (4.27-fold; $P \leq 0.05$). *Kissr2* (*Gpr54*) relative expression exhibited 7.83-fold; $P \leq 0.01$ in the wild compared to 5.36-fold; $P \leq 0.05$ in the captive. Furthermore, *Gnrh1* expression levels in the brains of captive females considerably decreased (5.64-fold; $P \leq 0.01$) compared to the levels in wild females (13.37-fold; $P \leq 0.001$). Furthermore, the relative expression levels of *Drd2* in the brains of captive females changed significantly, revealing a 7.94-fold change ($P \leq 0.05$) compared to a 1.86-fold change ($P \leq 0.05$) in the wild ones.

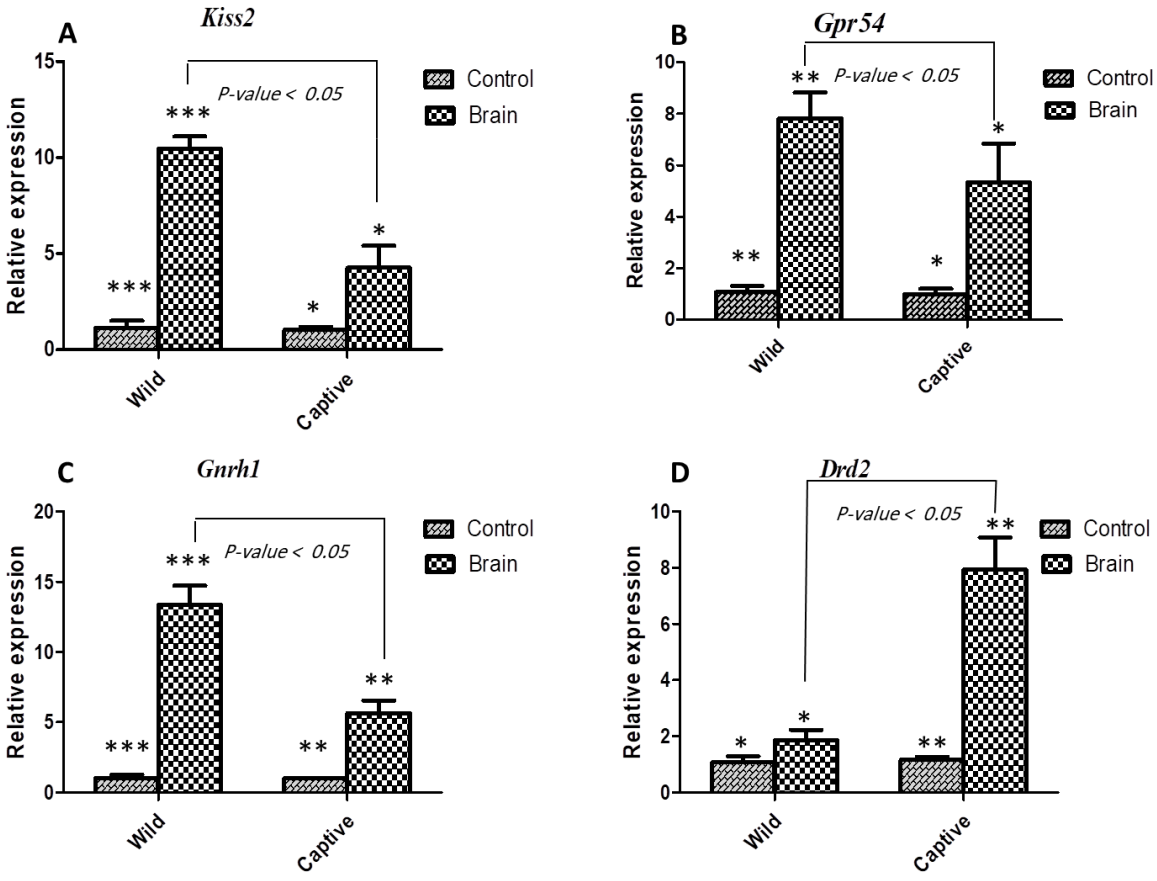

Fig.3 Relative gene expression levels of *Kiss2*: (A), *Gpr54*: (B), *Gnrh1*: (C) and *Drd2*: (D) were normalized to *β-actin* in the brain of wild and captive females of *M. cephalus* during the spawning season. Data are represented as mean ± SEM (n=3) for each group. *indicates significant differences ($P \leq 0.05$), ** indicates significant differences ($P \leq 0.01$) and *** indicates significant differences ($P \leq 0.001$).

2. Expression levels in the ovary for *Kiss2, Gpr54, Gnrh1* and *Drd2*

As illustrated in Fig. 4, mature females in both the wild and captivity had an increased transcription pattern of *Gnrh1, kiss2, Gpr54,* and *Drd2* in their ovaries compared to their expression in the immature ones, with distinct variations for each. In the ovary of wild females, *Gnrh1* expression was significantly higher (6.64-fold; $P \leq 0.01$) than in captive females (1.78-fold; $P \leq 0.05$). Likewise, *kiss2* expression in the wild females showed a significant increase (6.77-fold; $P \leq 0.05$) compared to the captive's (2.87-fold; $P \leq 0.05$). Inthe brains of wild females, *Gpr54* displayed a relative expression of (5.02-fold; $P \leq 0.05$) while in captive females, it was (4.07-fold; $P \leq 0.01$). Furthermore, the expression of *Drd2* in the ovaries was significantly lower in the wild females (1.35-fold; $P \leq 0.05$) than in the captive ones (4-fold; $P \leq 0.05$).

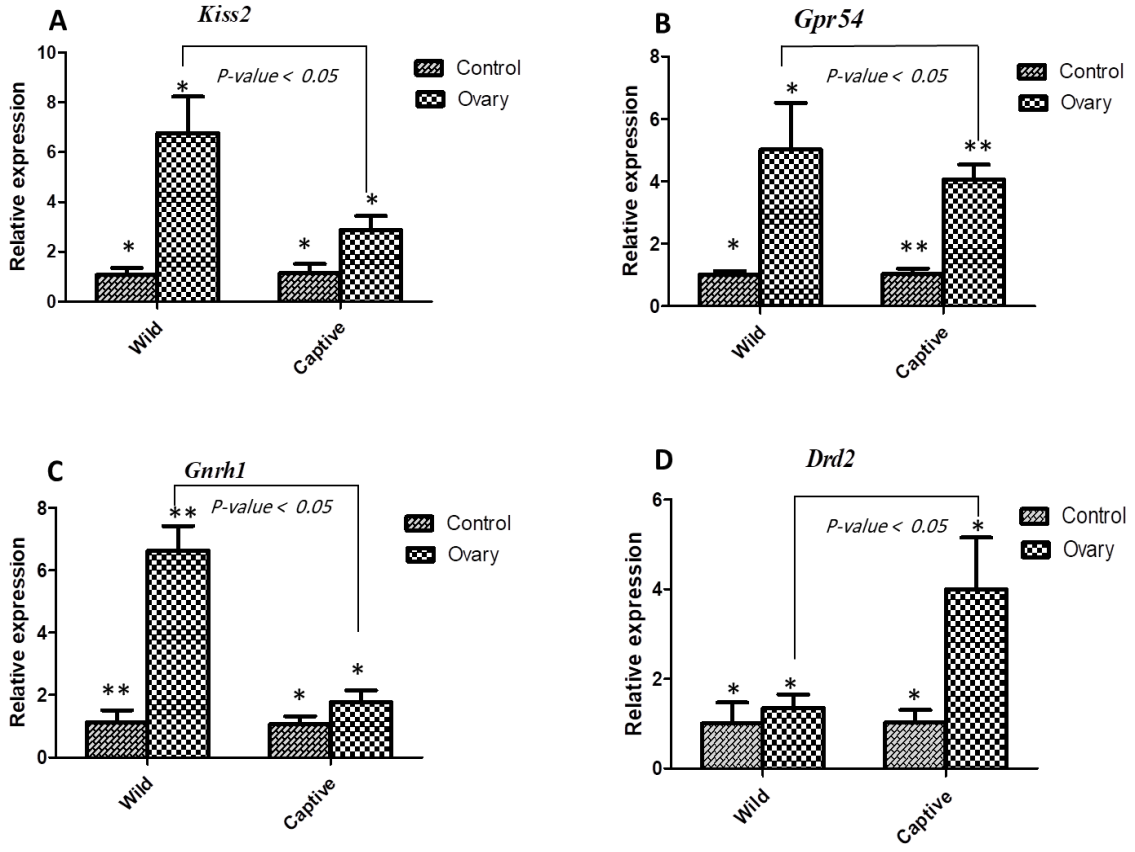

Fig.4 Relative gene expression levels of *Kiss2*: (A), *Gpr54*: (B), *Gnrh1*: (C) and *Drd2*: (D) were normalized to *β-actin* in the ovary of wild and captive females of *M. cephalus* during the spawning season. Data are represented as mean ± SEM (n=3) for each group. * indicates significant differences ($P \leq 0.05$), and ** indicates significant differences ($P \leq 0.01$).

3. Correlation between *Kiss2, Gpr54, Gnrh1* and *Drd2* at wild and captive environments

The various patterns of correlation between the analyzed gene expression profiles in the brain and ovary of wild females and captive ones are displayed in **Table (2)**. The investigated genes showed a direct Pearson association with significant values in the brains of wild females about the values of captives throughout the spawning period. Moreover, a direct correlation was observed in the ovaries of wild females for *Kiss2*, *Gnrh1*, and *Drd2* with their counterparts in the captive ones, while *Gpr54* revealed an inverse correlation in the ovaries within the two habitats.

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	***Kiss2***	0.79*	0.94*
	Gpr54	0.45*	-0.86*
	Gnrh1	0.95*	0.48*
	Drd2	0.99*	0.79*

*represents significance ($P \leq 0.05$)

4. Expression levels of *Fshß, Lhß,* and *Slα* in the pituitary gland

In the pituitaries of mature females, the genes under examination exhibited an increase in relative expression in comparison to the immature ones, as illustrated in **Figure 5**. Female wild pituitaries expressed *Fshß* at a higher relative expression level (14.39-fold; $P \leq 0.001$) than captive female pituitaries (6.32-fold; $P \leq 0.01$). Furthermore, *Lhß* in the pituitaries of wild females demonstrated a 12.23-fold increase; $P \leq 0.001$, whereas in the captive ones, it showed a 7.6-fold increase; $P \leq 0.01$. Additionally, the wild females exhibited a 3.38-fold increase in *Slα* expression ($P \leq 0.05$), while the captive females showed a slight variation (1.6-fold).

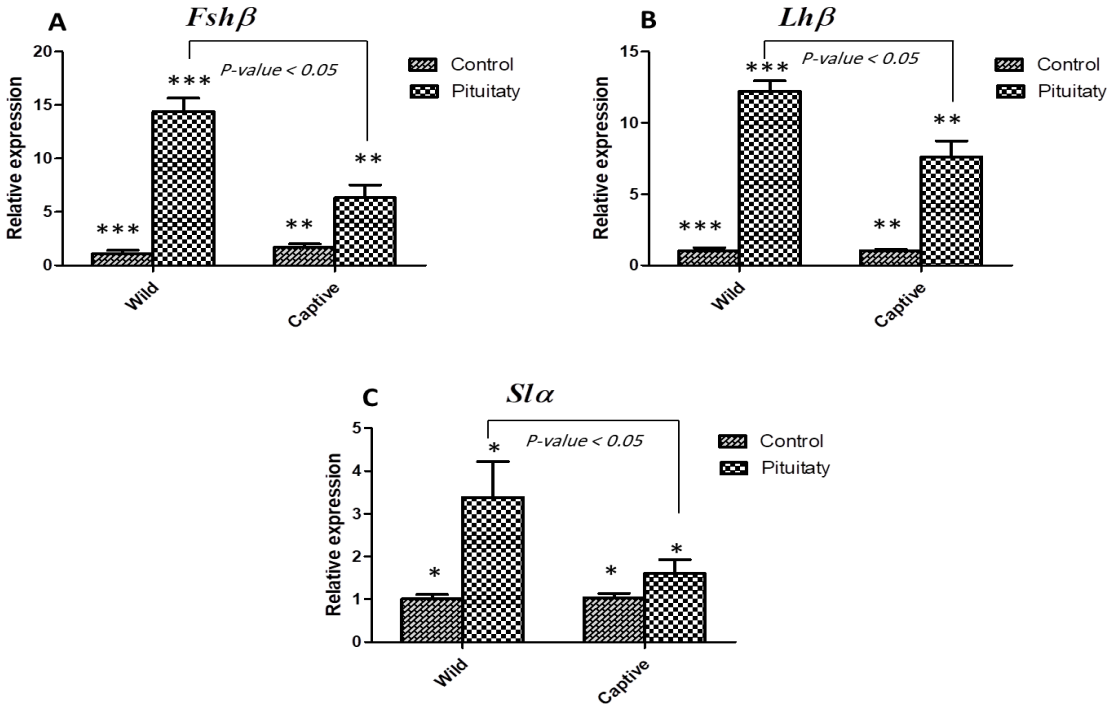

Fig. 5 Relative gene expression levels of *Fshβ:* (A), *Lhβ*: (B) and *Slα*: (C) were normalized to *β-actin* in the brain of wild and captive females of *M. cephalus* during the spawning season. Data are represented as mean ± SEM (n=3) for each group. *indicates significant differences ($P \leq 0.05$), **indicates significant differences ($P \leq 0.01$).

5. Expression pattern of *Fshß, Lhß,* and *Slα* in the ovary

As illustrated in Fig. 6, the expression levels of these genes in the ovaries of mature females demonstrated a noteworthy rise correlated with the expressions in immature females. The expression of *Fshß* was reduced in the captive population (3.8-fold; $P \leq 0.05$) compared to the wild females' ovaries, which showed an 11.62-fold increase ($P \leq 0.05$). In the ovaries of wild females, the relative expression of *Lhß* was 9-fold ($P \leq 0.001$), but in the captive females, it was 5.05-fold ($P \leq 0.05$). Lastly, compared to the expression of *Slα* in the ovaries of captive females 1.56-fold ($P \leq 0.05$), wild females displayed (2.58-fold; $P \leq 0.05$).

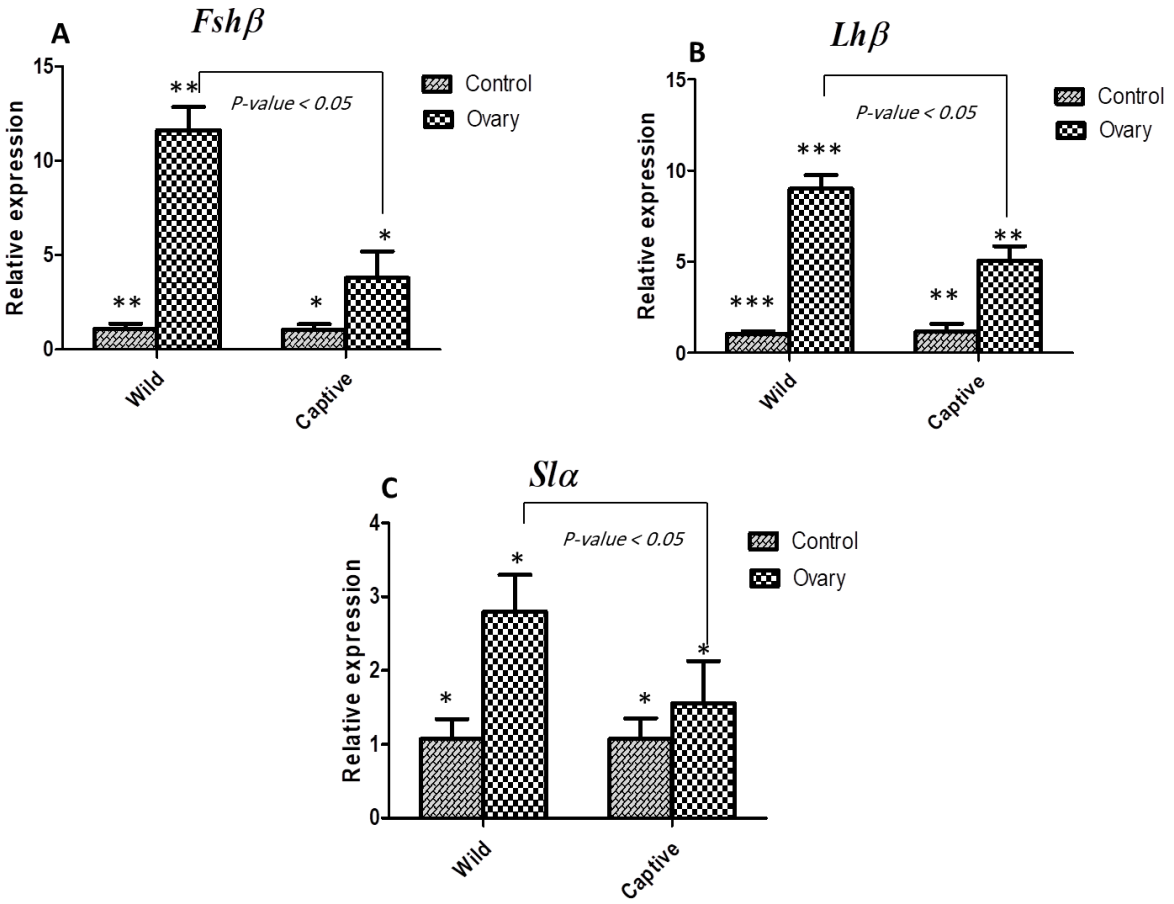

Fig.6 Relative gene expression levels *Fshβ:* (A), *Lhβ*: (B), and *Slα*: (C) normalized to *β-actin* in the ovary of wild and captive females of *M. cephalus* during the spawning season. Data are represented as mean ± SEM (n=3) for each group. *indicates significant differences ($P \leq 0.05$), **indicates significant differences ($P \leq 0.01$), and ***indicates significant differences ($P \leq 0.001$).

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 pituitaries and ovaries of wild females seem to be significantly correlated with those of their counterparts in captive females as shown in **Table (3)**. The direct relationship between *Fshβ* and *Lhβ* in the pituitaries and ovaries was observed. Furthermore, the direct association of *Slα* in the ovary was displayed as well, while, the negative correlation for *Slα* in the pituitary was observed.

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

Pearson correlation	Gene	Pituitary	Ovary
	Fshβ	0.2*	0.92*
	Lhβ	0.31*	0.65*
	Slα	-0.36*	0.99*

*represents significance ($P \leq 0.05$

DISCUSSION

By contrasting wild and cultured broodstocks during the spawning season, this work is the first report of the gene expression profiles by QRT-PCR in three separate tissues (brain, pituitary, and ovary) in female *M. cephalus*. Our research shed light on the potential effects ofconfinement on patterns of reproductive gene expression that differ from those found in the natural environment.

1. Expression pattern of *Kiss2, Gpr54, Gnrh1* and *Drd2* in the brain and ovary

Our research revealed significant variations in the patterns of *Kiss2, Gpr54,* and *Gnrh1* gene expressions in the brains and ovaries during spawning time for *M. cephalus* between wild and captive females. The relative expressions of these genes are higher in the wild than in the captive. Interestingly, also they express more in the brains than in the ovaries. In terms of *Drd2* expression, it appears that brains and ovaries of the wild females *M. cephalus* express less of the gene than do captive ones.

The study by **Shahjahan *et al.* (2010)** documented that, the *kiss2* has significantly elevated expression in the brain of adult grass puffer (*Takifugu niphobles*) through the pre-spawning and spawning periods in both males and females. They also reported the expressive increases in *Kiss2* and *Kissr2* mRNAs in the brain of both male and female grass pufferfish during the spawning period, representing an intense confident association between the extent of *Kissr2*, *Kiss2*, and *Gnrh1* mRNAs in the brain during the spawning season. The report by **Kanda *et al.* (2008)** represented that the kisspeptin system is critical for organizing reproduction in various fish species. **Ohga *et al.* (2013)** indicated that the kisspeptin in sexually mature fish has eight to ten times higher levels of mRNA than that observed in immature fish. As mammals, the kisspeptin system in fish is a key player in regulating the timing of puberty and controlling the secretion of gonadotropins in the various reproductive stages **(Han *et al.* 2005)**.

The significant increase in *Kiss2* transcript levels in wild females during maturation suggests its crucial role in the reproductive processes. The opposite trend in *Gpr54* in both wild and captive females may indicate a complex regulatory relationship between kisspeptin and its receptor during maturation. The kisspeptins are essential for coordinating reproduction in a variety of fish species which helps them control the release of gonadotropins during different stages of reproduction **(Kanda *et al.* 2008)**. Moreover, the study of **Ohga *et al.* (2013)** revealed that the levels of mRNA in kisspeptin are eight to ten times higher in sexually mature scombroid fish (*chub mackerel*) than in immature fish. According to a study by **Shahjahan *et al.* (2013)**, *kiss2* expression is markedly upregulated in the brains of adult grass puffers (*Takifugu niphobles*) throughout the pre-spawning and spawning phases in both males and females.

The significant increase in both *kiss1* and *kiss2* in the brains compared to juvenile and prepubertal fish, as well as their receptors, *kissr1* and *kissr2* (*Gpr54*) were reported by **Zmora *et al.* (2012)**, and **Fairgrieve *et al.* (2016)**.

Ohga *et al.* (2018) established a pattern that is maintained for a wide range of species: the expression of the kisspeptin system at the gonadal level is directly linked to the development of the gonadal organ. As grey mullet matured, there was an increasing tendency in the ovarian expression of *Kiss2* **(Nocillado *et al.* 2007)**. Additionally, *Kissr2*

expression increases quickly in both male and female goldfish **(Li *et al.* 2009)**, fathead minnows **(Filby *et al.* 2008; Song *et al.* 2016)**, and tongue soles throughout the gonadal differentiation.

Unlike the ovary, where *Gpr54* was found to be more highly expressed in the brain, with consistent of the majority of teleosts, such as zebrafish, fathead minnows, chub mackerel, and tongue sole, expressed significantly more *Gpr54* in their brains than in their gonads and other organs **(Wang *et al.* 2017; Ohga *et al.* 2018)**.

The *kiss-kissr-Gnrh* system in fish is essential for integrating environmental indications and metabolic signals and relaying this information to the reproductive axis, according to studies by **Faheem *et al.* (2019)**, and **Thakuria *et al.* (2017)**.

A positive correlation was reported between the amount of *Kiss2*, *Kissr2*, and *Gnrh1* mRNA in the brain during the spawning season**.** These studies are consistent with the study of **Kitahashi *et al.* (2009)** on Zebrafish. About the significant increase in *Gnrh1* transcript levels in wild females aligns with its role in stimulating gonadotropins release.

Other studies by **Pati and Habibi, (1998)** on the goldfish, the findings of **Nabissi *et al.* (2000)** in gilthead sea bream, and **Uzbekova *et al.* (2001)** on rainbow trout have proved that the ovarian expression of *Gnrh* is concerned with the development of oocyte. These outcomes are in agreement with our results.

Guzmán *et al.* (2009) found that there was a notable distinction of *Gnrh1* expression levels, in the captive females of Senegalese soles (*Solea senegalensis*) exhibiting lower levels than the wild counterpart. Our research revealed a significant differential expression pattern in *Gnrh1* of wild *M. cephalus*; which exhibited 13.37-folds compared to 5.64-folds in the captive.

As shown in mullet, dopamine also inhibits basal *Gnrh* and *LH* production in a variety of teleost species like tilapia (*Oreochromis mossambicus*) **(Yaron *et al.* 2003)**, rainbow trout (*Oncorhynchus mykiss*) **(Vacher *et al.* 2003)**, grey mullet (*M. cephalus*) (**Nocillado *et al.* 2007**) and in grass carp **(wang *et al.* 2011).**

Drd2 transcripts have been discovered in the ovaries of grey mullet **(Nocillado *et al.* 2007)**, tilapia **(Levavi-Sivan *et al.* 2005)**, and Anguilla **(Pasqualini *et al.* 2009)**, indicating a direct *DA* impact on the fish gonadal steroids that alter the expression of the *Drd2* gene. **Aizen *et al.* (2005)** discovered that in captive grey mullet, *M. cephalus*, dopaminergic inhibition is a major barrier along the reproductive neuroendocrine axis that prevents spontaneous spawning.

2. Expression pattern of *Lhβ, Fshβ,* and *Slα* genes in the pituitary and ovary

The findings of this research may report that the pituitaries and ovaries of wild female broodstocks have higher levels of *Fshβ, Lhβ*, and *Slα* mRNA in *M.* than their captive counterparts.

It is well known that throughout different stages of reproduction in different teleosts, *Fshβ* and *Lhβ* are expressed in the brain, pituitary, and gonads **(Chi *et al.* 2017)**. **Mechaly *et al.* (2012)** stated that from the beginning to the end of vitellogenesis, female mullets have higher expression levels of both *Fshβ* and *Lhβ* in the pituitary.

Moreover, reproductive events are known to be correlated with an increase in the *Fsh* level, and gametes require accurate management of this level for proper growth **(Nyuji *et al.* 2014)**. However, during the spawning time, female goldfish (*Carassius auratus*) had higher *Fshβ* mRNA levels than males, whereas *Lhβ* mRNA levels were equivalent in both sexes **(Sohn *et al.* 1999)**. Similarly, striped bass (*Morone saxatilis*), red seabream (*Pagrus major*), Japanese flounder (*Paralichthys olivaceus*), and red seabream (*Pagrus major*) have co- regulated levels of *Lhß* and *Fshß* mRNA (**Weltzien *et al.* 2004**; **Levavi-Sivan *et al.* 2010**).**Mechaly *et al.* (2012)** indicate that in female mullets, there are elevated expression levels of *Lhβ* and *Fshβ* in the pituitary as well starting from the initial to advance of vitellogenesis. They also mentioned a coordinated communication between the brain (kisspeptin system) and the pituitary in female mullets. The elevation of the kisspeptin system precedes the increase in gonadotropin gene expression, implying a potential role for kisspeptins in stimulating the release of *Lhβ* and *Fshβ* during reproductive stages, a pattern seen in various teleost species. It is well established that an upturn in the *Fsh* level is often associated with the onset of reproductive events, and its precise regulation is critical for the successful development of gametes **(Nyuji *et al.* 2014)**.

Remarkably, limited studies have been conducted on the transcription of *Gnrh1* in the brain of farmed fish compared to the more detailed attention given to *Lh* and *Fsh* in the pituitary. Additionally, in wild fish, there is a parallel elevation of the expression of these genes during reproductive stages **(Nyuji *et al.* 2012)**.

The interaction between the brain and pituitary was demonstrated by the up-regulation of the kisspeptin system and gonadotropins during the cortical alveolar stage **(Levavi-Sivan *et al.* 2010; Mechaly *et al.* 2012; Espigares *et al.* 2015)**. **Kusakabe *et al.* (2006)** and **Zmora *et al.* (2007)** have recorded that *Lh* and *Fsh* operate on the gonads to produce additional gonadal factors and sex steroids, which are essential for gamete formation and maturation.

The results published by **Bhandari *et al.* (2006)** indicated that the pituitary of *M. cephalus* expresses more *Slα* mRNA with the maturation. Additionally, both *Slα* and *Slβ* were significantly improved before and during spawning, going from two-folds for *Slα* and seven-folds for *Slβ* **(Benedet *et al.* 2008)**. This advocates *Slα* with a further fundamental role in the endocrine regulation of physiological procedures in teleost fish. **Degani, (2015)** found that *Sl* may be involved in vitellogenesis rather than maturation in female blue gourami. Our results, which showed that *Slα* was expressed 3.38 times in the pituitaries of wild *M. cephalus*, are in agreement with these findings. The findings of **Taniyama *et al.* (2000)** also showed that the expression of the *Sl* gene is influenced or improved by *Gnrh1* in the pituitary of maturing sockeye salmon.

Selvaraj *et al.* 2021, proposes that the little actions of the BPG axis, including kisspeptins, *Gnrh*, and sex steroids, have been mainly implicated in the dysfunction of the reproductive system in captivity. According to **Nyuji *et al.* (2020)**, two factors affect the expression of the BPG axis gene: external factors such as feeding schedule, dosage, and water temperature, and internal factors such as fish development and age. According to **Guzmán *et al.* (2009)**, the reproductive process will be negatively impacted if the BPG system is likely to be compromised in captivity. In captivity, jack mackerel may

experience reproductive impairment due to blockage of the BPG-axis, as proposed by **Imanaga *et al.* (2014)**.

In conclusion, it was shown that fish kept in captivity expressed the least amounts of *Kiss2*, *Gpr54*, *Grnh1*, *Fshβ*, and *Lhβ*. When compared to the wild female *M. cephalus*. *Gnrh1* expressions in the brains were higher and tended to correlate with the expression of *Fshβ, Lhβ*, and *Slα* in the pituitary of wild females, while the expression of *Lhβ* and *Fshβ* in the pituitaries of captive fishes may be inversely correlated with *Gnrh1* transcription levels in the brains. Whereas the expression levels of *Drd2* are more likely to be stumpy in the wild fishes than in the captive ones.

We suggest that this study clarify the function of the kisspeptin system (*Kiss2* and *Gpr54*) and how it contributes to spawning because of the observed variations in its gene expression in the two habitats and *kiss2* relation to *Lhβ* secretion. Consequently, it is important to investigate it in a lab setting by injecting it into captive mullet fish, *M. cephalus* determining how much of their impact on the spawning process. According to the study, *Gnrh1* and *Drd2* have a significant impact on reproduction, and their inverse relationship in the wild helps the spawning process to be completed, whereas direct correlation in captivity prevents it from happening. The research provides additional context for the involvement of *Slα* since it was found to have statistically significant relationships with *Gnrh1* and to have a noticeably less increase in gene expression in the captive.

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