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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 current research work provided insights into the expression patterns of key genes in the BPG axis of *Mugil cephalus* females, highlighting differences between wild and captive environments during the spawning season. The investigated genes include *Kiss2*, *Gpr54*, *Gnrh1*, and *Drd2* in the brain and ovary, as well as *Lhβ*, *Fshβ*, and *Slα* in the pituitary and ovary. The finding indicated that the relative expression of all examined genes is elevated in mature females compared to immature ones across all examined tissues (brain, pituitary, and ovary). The observation that the expressions of the examined genes in wild females of *M. cephalus* are highly correlated, either directly or inversely, with their counterparts in captive females, indicated a connection between gene expression patterns and the different environments (wild vs. captive). The results between wild and captive females were in significant values ($P \leq 0.05$). Overall, the observed correlations and significant differences in gene expression proposed potential impacts of captivity on the reproductive physiology of these fish.

INTRODUCTION

Mugil cephalus is an 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 toward 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 of **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, they 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 definitely 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 & 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 aimed 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. These genes are also examined in the ovary. This study involved comparing individuals raised in captivity with those captured from the wild. This approach was 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 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 47cm, and the whole weight ranged from 784.4 to 1141.1g. 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 50.5cm, and total weights ranging from 919 to 1544g. 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 100mg 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 exhibited 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, an amount of 2µg of total RNA was 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 60min and 70°C for 5min.

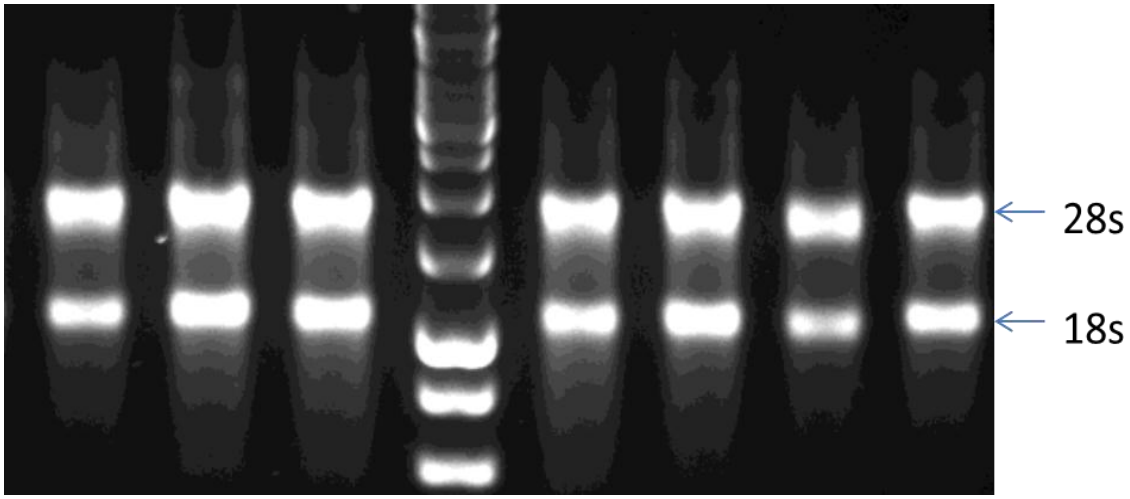

Fig. 1. The examined integrity of RNA

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 mRNA of actin is 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 they are scanned, as displayed in Fig. (2). Primers were specifically designed (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 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. The cycling parameters were as follows: an intial step at 50°C for 2min, followed by 10min at 95°C, then 40 cycles of denaturation at 95°C for 15s, annealing at 60°C for 30s, and a final extension step at 72°C for 30s.

The cDNA from immature female mullet was used as a calibrator. The data were 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 following **Livak and Schmittgen (2001)** protocol, with normalization carried out according to the method of **Pfaffl (2001)**.

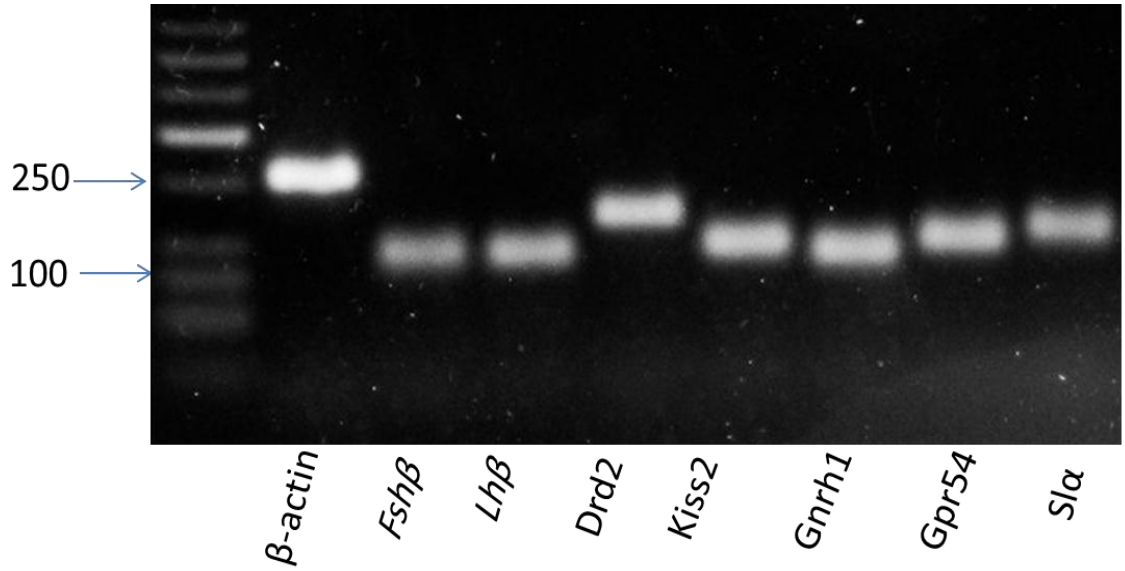

Fig. 2. The specific gene sequences with the primers

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 (GraphPad prism 5) that 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*

The investigated genes in the brain of wild and captive females showed temporal variations in the expression levels with maturation, as shown in Fig. (3). Significantly higher levels of *Kiss2* were recorded in the brain of wild females (15.33fold; $P\leq 0.001$), with a less pronounced difference of its expression in captive female (11fold; $P\leq 0.05$). The relative expression of *kissr2* (*Gpr54*) was shown in the wild (8.96fold; $P\leq 0.01$) in opposition to

(4.63fold; $P \leq 0.05$) the captive. Moreover, the relative expression levels of *Gnrh1* in the brain declined significantly in the captive female (4.54folds; $P \leq 0.05$) relative to levels in the wild in the female (9.18fold; $P \leq 0.05$). The relative expression levels of *Drd2* in the brain of wild females exhibited significant changes with the maturation (8.51fold; $P \leq 0.05$) in contrast to (5.34fold; $P \leq 0.05$) the captive ones.

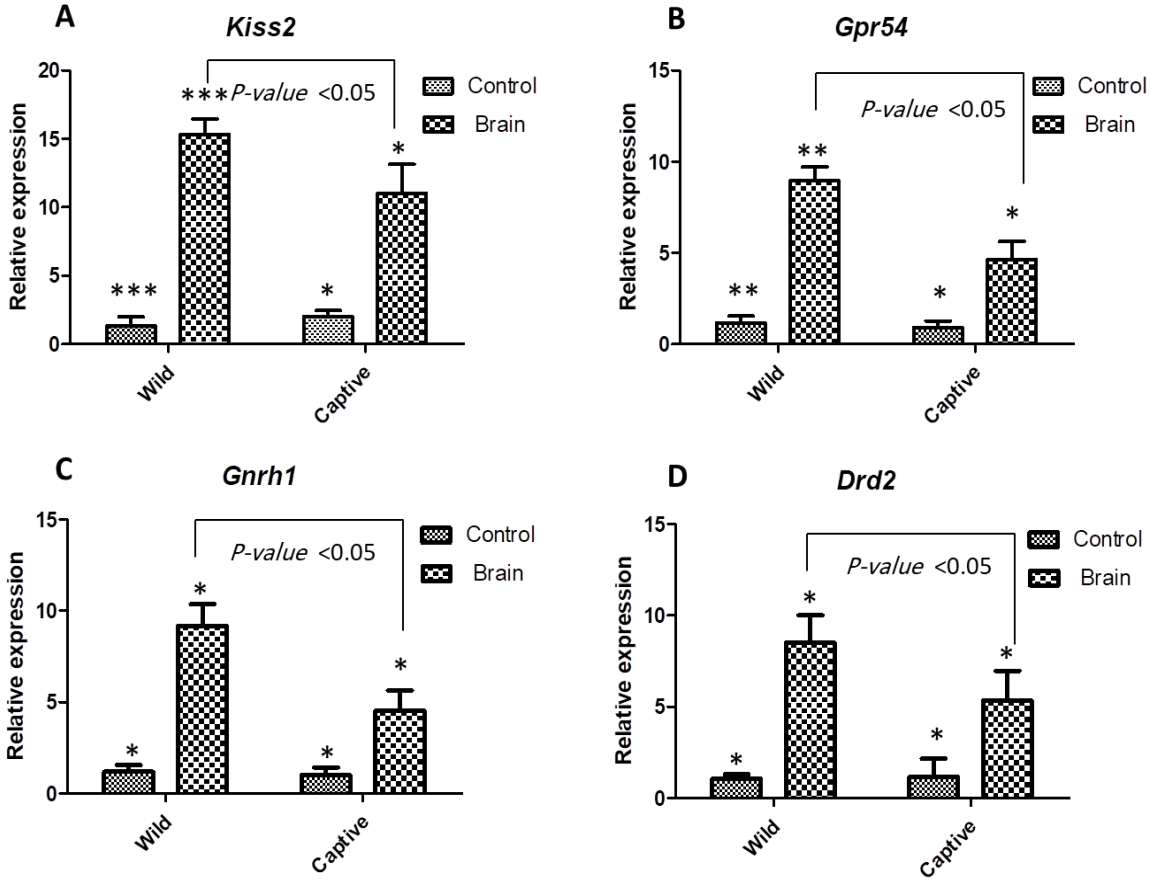

Fig. 3. Relative gene expression levels of *Kiss2* **(A)**, *Gpr54* **(B)**, *Gnrh1* **(C)**, and *Drd2* **(D)** were normalized to *β-actin* in the brain of wild and captive females of *M. cephalus* during the spawning season

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*

Wild mature females showed an increased transcription pattern of *Gnrh1, kiss2, gpr54,* and *Drd2* in the ovary than their expression in the immature females, as displayed in Fig. (4). A significant increase was detected for *Gnrh1 (*14.65fold; $P \leq 0.05$) in the ovary of wild females with a reduced expression in captive (6.5fold; $P \leq 0.05$). Moreover, the *kiss2* revealed a marked increase in the wild (11.34fold; $P \leq 0.01$) in contrast to (5.88fold; $P \leq 0.05$) that recorded in the captive. The relative expression of *Gpr54* is 9.53fold ($P \leq 0.01$) for the wild females and it showed 6.5 fold ($P \leq 0.01$) in the captive ones. Finally, the wild females

expressed 6.14fold ($P \leq 0.05$) for *Drd2* in the ovary, while a value of 4.16fold ($P \leq 0.001$) was recorded in the ovary of captive females.

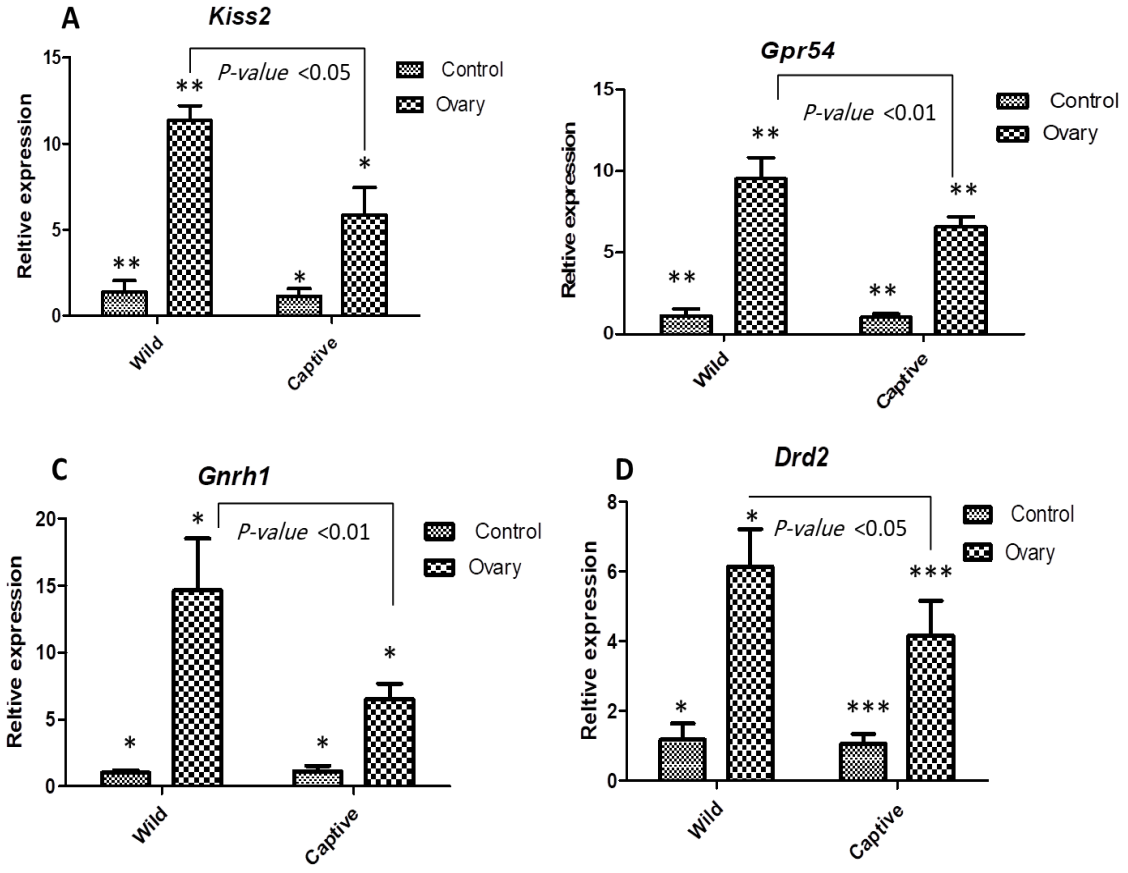

Fig. 4. Relative gene expression levels of *Kiss2* **(A)**, *Gpr54* **(B)**, *Gnrh1* **(C)**, and *Drd2* **(D)** were normalized to *β-actin* in the ovary of wild and captive females of *M. cephalus* during the spawning season

Data are represented as mean ± SEM (n= 3) for each group. * indicates significant differences ($P \leq 0.05$), ** indicates significant differences ($P \leq 0.01$), and *** indicates significant differences ($P \leq 0.001$).

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

The different correlation patterns in the expression profiles of examined genes between the brain and ovary of wild females compared to captive females are shown in Table (2). The direct Pearson correlation with significant values was observed in the brains of wild females related to the values of captives during the spawning season, whereas the ovary showed a reverse correlation for *Gpr54* and *Gnrh1*, and a direct correlation of *Kiss2* and *Drd2* was represented. Notably, the correlations are significant, as displayed in Table (2).

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***	0.9*	-0.24**
	Kiss2	0.99*	0.12*
	Gnrh1	0.85*	-0.94**
	Drd2	0.005*	0.99*

*represents significance (P≤ 0.05), **represents significance (*P*≤ 0.01).

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

The examined genes in the pituitary of mature females showed an increment of their relative expression corresponding to the immature ones, as represented in Fig. (5). *Fshß* showed (15.34fold; *P*≤ 0.01) relative expression compared to (12.16 fold; *P*≤ 0.01) captive females. Moreover, wild females showed the same transcription pattern for both *Lhß* and *Slα* (12.9 fold; *P*≤ 0.01) in the pituitary, whereas a substantial expression was recorded (11.05fold; *P*≤ 0.05) for *Lhβ* ,and (7.26fold; *P*≤ 0.05) for *Slα* in the captive females.

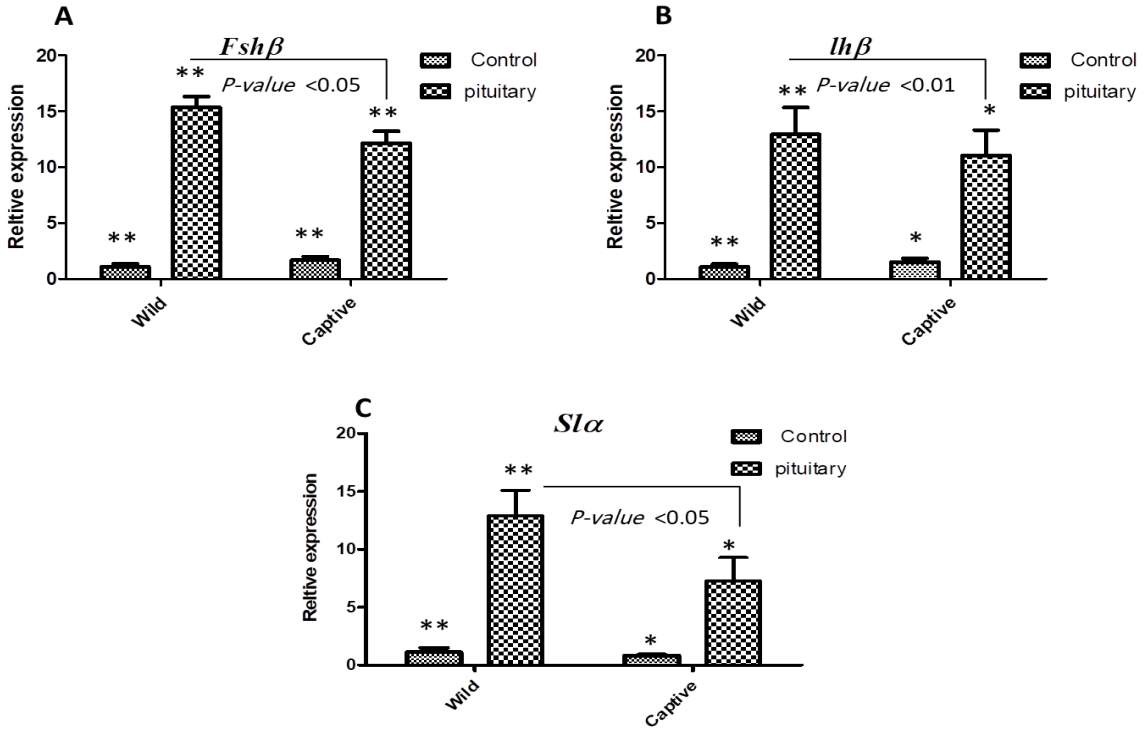

Fig. 5. Relative gene expression levels of *Lhβ* **(A)**, *Fshβ* **(B)**, and *Slα* **(C)** were normalized to *β-actin* in the brain of wild and captive females of *M. cephalus* during the spawning season

Data are represented as mean ± SEM (n= 3) for each group. * indicates significant differences (*P*≤ 0.05), and ** indicates significant differences (*P*≤ 0.01).

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

The expression levels of these genes in the ovary showed a significant elevation in their expression related to the expression of immature females, as shown in Fig. (6). The ovary of wild females revealed 10.48fold ($P \leq 0.05$) for *Fshß* with a reduction in its expression in the captive (7.08fold; $P \leq 0.05$). The relative expression of *Slα* in the wild is 8.65fold ($P \leq 0.05$), nevertheless it expressed 6.12fold ($P \leq 0.05$) in the captive. Finally, the ovary of wild females showed (7.84fold; $P \leq 0.01$) for *Lhß* in contrast to (4.9fold; $P \leq 0.05$) in the captive ones.

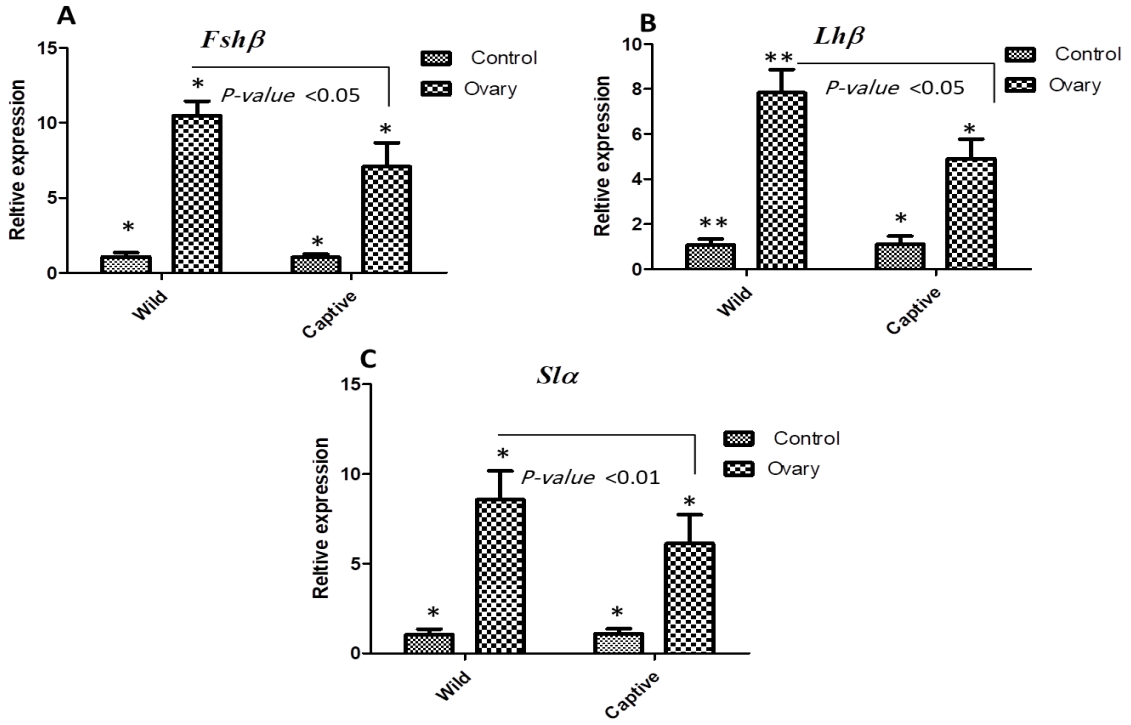

Fig. 6. Relative gene expression levels *Lhβ* **(A)**, *Fshβ* **(B)**, and *Slα* **(C)** normalized to *β-actin* in the ovary of wild and captive females of *M. cephalus* during the spawning season

Data are represented as mean ± SEM (n= 3) for each group. *indicates significant differences ($P \leq 0.05$), and **indicates significant differences ($P \leq 0.01$).

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

It appears that there is a significant correlation between the expression patterns of these genes (*Fshβ, Lhβ,* and *Slα*) in the pituitary and ovary of wild females and their counterparts in captive females. A reverse correlation was shown for *Fshβ* in the pituitary and ovary. *Lhβ* also showed a reverse correlation only in the pituitary. Moreover, the relations of *Lhβ* in the ovary and *Slα* in the pituitary and ovary exhibited a direct correlation, as shown in Table (3).

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

Pearson correlation	Gene	Pituitary	Ovary
	Fshβ	-0.14*	-0.19*
	Lhβ	0.99**	0.41*
	Slα	-0.15*	0.2**

*represents significance ($P \leq 0.05$), and **represents significance ($P \leq 0.01$).

DISCUSSION

By investigating the gene expression profiles in different tissues (brain, pituitary, and gonads), our study provided insights into how captivity might influence the reproductive gene expression patterns compared to those observed in the natural habitat. Understanding these patterns is essential for comprehending the reproductive physiology of grey mullets, which has implications for both natural populations and artificial fertilization practices in fish species.

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

Our findings advocate that the distinct patterns of gene expression in the brain are associated with the spawning season, and there are notable differences between wild and captive females. The increase in *Kiss2* expression in the brains of wild females, along with the concurrent increases in *Gpr54*, *Gnrh1*, and *Drd2*, implies a potential regulatory relationship among these genes during puberty in the natural habitat. Furthermore, the reduction in the expression of these genes in the brains of captive females has an impact on the reproduction process. The differences observed between wild and captive females could be attributed to environmental factors, captivity conditions, or other variables influencing gene regulation.

The study by **Shahjahan *et al.* (2010)** documented that *kiss2* exhibits significantly elevated expression in the brain of adult grass puffer (*Takifugu niphobles*) through the pre-spawning and spawning periods in both males and females. Additionally, they 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)** presented 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 levels of mRNA more than that observed in immature fish. Similar to 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 current study showed a significant correlation in the relative expression patterns of *Gpr54* and *drd2* in the brain, aligining with the findings of **Nocillado *et al.* (2007)**, who documented an inhibition impact of dopaminergic signaling on *Gpr54* in grey mullet. This

represents a potential connection or coordinated regulation between these two genes in the context of the reproductive processes.

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 significant increase in *Gnrh1* transcript levels in wild females aligns with its role in stimulating gonadotropin release.

The decline in captive females may reflect differences in environmental cues affecting the regulation of *Gnrh1* during maturation. The increase in *Drd2* transcript levels in wild females submits a potential role in the maturation process. The less pronounced increase in captive females may be indicative of environmental influences on dopamine receptor expression during maturation.

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

Overall, it appears that in wild females, the specified genes show increased expression in the ovary during the spawning period. Notably, *Gnrh1* and *Gpr54* have higher expression levels in the ovary compared to the brain. Oppositely, in captive females, the relative expression levels for these genes are lower compared to wild females.

The findings from **Nocillado *et al.* (2007)** supported the crucial role of *Gnrh1* in the ovary during the puberty stages of *Mugil*. Additionally, they identified that the increased expression of *Gnrh1* in the ovary is linked to its involvement in sustaining gonadal development. 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. Consequently, these outcomes are in agreement with our results.

The findings of **Ohga *et al.* (2018)** highlighted a consistent pattern across various species, where the expression of the kisspeptin system at the gonadal level is directly correlated with the progress of gonadal development. Ovarian expression of *Kiss2* exhibited an increment trend in the early development of the grey mullet **(Nocillado *et al.*, 2007)**. Furthermore, **Zmora *et al.* (2012)** indicated that in females of striped bass, there are significant changes in the expression levels of *Kiss1* and *Kiss2*, as well as their receptors, with a notable difference between mature females and immature females.

Dopamine frustrates basal *Gnrh* and *Lh* secretion as documented in mullet and numerous teleost species **(Yaron *et al.*, 2003; Aizen *et al.*, 2005)**. Previous studies by **Levavi-Sivan *et al.* (2004)** and **Dufour *et al.* (2005)** reviewed that dopamine is expressible through the *Drd2 in vivo* and *in vitro*, as well as inhibiting and preventing the synthesis and release of *Gnrh* and its receptors. Studies on the Nile tilapia have shown that increasing concentrations of 17b- estradiol resulted in increased *Da* receptor 2 (*Drd2*) mRNA levels both *in vivo* and *in vitro*, inhibition of *Lh* and *Fsh* release, and inhibition of *lhb* mRNA levels *in vivo* **(Levavi-Sivan *et al.*, 2006)**.

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

The results suggest that the relative expression levels of *Fshβ, Lhβ*, and *Slα* in the pituitary of female *M. cephalus* are influenced by the environment (wild vs captive). Wild individuals displayed a more balanced and parallel expression pattern, while captive individuals showed a decline in the expression of these genes, with *Fshβ* maintaining the highest expression level. This information provides insights into the potential impact of captivity on the pituitary gene expression related to reproductive processes in female *M. cephalus*.

Mechaly *et al.* (2012) elucidated that, in female mullets, there are elevated expression levels of *Lhβ* and *Fshβ* in the pituitary, starting from the initial to advance of vitellogenesis. Additionally, they 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 increase in the expression of *Slα* mRNA in the pituitary of *Mugil cephalus* was reported in the study of **Bhandari *et al.* (2003)**. Additionally, the previous authors suggested a dynamic amplified pattern of *Sl* mRNA levels about the reproductive stages of salmon, particularly masu salmon and chum salmon. Furthermore, *Slα* and *Slβ* improved considerably before and through spawning to be 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.

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) proposed 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.

In conclusion, the lowest expression of *Grnh1* and *Gpr54* in captive fish was observed. Moreover, the transcription levels of *Gnrh1* could have an inverse relation to *Lhβ* and *Fshβ* expression in captive *M. cephalus*. Compared to the wild female *M. cephalus*, the expression of *Kiss2*, *Gpr54* and *Drd2* in the brains of the captive fish was dramatically lower, while *Gnrh1* revealed a higher expression that tended to correlate with the expressions of *Fshβ*, *Lhβ*, and *Slα* expression in the ovary of wild females, whereas the expression levels of these genes are inclined to be stumpy in the captive fish. The findings suggest that optimizing

aquaculture conditions to better mimic the natural environment could lead to improvements in reproductive success and breeding programs for this species.

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