Hormone-Secreting Cells in the Hypophysis of *Mugil cephalus*

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ABSTRACT

Understanding the anatomy of endocrine glands in fish allows for the formulation of a strategy for their reproduction. The current investigation aimed to investigate the immunohistochemical identification of hormonal-secreting cells in *Mugil cephalus* pituitary gland. Antisera against both piscine and mammalian hormones were used to complete the immunohistochemistry of the cell types in *M. cephalus* pituitary gland. The adenohypophysis and the neurohypophysis made up the two main sections of *M. cephalus*'s hypophysis. Moreover, the adenohypophysis obtained exhibited three subdivisions: rostral pars distalis (RPD), proximal pars distalis (PPD), and pars intermedia (PI). In the glandular portion, seven cell types were identified according to their distribution, immunoreactivity, and grouping. The cells of prolactin (PRL) were acidophilic and occupied most of the RPD. They responded positively and specifically to antiserum against salmon PRL. Positive immunoreactivity was observed by antiserum of human adrenocorticotrophic (ACTH), within the lead hematoxylin-positive (PbH+) cells that surrounded the RPD neurohypophysis. Salmon growth hormone (GH) antiserum produced a specific and intense immunoreactivity in the PPD's acidophilic cells. Furthermore, basophilic cells, exhibiting a strong and distinct immunoreactivity to antisera against the subunits Iβ and IIβ of salmon gonadotropin (GTH), were localized in PPD's central area. The cells thought to secrete thyrotropin (TSH) were located between the PPD and RPD in the neurohypophysis. They underwent an adverse immunoreactivity when reacted to rat thyrotropin (rBetaTSH) antiserum. Within the PI, two cell types were identified: PbH+ cells that immunoreacted with alpha-melanin-stimulating hormone (α-MSH) antiserum and cells that displayed a positive immunoreaction to anti-salmon somatolactin (SL) and were classified as Periodic Acid-Schiff-positive (PAS+). Current pituitary gland immunohistochemistry of *M. cephalus* may help to explain hormonal alterations that take place during reproduction in the nature, in captivity, or when this fish is receiving hormonal treatments.

INTRODUCTION

Fish reproduction is primarily managed by the hypophyseal-gonadal axis. Every species of vertebrate has two major parts to the pituitary gland: the adenohypophysis (glandular) and the neurohypophysis (nervous). The teleostean adenohypophysis is generally separated into three sections: the PPD, which contains somatotropic, gonadotropic, and thyrotropic cells; the RPD, which contains prolactin (PRL) and adrenocorticotropic cells; and the PI, which contains melanotropic and somatolactin (SL)

cells **(Follénius *et al*., 1978; Farbridge & Leatherland, 1986; Mousa *et al*., 2021; Trudeau *et al*., 2023; Zhu & Li, 2024)**. Histochemical and immunocytochemical techniques have been employed to describe the cells of adenohypophysis in some species of fish. Based on molecular biochemistry, the eight or nine hormones recognized to be produced by teleosts' adenohypophysis are categorized into three groups. They originate from three main origins: (i) The GH/PRL group, which consists of the cells that produce PRL, GH, and SL; (ii) GTHs and TSH glycopeptide hormones; and (iii) hormones derived from proopio melanocortin (POMC), such as MSH and ACTH **(Mousa, 1998; Xu *et al*., 2019; Zhu & Li, 2024)**. All these hormones are found in other vertebrates, with the exception of SL **(Rand-Weaver *et al*., 1991; Kaneko, 1996)**. Gonadotropic cells and/or GTHs in two different forms are observed in some fish species **(Kawauchi *et al*., 1989; Nozaki *et al*., 1990; Mousa, 2002; Mousa *et al*., 2021)**.

The grey mullet, *M. cephalus*, is a euryhaline economic teleost that inhabits a variety of salinities and culture systems. The current natural application of juvenile *M. cephalus* is insufficient to meet the growing demand for its juveniles. Mature female breeders' resources are extremely valuable, particularly when combined with the large-scale hatchery production of fish juveniles. Nevertheless, prespawning females would have made the traditional migration to spawn in the sea, making it impossible to harvest mature mullet females from their natural habitat **(Mousa, 1994)**. In such cases, continuous maintenance of broodstock in captivity and the application of unique methods to regulate gonadal development are, therefore of outmost importance.

The reproductive physiology of this species, including its propagation in captivity or under hormonal therapy, additionally to the arrangements and variations of hypophysial hormones throughout the annual cycle, is, however, poorly understood at this time. Comparative data collection is critical in the teleost reproduction investigation since different species exhibit various physiological and behavioral strategies **(Royan *et al*., 2021)**. This study sought to identify immunoreactive adenohypophysial hormone locations within *M. cephalus*'s pituitary gland. As immunohistochemical probes, antisera against fish and mammalian pituitary hormones were employed to this end.

MATERIALS AND METHODS

Sample collection:
From the Mediterranean Sea near the shore of Damietta, fifty mature *M. cephalus* breeders, averaging 30cm in length and 800g in weight, were observed during the months of their development (September and October).

Histochemistry and histology
Before dissection live fish were anesthetized in a 40mg/ l solution of clove oil (Sigma). Immediately after dissection, the pituitary gland-attached brain was preserved for 72 hours at 4°C using Bouin's fixative. The fixed pituitaries and brains were prepared for histology as previously described **(Mousa & Mousa, 1999)**. Sagittal pituitary serial

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sections were obtained at a 4µm thickness. The following methods were used to stain the selected sections of each pituitary:

1- Harris's alum hematoxylin and eosin **(Conn, 1953)**.

2- Mallory's triple stain, according to **Mallory (1938)**.

2- Periodic Acid-Schiff-Lead hematoxylin-Orange G (PAS-PbH-OG) **(McConial, 1947; Pearse, 1949)**.

3- Performic acid-Alcian blue-PAS-Orange G (AB-PAS-OG) stains **(Heath, 1965)**.

Immunohistochemical procedures

Antibodies: Antisera of the present investigation are illustrated in Table (1) and used as indicated in a prior study **(Mousa, 1998)**.

Immunohistochemical reactions: Using immunohistochemistry, the sections of pituitaries was performed using the ABC Kit (Avidin-Biotin Complex) as previously mentioned **(Mousa, 1998; Mousa & Mousa, 1999)**. In summary, sections after hydration were washed twice for ten minutes each in phosphate-buffered saline (pH 7.4). All steps of staining were done at laboratory temperature. Washing of slides in PBS was done following each step. The primary antibodies were left on the sections for an overnight incubation period. The dilutions of primary antibodies were experimentally obtained (Table 1). The sections were then left to incubate with the secondary antibody for 1 hour and 45 minutes with AB-conjugated peroxidase. Moreover, 3, 3-diaminobenzidine tetrahydrochloride (DAB) was employed to display the immunoreaction. At the end, the immuno-stained slides were dehydrated and completed as previously mentioned **(Mousa & Mousa, 1999)**.

To ascertain the current antibodies' specificity, pituitary control sections were stained without the antisera of hormones or by substituting the primary antiserum with bovine serum. There wasn't any positive reaction in any sections.

RESULTS

The neurohypophysis and the adenohypophysis made up the two main elements of the *M. cephalus* pituitary gland. RPD, PPD, and PI are the three subdivisions that compose the adenohypophysis, as indicated in Fig. (1a). In the glandular section of *M. cephalus*, three cell type groups were identified based on their immunoreactivity, distribution, and grouping. First group consists of proopiomelanocortin (POMC)-related peptides, which include MSH and ACTH. Single protein hormones, which include GH, PRL and SL, make up the second group. GTHs and TSH are among the glycoprotein hormones that make up the final group (Table 1 & Figs. 1- 3).

POMC-related peptides

By using an antiserum to human ACTH, identification of ACTH-immunoreactive (ACTH-ir) cells was restricted to cords encircling the RPD's neurohypophysis (Fig. 2a, b). Additionally, the PI contained aggregates of ACTH-ir cells (Fig. 2a). The ACTH-ir

cells were specifically stained with lead hematoxylin (PbH+) and had a round or fusiform shape with round nuclei (Fig. 1b, c).

Anti-α MSH was used to specifically immunostain the MSH-like cells that are discovered in the pars intermedia (Fig. 2c, d). Moreover, anti-human ACTH cross-reacted with these cells (Fig. 2a). MSH-ir cells had a triangular or fusiform shape, were in touch with the neurohypophyseal tissue and were mixed with immunonegative cells that may be SL-ir cells (Fig. 1d). Lead hematoxylin (PbH+) was used specifically to stain MSH-ir cells (Fig. 1d).

GH/PRL family

In the pituitary gland of *M. cephalus*, three distinct subtypes of GH/PRL family positive cells were identified: PRL-ir, GH-ir, and SL-ir cells (Table 1).

The anti-chum salmon PRL utilized to specifically and intensely immunostain the cells of PRL in the RPD, as shown in Fig. (2e, f). The PRL-producing acidophilic cells were acid stained with acid fuchsin, eosin or orange G (Fig. 1b, c, e, f). Around the boundaries of the follicles, PRL-ir cell aggregates were discovered. They had round nuclei and an elongated or ovoid form (Figs. 1b, e and 2f).

GH-ir cells were particularly immunostained with anti-chum salmon GH in the PPD (Fig. 3a, b). These cells were observed being in proximity to and communicating with the neurohypophyseal processes (Fig. 1b, c, e, f). GH-ir cells were acid stained and displayed round or oval shapes with oval-shaped nuclei (Fig. 1b, c, e, f).

The PAS+ SL-ir cells were located in the PI either singular or in clusters (Figs. 1d, 3c, d). Salmon SL antiserum was utilized to specifically immunoreact with these cells (Fig. 3c, d). Around the neurohypophyseal tissue, SL-ir cells were observed mingling with immunonegative cells that might be MSH cells (Figs. 1d and 3d). They had central nuclei that were ovoid in form and could exhibit either elongated or ovoid shapes (Figs. 1d, 3d).

Glycoprotein hormone family

The hypophysis of *M. cephalus* contained two different varieties of glycoprotein hormone-secreting cells: TSH- and GTH-ir cells (Table 1; Figs. 1b, c, e, f and 3e, 3f).

Basophilic TSH-cells have been observed in the neurohypophysis region between the PPD and the RPD (Fig. 1b, c, e, f). They were comparatively scarce and exhibited moderate to mild basic staining (Fis. 1b, e, f). These cells are angular or elongated, with a tiny nucleus encircled by a tiny rim of cytoplasm (Fig. 1c, f). When antiserum to the subunit of TSHβ in rats was used, TSH-producing cells produced an adverse immunoreactivity (Table 1).

The PPD's central region is home to the cells that produce GTHs (Figs. 1b, c, e, f and 3e, f). Alcian blue, aniline blue and PAS were applied to GTH-ir cells staining (Fig. 1b, e, f). These cells had spherical nuclei and varied in size and shape (Fig. 1c, f). GTH-ir cells were specifically and intensely immunostained with anti-chum salmon GTHIβ and anti-chum salmon GTHIIβ (Table 1 & Fig. 3e, f). The GTHs-ir cells showed unstained

intracytoplasmic vacuoles and a sizable amount of immunoreactive granules (Figs. 1, 2 and 3f).

Table 1. Immunohistochemical staining of the hypophysis of *M. cephalus*

Antiserum to	Dilution	RPD		PPD		PI	
		P	C	S	G	PAS$^+$	PbH$^+$
POMC-related peptides:							
Human ACTH	1:500	-	++	-	-	-	+
α-MSH	1:1000	-	-	-	-	-	++
GH/PRL family:							
Chum salmon PRL	1:5000	++	-	-	-	-	-
Chum salmon GH	1:5000	-	-	++	-	-	-
Chum salmon SL	1:5000	-	-	-	-	++	-
Glycoprotein hormone family:							
Chum salmon GTH Iβ	1:1000	-	-	-	++	-	-
Chum salmon GTH IIβ	1:5000	-	-	-	++	-	-
Rat βTSH	1:500	-	-	-	-	-	-

Note. RPD, rostral pars distalis; PPD, proximal pars distalis; PI, pars intermedia; P, PRL cells; C, ACTH cells; T, thyrotrops; S, somatotrops; G, gonadotrops; PAS$^+$, periodic acid-Schiff reaction positive cell; PbH$^+$, lead hematoxylin positive cell; PRL, prolactin; ACTH, adrenocorticotropin; TSH, thyrotropin; GH, growth hormone; GTH, gonadotropin; SL, somatolactin; -, +, ++, negative, weak and strong immunostaining responses, respectively.

Fig. (1): Section Sagittal in *M. cephalus* pituitary gland stained with: **a)** Mallory's triple stain, displaying the NH and the glandular portion, which includes PPD, RPD, and PI. X40. **b)** PbH-PAS-OG technique, showing the PbH-stained ACTH cells (arrow), PRL and GH cells stained with OG, and PAS-stained GTH cells. Beside TSH cells moderately PAS –marked. X100. **c)** Hematoxylin and eosin stain, displaying the cells of GTH, TSH, GH, PRL and ACTH which differ in size and form, and NH. X400. **d)** PbH and PAS to highlight the PAS+ GTH-cells, and the PI_1 cells; the PAS-positive cells and the PI_2 cells; PbH-positive cells. X400. **e)** AB-PAS-OG, showing the secreting cells of adenohypophysis; the cells of GTH marked with both PAS and alcian blue, TSH and ACTH cells moderately stained with PAS, and both PRL and GH marked with OG. Also, part of PI was occurred. X100. **f)** Mallory's triple stain, showing GTH and the cells of TSH marked with aniline blue, GH and PRL stained with acid fuchsin. X400

Fig. 2. Sagittal section of pituitary gland of *M. cephalus* immunoreacted with: **a)** hACTH antiserum, displaying the immunoreaction of ACTH cells (arrows) and the PbH+ cells cross-reactivity in the PI (arrowheads). X100. **b)** Enlarged section of Fig. (a) demonstrating the ACTH cells' intense immunoreaction. X400. **c)** Anti- α-MSH. Note the intensive immunoreactions of PI-MSH cells. X40. **d)** Enlarged section of Fig. (c) indicating intense immunoreactivity of α-MSH cells. X400. **e)** Antiserum against salmon PRL. The RPD contains PRL cells. X40. **f)** A magnified enlarged portion of Fig. (e) illustrating intense immunoreactivity of PRL-secreting cells. X400

Fig. (3): Section Sagittal of the hypophysis of *M. cephalus* immunostained with **a)** Antiserum against salmon GH. The PPD contains the GH-positive cells. X40. **b)** Enlarged part of Fig. (a) indicating strong immunostaining of the GH cells was achieved using anti-chum salmon GH antiserum. X400. **c)** Salmon SL antiserum. The immunoreactive SL cells in PI are visible. X40. **d)** A magnified enlarged portion of Fig. (c), illustrating the cells of SL immunostained positively in the PI. X400. **e)** Antiserum against salmon GTH II*β*. The PPD contains the cells that produce GTH IIβ. X40. **f)** Section of Fig. (e) that has been magnified to show the GTH IIβ cells strongly immunostained with salmon GTH IIβ subunit antiserum. X400

DISCUSSION

The RPD, PPD, and PI region were the three main elements of *M. cephalus*'s adenohypophysis. The adenohypophysis is also separated into the same three distinct zones in many other teleosts (**Segura-Noguera *et al*., 2000; Mousa, 2002; Weltezein *et al*., 2003; Mousa *et al*., 2021**). Based on their distribution, grouping, and immunoreactivity, seven distinct immunoreactive cells were obtained in the hypophysis of *M. cephalus*: the immunoreactive cells of MSH- and SL-ir in the PI and ACTH-, PRL-, GH-, TSH-, and GTHs-ir cells in the PD.

ACTH- and α-MSH-ir cells

POMC in teleost is ACTH's progenitor in the corticotropic cells located the RPD and α-MSH in the melanotropic cells found the PI (**Dores, 1990**). In the present investigation, the ACTH cells of *M. cephalus* were localized using an antiserum to human ACTH. Earlier studies on different teleost species employed the same antiserum (**Segura-Noguera *et al*., 2000; Weltzein *et al*., 2003; Mousa & Khalil, 2004; Mousa *et al*., 2021**). ACTH and MSH have the same first 13 amino acids, according to sequence analysis. Thus, in a number of teleost species, there has been a strong cross-reaction between native α-MSH and the anti-hACTH (1–24) serum (**Follénius & Dubois, 1980; Rendón *et al*., 1997; Mousa, 2002; Mousa & Khalil, 2004; Mousa *et al*., 2021**). According to the immunohistochemical results obtained in *M. cephalus*, ACTH-ir cells were obtained as cords bordering the RPD's neurohypophysis. While MSH-ir cells were obtained interacting with the neurohypophyseal tissue and mixing with the SL cells in the PI. This distribution agrees with earlier findings in teleosts (**Mousa, 2002; Sánchez Cala *et al*., 2003; Mousa & Khalil, 2004; Mousa *et al*., 2021**).

Within the PI, two distinct cell types were identified: PbH+ cells and PAS+ cells. Immunohistochemistry demonstrated that the PAS-positive cells produced SL, whereas the PbH-positive cells produced MSH. Anti-α-MSH was used to immunostain MSH cells. Previous researches on other teleosts have shown that the same antiserum is helpful in identifying MSH cells (**Mousa, 2002; Sánchez Cala *et al*., 2003; Mousa & Khalil, 2004; Mousa *et al*., 2021**). The adaptations to background color and stress response have been linked to the functions of ACTH and α-MSH (**Baker *et al*., 1984; Wendelaar-Bonga, 1997**).

GH/PRL/SL family

Members of the pituitary hormone family, GH, PRL, and SL, are thought to have diverged and then duplicated from an ancestral gene (**Rand-Weaver *et al*., 1993**). Three distinct GH family positive cell types; PRL-, GH-, and SL-ir cells were identified within the hypophysis of *M. cephalus* in the current investigation.

The salmon PRL antiserum seems to elicit a particular immunoreaction with *M. cephalus* putative PRL cells. Previously, PRL cells in different teleosts utilized the same

antiserum to identify (**Rendón *et al*., 1997; Mousa, 2002; Sánchez Cala *et al*., 2003; Mousa & Khalil, 2004; Mousa *et al*., 2021**). According to the present data, the RPD was the primary location for *M. cephalus* PRL-ir cells. PRL is crucial for osmoregulation in freshwater and euryhaline teleosts (**Manzon, 2002; Malintha, 2023; Zhu & Li, 2024**). It is known that PRL plays a role in fish reproduction and stress furthermore to its osmoregulatory function (**Avella *et al*., 1991; Wendelaar Bonga, 1997; Bozhkov *et al*., 2023; Zhu & Li, 2024**). Additionally, there is growing proof that in certain teleosts, like *Fundulus heteroclitus* and *Pleuronectes platessa*, GH and PRL have gonadotropic and steroidogenic properties (**Singh *et al.*, 1988; Power, 1992**).

Specifically, antiserum to chum salmon GH was used to immunostain the GH-ir cells located in the PPD of *M. cephalus*. These findings are consistent with earlier research in respect to other teleost species (**Kawauchi *et al*., 1986; Mousa, 2002; Weltzein *et al*., 2003; Mousa & Khalil, 2004; Mousa *et al*., 2021**). In teleosts, the physiological function of growth hormone (GH) as a growth-promoting hormone is well-established (**McLean & Donaldson, 1993**). GH is also connected to immunological response, metabolism, and reproduction [38]. Furthermore, it has been documented that GH plays an osmoregulatory function in various fish species (**Sakamoto *et al*., 1993; Mancera & McCormick, 1998; Mousa *et al*., 1999**).

Only SL-ir cells were immunostained with anti-chum salmon SL in the current investigation. In the PI, SL-ir cells mixed with MSH-ir cells and encircled the neurohypophyseal tissue. The location and dispersion of SL-ir cells matched earlier findings in teleosts (**Mousa & Mousa, 1999, Segura-Noguera *et al*., 2000; Mousa, 2002; Sánchez Cala *et al*., 2003; Mousa *et al*., 2021**). It's still unclear how SL operates physiologically. This hormone has been linked to background adaptation, osmoregulation, acid-base regulation, stress, fat metabolism, and reproductive maturation (**Kaneko, 1996; Mousa & Mousa, 2000**). Via immunocytochemistry, changes in cell number and location in addition to staining intensity were correlated with gonadal maturation and the spawning of *O. niloticus*, *M. cephalus*, and *L. niloticus*. This implied that SL might be associated with gonadal maturation (**Mousa & Mousa, 1999, 2000; Khalil *et al*., 2007**). Furthermore, biochemical studies supporting the immunocytochemical results demonstrated that SL stimulates gonadal steroidogenesis *in vitro* in *Oncorhynchus kisutch* (**Planas *et al*., 1992**). Moreover, plasma SL levels rose in *O. kisutch* during the gonadal growth phase and were strongly connected with female oestradiol levels and male 11-ketotestosterone levels (**Rand-Weaver *et al*., 1992, 1995**).

GTHs- and TSH-ir cells

TSH and GTHs (GTH I and II) are members of adenohypohyseal glycoprotein hormones. Our research showed that the TSH cells situated between the PPD and RPD of the *M. cephalus* pituitary gland exhibited a negative immunoreaction to anti-rat β-TSH. Nonetheless, studies have indicated that antiserum produced against rat or human βTSH

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will cross-react with numerous teleost species' TSH-producing cells **(Segura-Noguera *et al*., 2000; Mousa, 2002; Weltzein *et al*., 2003; Mousa & Khalil, 2004; Mousa *et al*., 2021)**. On the other hand, the gonadotropes of certain teleosts showed a weak immunoreactivity to this antiserum **(Nozaki *et al.,* 1990)**.

In teleost species, two distinct GTHs; GTH I and GTH II have been documented **(Nozaki *et al.*, 1990; Naito *et al.*, 1991; Mitparian *et al*., 2023)**. The results of the current study showed that the immunoreactivities of both GTH IIβ and GTH Iβ colocalized in one cell type within the *M. cephalus* pituitary gland's PPD. Similarly, the chum salmon βGTHI and βGTHII immunoreactivities were colocalized in same cells of *L. niloticus* and *S. solea* **(Mousa, 2002; Mousa & Khalil, 2004; Mousa *et al*., 2021)**. GTH cell distribution in *M. cephalus* is similar to that which can be discovered in other telesosts **(Toubeau *et al.*, 1991; Mousa, 1998, 2002; Mousa & Khalil, 2004; Mousa *et al*., 2021)**. In particular, gonadotropin, which is generated by gonadotrops, is intimately linked to reproduction since it promotes the production of steroids, vitellogenin absorption, ovulation, maturation of eggs, and spermiation **(Wallace & Selman, 1981; Goetz, 1983)**. Gonadotropin-induced elevation of plasma estradiol-17β (E1) levels in female teleosts drives sexual maturation **(Nagahama, 1987; Fakriadis *et al*., 2024)**. Gonadotropin (s) promotes the oocyte's uptake of vitellogenin, whereas E1 activates the liver's vitellogenin (VTG) synthesis and secretion, a precursor to yolk protein **(Mommsen & Walsh, 1988; Ferdinand *et al*., 2023)**.

In conclusion, the current study identified seven distinct adenohypophysial hormone-ir cells in the hypophysis of *M. cephalus*. These cell types may offer the morphological foundation for a deeper comprehension of the distribution and fluctuations of hypophysial hormones throughout the yearly cycle, during reproduction in captivity, and during hormonal treatments for this teleost species.

Conflict of interest
According to the authors there aren't any conflicts of interest.
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REFERENCES

Avella, M.; Schreck, C.B. and Prunet, P. (1991). Plasma prolactin and cortisol concentrations of stressed coho salmon, *Oncorhynchus kisutch*, in fresh water or salt water. Gen. Comp. Endocrinol., 81 (1): 21-27.

Baker, B.I., Wilson, J.F., Bowley, T.J. (1984). Changes in pituitary and plasma levels of MSH in teleosts during physiological color change. Gen. Comp. Endocrinol., 55: 142-149.

Björnsson, B.Th. (1997). The biology of salmon growth hormone: from daylight to dominance. Fish Physiol. Biochem., 17: 9-24.

Bozhkov, A.; Nikolov, G.; Ürku Atanasov, Ç.; Zarpyanova, D.; Atanasoff, A.; Seçer, F. and Kandir, S. (2023). Effect of different light intensities on prolactin and cortisol plasma concentration in farming African catfish (*Clarias gariepinus*) in RAS with low-water exchange. Israeli Journal of Aquaculture-Bamidgeh, 75.

Conn, H.J. (1953). "Biological stains" (Williams and Wilkins Company, Baltimore).

Dores, R.M. (1990). The proopiomelanocortin family. In: Epple, A.; Scanes, C.G. and Stetson, M.H. (eds.), *Progress in comparative endocrinology*, New York: Wiley-Liss, pp. 22-27.

Fakriadis, I.; Meiri-Ashkenazi, I.; Bracha, C.; Rosenfeld, H.; Corriero, A.; Zupa, R.; Pousis, C.; Maria Papadaki, M. and Mylonas, C. C. (2024). Gonadotropin expression, pituitary and plasma levels in the reproductive cycle of wild and captive-reared greater amberjack (*Seriola dumerili*). General and Comparative Endocrinology, 350, 114465.

Farbridge, K.J. and Leatherland, J.F. (1986). A comparative immunohystochemical study of the pars distalis in six species of teleost fishes. Fish Physiol. Biochem., 1: 63-74.

Ferdinand, B.R.; Venci Candida, X.; Suganthi, A.S. and Padua, J.C. (2023). Functional Aspects of Vitellogenin in Oogenesis and Its Regulatory Mechanism. In *Vitellogenin in Fishes-Diversification, Biological Properties, and Future Perspectives* (pp. 75-91). Singapore: Springer Nature Singapore.

Follénius, E.; Doerr-Schott, J. and Dubois, M.P. (1978). Immunocytology of pituitary cells from teleost fishes. Int. Rev. Cytol., 54: 193-233.

Goetz, F.W. (1983). Hormone control of oocyte final maturation and ovulation in fishes. In "Fish Physiology" (W.S. Hoar, D.J. Randall, and E.M. Donaldson, Eds.), Vol. *9,* Part B, pp. 117-170. Academic Press. New York.

Heath, E.H. (1965). Application of the performic acid-alcian blue periodic acid-Schiff-orange G stain to sections of pituitary glands from domestic mammals. Am. J. Vet. Res., 26 (III): 36.

Kaneko, T. (1996). Cell biology of somatolactin. Int. Rev. Cytol., 169: 1-14.

Kawauchi, H.; Moriyama, S.; Yasuda, A.; Yamaguchi, K.; Shirahata, K.; Kato, J. and Hirano, T. (1986). Isolation and characterization of chum salmon growth hormone. Arch. Biochem. Biophys., 244: 542-552.

Kawauchi, H.; Suzuki, K.; Itoh, H.; Swanson, P.; Naito, N.; Nagahama, Y. and Nozaki, M. (1989). The duality of teleost gonadotropins. Fish Physiol. Biochem., 7: 29-38.

Khalil, N.A.; El-Gamal, A.S.; Gaber, S.A.; Mousa, M.A. (2007). Immunohistochemical Localization of Gonadotropin-Releasing Hormone and

_____

Somatolactin During Sexual Maturation and Spawning of *Lates niloticus*. J. Biol. Sci., 7 (7): 1102-1111.

Malintha, G. H. T. (2023). Environmentally Sensitive Transcription Factors in the Prolactin Cell, Gill and Kidney of Mozambique Tilapia (*Oreochromis mossambicus*) (Doctoral dissertation, University of Hawai'i at Manoa). Mallory FB. Pathological technique. Philadelphia: WE; Saunders, 1938.

Mancera, J.M. and Mccormick, S.D. (1998). Osmoregulatory actions of the GH/IGF axis in non-salmonids teleosts. Comp. Biochem. Physiol., 121B: 43-48.

Mallory, F.B. (1938). Pathological technique, Philadelphia and London, (1938) pp. 257.

Manzon, L.A. (2002). The role of prolactin in fish osmoregulation: a review. Gen. Comp. Endocrinol., 125: 291-310.

McConial, M.A. (1947). Staining of the central nervous system with Lead Hematoxylin. J. Anat., 81:371-372.

Mclean, E. and Donaldson, E.M. (1993). The role of growth hormone in growth of poikilotherms. In: Schreibman, M. P.; Scanes, C. G. and Pang, P. K. T., eds. The Endocrinology of Growth, Development and Metabolism in Vertebrates. New York: Academic Press, pp. 43-71.

Mitparian, T.; Kettratad, J.; Kangwanrangsan, N.; Ampawong, S.; Kaneko, G.; Iida, A.; Senarat, S. and Charoenphon, N. (2023). Brain structure and ultrastructure of gonadotropic cell in female grunting toadfish, *Allenbatrachus grunniens* (Linnaeus 1758). Agriculture and Natural Resources, 57 (2): 281-288.

Mommsen, T.P. and Walsh, P.J. (1988). Vitellogenesis and oocyte assembly. In: W.S. Hoar and D.J. Randall (Editors), Fish Physiology, Vol. XIA. Academic Press, Inc., New York, NY, pp. 347-406.

Mousa, M.A. (1994). Biological studies on the reproduction of mullet (*Mugil cephalus* L.) in Egypt. Ph.D. Thesis, Ain Shams University, pp. 278.

Mousa, M.A. (1998). Immunocytochemical and histochemical studies on the reproductive endocrine glands of the Nile tilapia ,*Oreochromis niloticus* (Teleostei, Cichlidae). J. Egypt. Ger. Soc. Zool., 27 (C): 109-134.

Mousa, M.A. (2002). Immunocytochemical identification and distribution of the cell types in the pituitary gland of the Nile perch, *Lates niloticus* (Teleostei, Centropomidae). Bull. Nat. Inst. of Oceanogr. & Fish., A.R.E., *28*: 157-178.

Mousa, M.A. and Khalil, N.A. (2004). Immunocytochemical study of cell type distribution in the pituitary gland of the common sole, *Solea solea* (Linnaeus, 1758). J. Union Arab Biol. Cairo, 22 (A) Zool.:51-74.

Mousa, M.A. and Mousa, S.A. (1999). Immunocytochemical study on the localization and distribution of the somatolactin cells in the pituitary gland and the brain of *Oreochromis niloticus* (Teleostei, Cichlidae). Gen. Comp. Endocrinol. 113: 197-211.

Mousa, M.A. and Mousa, S.A. (2000). Implication of somatolactin in the regulation of sexual maturation and spawning of *Mugil cephalus*. J. Exp. Zool., 287: 62-73.

Mousa, M.A.; El-messady, F.A. and Khalil, N.A. (2021). Immunohistochemical description and dispensation of the hypophyseal cell types in *Liza ramada*. Egypt. J. Histol., 45 (4): 1068-1078.

Mousa, M.A.; El-Shebly, A.A. and Khalil, M.-B.A. (1999). Effect of salinity on prolactin and growth hormone cell activity in *Mugil cephalus*. Egypt. J. Aquat. Biol. & Fish., 3 (2): 85-101.

Nagahama, Y. (1987). Gonadotropin action on gametogenesis and steroidogenesis in teleost gonads. Zoological Science, 4: 209- 222.

Naito, N.; Hyodo, S.; Okumoto, N.; Urano, A. and Nakai, Y. (1991). Differential production and regulation of gonadotropins (GTH I and GTH II) in the pituitary gland of rainbow trout *Oncorhynchus mykiss*, during ovarian development. Cell Tissue Res., 266: 457-467.

Nozaki, M.; Naito, N.; Swanson, P.; Mijata, K.; Nakai, Y.; Oota, Y.; Suzuki , K.and Kawauchi, H. (1990). Salmonid pituitary gonadotrophs I. Distinct cellular distributions of two gonadotropins, GTH I and GTH II. Gen. Comp.Endocrinol., 77: 348 –357.

Pearse, A.G.E. (1949). The cytochemical demonstration of gonadotropic hormone in the human anterior hypophysis. J. Pathol. Bacteriol., 1949, 61: 195.

Planas, J.V.; Swanson, P.; Rand-Weaver, M. and Dickhoff, W.W. (1992). Somatolactin stimulates   *in vitro* gonadal steroidogenesis in coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol., 87:1-5

Power, M.D. (1992). Immunocytochemical identification of growth hormone, prolactin, and gonadotropin cells in the pituitary of male Plaice (*Pleuronectes platessa*) during gonadal maturation. Gen. Comp. Endocrinol. *,* 85: 358.

Rand-Weaver, M. and Kawauchi, H. (1993). Growth hormone, prolactin and somatolactin: a structural overview. In: Hochachka, P. W. and Mommsen, T. P., eds. *Biochemistry and molecular biology of fishes*. Amsterdam: Elsevier, vol. 2, pp. 39-56.

Rand-Weaver, M.; Baker, J.B.and Kawauchi, H. (1991). Cellular localization of somatolactin in the pars intermedia of some teleost fishes. Cell Tissue Res., 263: 207-215.

Rand-Weaver, M.; Swanson, P.; Kawauchi, H. and Dickhoff, W.W. (1992). Somatolactin, a novel pituitary protein : purification and plasma level during reproductive maturation of coho salmon. J. Endocrinol., 133 : 393 -403 .

Rand-Weaver, M.; Pottinger, T.G. and Sumpter, J.P. (1995). Pronounced seasonal rhythms in plasma somatolactin levels in rainbow trout. J. Endocrinol. , 146 :113-119.

_____

Rendón, C.; Rodríguez-Gómez, F.J.; Muñoz-Cueto, J.A.; Piñuela, C. and Sarasquete, C. (1997). An immunocytochemical study of pituitary cells of the Senegalese sole, *Solea senegalensis* (Kaup, 1858). Histochem. J., 29: 813-822.

Royan, M. R.; Siddique, K.; Csucs, G.; Puchades, M. A.; Nourizadeh-Lillabadi, R.; Bjaalie, J. G.; Henkel, C.V.; Weltzien, F. A. and Fontaine, R. (2021). 3D atlas of the pituitary gland of the model fish medaka (Oryzias latipes). Frontiers in Endocrinology, 12, 719843.

Sakamoto, T.; McCormick, S.D. and Hirano, T. (1993). Osmoregulatory actions of growth hormone and its mode of action in salmonids:A review. Fish Physiology and Biochemistry,11: 155-164.

Sánchez Cala, F.; Portillo, A.; Martín del Río, M.P. and Mancera, J.M. (2003). Immunocytochemical characterization of adenohypophyseal cells in the greater weever fish (*Trachinus draco*). Tissue & Cell, 35:169-178.

Segura-Noguera, M.M.; Laíz-Carrión, R.; Martín del Río, M.P. and Mancera, J.M. (2000). An immunocytochemical study of the pituitary gland of the white seabream (*Diplodus sargus*). Histochem. J., 32: 733-742.

Singh, H.; Griffith, R.W.; Takahashi, A.; Kawauchi, H.; Thomas, P. and Stegeman, J.J. (1988). Regulation of gonadal steroidogenesis in *Fundulus heteroclitus* by recombinant salmon growth hormone and purified salmon prolactin. Gen. Comp. Endocrinol., 72: 144-153.

Toubeau, G.; Poilve,A.; Baras, E.; Nonclercq, D.; De Moor, S.; Beckers, J.F.; Dessy-Doize, C. and Heuson-Stiennon, J.A. (1991). Immunocytochemical study of cell types distribution in the pituitary of *Barbus barbus* (Teleostei, Cyprinidae). Gen. Comp. Endocrinol., 83*:* 35.

Trudeau, V. L.; Shaw, K.; Spadacini, V., and Hu, W. (2023). Reproductive Neuroendocrinology in Teleost Fishes. Available at SSRN 4661396.

Wallace, R.A. and Selman, K. (1981). Cellular and dynamic aspects of oocyte growth in teleosts. Am. Zool., 21: 325-343.

Weltzein, F-A; Norberg, B.; Helvik, J.V.; Andersen, Ø.; Swanson, P. and Andersson, E. (2003). Identification and localization of eight distinct hormone-producing cell types in the pituitary of male Atlantic halibut (*Hippoglossus hippoglossus* L.). Gen. Comp. Endocrinol., 134: 315-327.

Wendelaar Bonga, S. E. (1997). The stress response in fish. Physiol. Rev., 77: 591–615.

Xu, W-g.; Amagai, T.; Morita, Y.; Chuda, H.; Soyano, K. (2019). Pituitary structures and immunohistochemical identification of FSHβ and LHβ expressing cells in four species of the adult groupers. Aquacult. Sci., 67 (3): 249-256.

Zhu, T., and Li, W. (2024). The regulation of prolactin secretion and its targeting function of teleost. Gen. Comp. Endocrinol. , 354, 114530.