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Comparative histological and functional studies on the brain of some freshwater fishes during prespawning and spawning seasons

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ABSTRACT

Four adult female freshwater teleost fish species; *Malapterurus electricus*, *Synodontis schall*, *Labeo niloticus*, and *Lates niloticus* (n=7) were collected in both pre-spawning and spawning seasons. The brain was dissected and the cerebellum was separated. The cerebellum was examined histologically by light and transmission electron microscope. cerebellar immunohistochemical stain was performed to investigate follicle-stimulating hormone (FSH) and glial fibrillary acidic protein (GFAP) for the assessment of the level of sex hormones and oxidative stress respectively. Biochemical measurements were executed to measure the brain contents of serotonin, dopamine and fatty acids and the activity of aromatase, Glucose 6 phosphate dehydrogenase (G6PD), Adenosine triphosphate (ATP) and *lactate dehydrogenase* (LDH). During spawning season, the cerebellar layers appeared fragile except of *Malopterurus electricus* compared to prespawning season. The cerebellar cortex of the studied fishes with the exception of *Malopterurus electricus*, is extremely vulnerable to oxidative stress during spawning season. In *Synodontis schall* and *Lates niloticus*, many of the cerebellar neurons have lost their myelinated coating. In *Lates niloticus*, some Purkinje cells exhibited fragmented rough endoplasmic reticulum and some granular cells appeared damaged and pyknotic, while others were surrounded with nerve fibrous sheath. Dense expression of FSH was evidenced during prespawning season, which refers to the increased level of sex hormones if compared with the spawning period. In the studied species, serotonin levels increased during prespawning season while dopamine levels decreased. The spawning period appeared to require higher energy, which was assessed by increasing activity of brain ATPase and G6PD. While LDH activity decreased during spawning that may reflect the absence of some myelinated sheaths. It was concluded that the brain of the studied species exhibited high sensitivity to hormonal alterations during pre-spawning and spawning seasons except for *Malapterurus electricus* that showed the highest resistance.

INTRODUCTION

Fishes are considered as the largest vertebrate class that exhibit different strategies for reproduction **(Kotrschal *et al*., 1998).** Brain and sensory organs of fishes have great variations among species. The brain, as the integrator of both inside and outside information affecting the body, is involved in all stages of sexual cycle **(Kah *et al*.,**

1993). The cerebellum is the main organ associated with emotional conditioning, cognition, memory, and sensory-motor learning **(Strata, 2015)**. Also, it is the main center involved in sensory inputs. It collects sensory information from all the receptors of limbs, head, and cerebral cortex **(Baumann *et al*., 2015)**. The cerebellar Purkinje cells play a crucial role in the biosynthesis of neurosteroids from brain cholesterol such as sex steroids (**Tsutsui and Haraguchi, 2020)**. Synaptic transmission between cerebellar Purkinje cells and parallel fibers is facilitated by estrogen hormone **(Hedges *et al*., 2018)**. In addition, the expression of estrogen receptor in the neurons of the cerebellum reflects the neurodevelopmental regulation made by estradiol **(Hedges *et al*., 2012)**. Therefore, Purkinje cells is considered an ideal model for studying the steroid actions on the organization of cerebellar neurons **(Tsutsui, 2008)**.

The releasing of gametes during spawning is carried out under the control of internal hormonal factors and external aspects of the environment including photoperiods, pheromones, spawning substrate, and temperature **(Kobayashi *et al*., 2002)**. These factors are transferred in fishes' brain into neural signals to regulate the releasing of GnRH that stimulate the production of gonadotropins FSH and LH from the pituitary gland **(Yousefian and Mousavi, 2011)**. One of the endogenous main factors controlling reproductive functions in vertebrates is feedback system of sex steroids exerted by the gonads to the hypothalamus and pituitary gland **(Fontaine *et al*., 2020)**. In addition, neurotransmitters play a crucial role in the releasing of gonadotropin **(Genazzani *et al*., 2000; Zohar *et al*., 2010)**. In all vertebrates, sex steroid hormones are important for the development, growth, differentiation, and functions of the central nervous system **(Balthazart and Ball, 2006)**. Also, they inform the brain about the sexual status **(Zohar *et al*., 2010)**.

While neuroanatomical and neurophysiological evidence suggest that the cerebellum organization is especially well preserved in vertebrates, little is known about the cerebellar relation to reproduction of fishes. The present study aimed to illustrate the structural and functional alteration of the cerebellum in some teleost fishes and its correlations with the sex hormonal variation during prespawning and spawning seasons.

MATERIALS AND METHODS

56 fresh water samples of four adult female teleost fish species (n=7 prespawning and n= 7 spawning): *Malapterurus electricus* (Order: Siluriformes, Family: Malapteruridae, **Gmelin, 1789**), *Synodontis schall* (Order: Siluriformes, Family: Mochokidae, **Bloch and Schneider, 1801**), *Labeo niloticus* (Order: Cypriniformes, Family: Cyprinidae, **Forsskål, 1775**) and *Lates niloticus* (Order: Perciforms**,** Family: Centropomidae, **Linnaeus, 1758**) were collected from the River Nile in Dakahlea Governorate, Egypt during both prespawning and spawning seasons. The studied fishes were euthanized by using 1%

clove oil and sacrificed. The handling of the animals was in accordance with the committee guidelines of experimental animal ethics of Mansoura University, code number: Sci-Z-P-2021-46. The samples ovaries were dissected for the confirmation of prespawning or spawning periods. In addition, the brain was removed, photographed and examined macroscopically. Also, the cerebellum was separated then the following investigations were processed:

1. Histological investigation

The separated ovaries and cerebellums of the studied fishes were fixed in 10% phosphate buffered formalin (PH 7.4) then dehydrated in ascending series of ethyl alcohol, cleared in xylene and mounted in molten paraffin wax at 58-62°C. Serial 5µm transverse and sagittal histological sections of brain and ovary, respectively, were cut, stained with hematoxylin and eosin, and examined by using bright- field light Olympus microscope.

2. Transmission electron microscopic investigation

Fresh specimens of the cerebellums were fixed in 2% phosphate buffered glutaraldehyde (PH 7.4), washed and post-fixed in 1% osmium tetroxide. The specimens were then dehydrated in ascending concentration of ethanol, cleared in propylene oxide, and mounted in epoxy resin. Ultrathin sections were cut, mounted on grids, stained with uranyl acetate and lead citrate and examined at a Joel 100 CX1 transmission electron microscope (Mansoura University).

3. Immunohistochemical staining for GFAP and FSH

The paraffin-embedded cerebellar sections were dewaxed, hydrated, and incubated in 2% hydrogen peroxidase to block the activity of peroxidase. Antigen retrieval was carried out by microwaving the sections for 10 min at 95–100°C in 10 mM citrate buffer (pH 6.0). Then, the slides were incubated overnight with the primary antibodies of GFAP (mouse, Santa Cruz) and FSH (mouse, Santa Cruz) in a humidified chamber at 4°C followed by incubation at room temperature in biotinylated secondary antibody for 50 minutes. Then conjugation with Avidin–Biotin– horseradish peroxidase was executed for 30 minutes. Sections were stained with 0.04% 3, 3- diamino-benzidine tetrahydrochloride followed by using hematoxylin as a counterstain. Digital image analysis was done for both FSH and GFAP reactions by using a computer (Intel Core I3) with Video Test Morphology software (Russia) for area percentage measurement.

4. Biochemical Analysis

The brains of the different studied fishes were homogenized with phosphate buffer, centrifuged and their supernatants were kept in refrigerator at -20C for biochemical assay. Eliza Kit of Eagle Bioscience Company (USA, catalog No: DOU 39-K01) was used for

the determination of dopamine. Also, Eliza kits of Biovision Company were used for assessments of serotonin (USA, catalog No. E4294-100), aromatase (USA, Catalog No. K983-100), LDH (Catalog No. K726-500), G6PD (Catalog No. K751-100), ATPase (Catalog No. K417-100). Concerning fatty acids, it was determined by gas chromatograph Varian® (model 3800) fitted with flame ionization detector, injector type split/split-less, software for controlling the analysis, capillary column of polyethylene glycol (Ohio Valley®) with 30 m long and 0.25 mm internal diameter, stationary phase non-bonded, thickness 0.25μm (Carbowax 20M). The identification of fatty acids was done using standard fatty acids (Supelco, template 37 mix components FAME), comparing the retention times of the fatty acids of the sample with the retention times of the standards.

5. Statistical Analysis

The data were presented as means ± standard deviation by using T test analysis in SPSS (version 26) for the comparison between prespawning and spawning seasons of the studies species and considered statistically significant at $P < 0.05$.

RESULTS

1. Gross morphology of brain

The brain of studied teleost fishes exhibited a characteristic structure. It is composed of five main regions; olfactory lobes, cerebral hemisphere, optic lobe, cerebellum, and medulla oblongata. Macroscopic observation of dorsal brain regions revealed some variation. *Synodontis schall* possessed characteristic olfactory lobe with long stalk attached to the cerebral hemisphere while, it was sessile in the other species. The cerebral hemisphere appeared more enlarged in both *Malapterurus electricus* and *Synodontis schall*. The optic lobe is also bulged and well developed in the studied fishes. The cerebellum forms a large conical fold expanding rostrally until reaching the cerebral hemispheres and covering the optic lobes in both *Malapterurus electricus* and *Synodontis schall*. Electro-sensitive lateral line lobe was well detected in *Malapterurus electricus* and *Synodontis schall* and absent in the other studied fishes (Fig. 1).

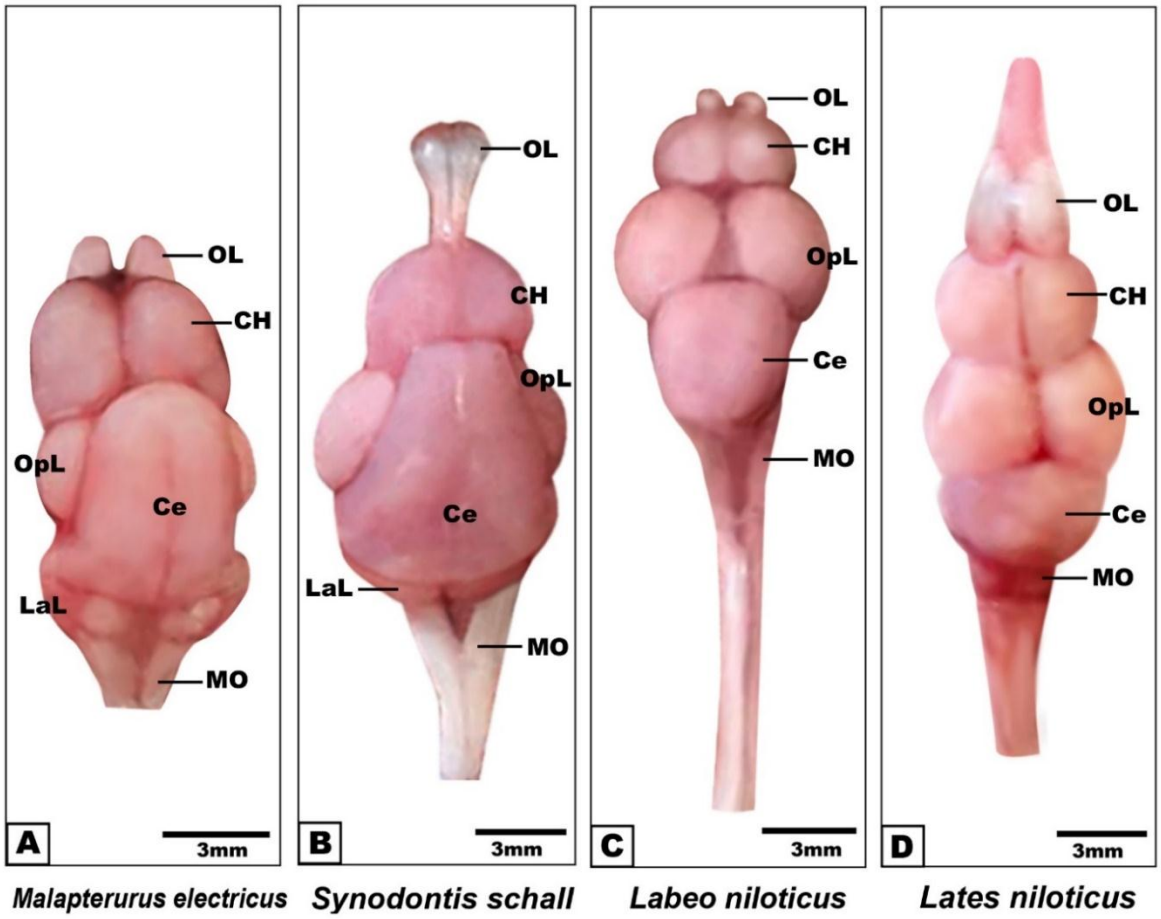

Fig. 1. Photomacrograph of dorsal view of brain of the studied teleost fishes showing varied structural organization. Note, the stalked olfactory lobe of *Synodontis schall*, the presence of lateral line lobe and the expanded cerebellum over the optic lobes in both *Malapterurus electricus* and *Synodontis schall*. Abbreviations: Ce, cerebellum; CH, cerebral hemisphere; OL, olfactory lobe, Op L, optic lobe; La L, lateral line lobe; MO, medulla oblongata.

2. Histological observations

At light microscopic level, the cerebellar cortex of all studied fishes is composed of three layers; molecular, granular and Purkinje cells lied in between. The molecular layer is composed of axons, nerve fibers and finely dispersed cells. The Purkinje cells are distributed in haphazard fashion and dispersed through the lower portion of the molecular layer. It acquired spindle-shaped structures with vesicular nuclei, large cytoplasm and dendritic axons. The granular cells are rounded-shaped structure, and densely grouping. In the studied fishes, the histological structure of the cerebellar cortex is closely similar in the studied fishes except *Synodontis schall* which possessed highly densely compacted granular cells during prespawning season (Fig. 2D). Also, in prespawning season, the

Purkinje cells exhibited slightly decreased number in *Labeo niloticus* (Fig. 2F). However, during spawning season the molecular layer of the studied fishes appeared fragile except of *Malopterurus electricus* which exhibited a high resistance to the stress of hormonal alterations during prespawning and spawning season. In addition, the Purkinje cells appeared surrounded by wide zones in both *Labeo niloticus* and *Lates niloticus* and became hypertrophied in *Lates niloticus*. The change of the granular layer during spawning season varied between the studied fishes and ranged between increased spaces between granular cells in *Syondontis schall* and increased infiltration of nerve fibers in between the granular cells in *Lates niloticus*. The structural organization of the cerebellar cortex is otherwise similar in the studied seasons (Figs. 2&3).

3. Transmission electron microscopic observations

During prespawning season, the cerebellar cortex of the studied fishes is characterized by large Purkinje cells with peculiar central nuclei. The nucleus is enclosed by abundant euchromatin and peripheral margination of heterochromatin at the nuclear envelope. The cytoplasm showed abundant distribution of ribosomes, rough endoplasmic reticulum, and mitochondria especially close to the nuclear envelope. The granular cells acquired rounded-shaped structure with centrally located nucleus enclosed with a thin coat of the cytoplasm. In between the granular cells, nerve fibers and myelinated axons were observed (Fig. 4).

During spawning season, the cerebellar neurons of the studied fishes undergoing cytological alterations. The Purkinje cells possessed nuclei with moderately condensed heterochromatin materials and convoluted nuclear envelope. The cytoplasm possessed fragmented rough endoplasmic reticulum and electron dense compacted mitochondria in *Synodontis schall* (Fig. 4B2). Electron-dense heterochromatin was highly detected within the nucleus of the granular cells of *Synodontis schall* and *Lates niloticus* (Fig. 4 B3&D4). Demyelination of the nerve axons was also remarked in *Synodontis schall* and *Lates niloticus* (Fig. 4 B3&D6) in addition to the appearance of swollen and pyknotic granule cells in *Lates niloticus* (Fig. 4 D3&D4) and degenerated granule cells in *Labeo niloticus* (Fig. 4 C3). In *Lates niloticus*, a striking observation was found. This was characterized by ensheathing the damaged granular cells by a thin coat of nerve fibers (Fig. 4 D4&D5). *Lates niloticus* was highly susceptible to hormonal alterations during spawning season and exhibited increased cytological alterations in neuronal cells, while *Malapterurus electricus* showed the highest resistance to hormonal alteration (Fig. 4).

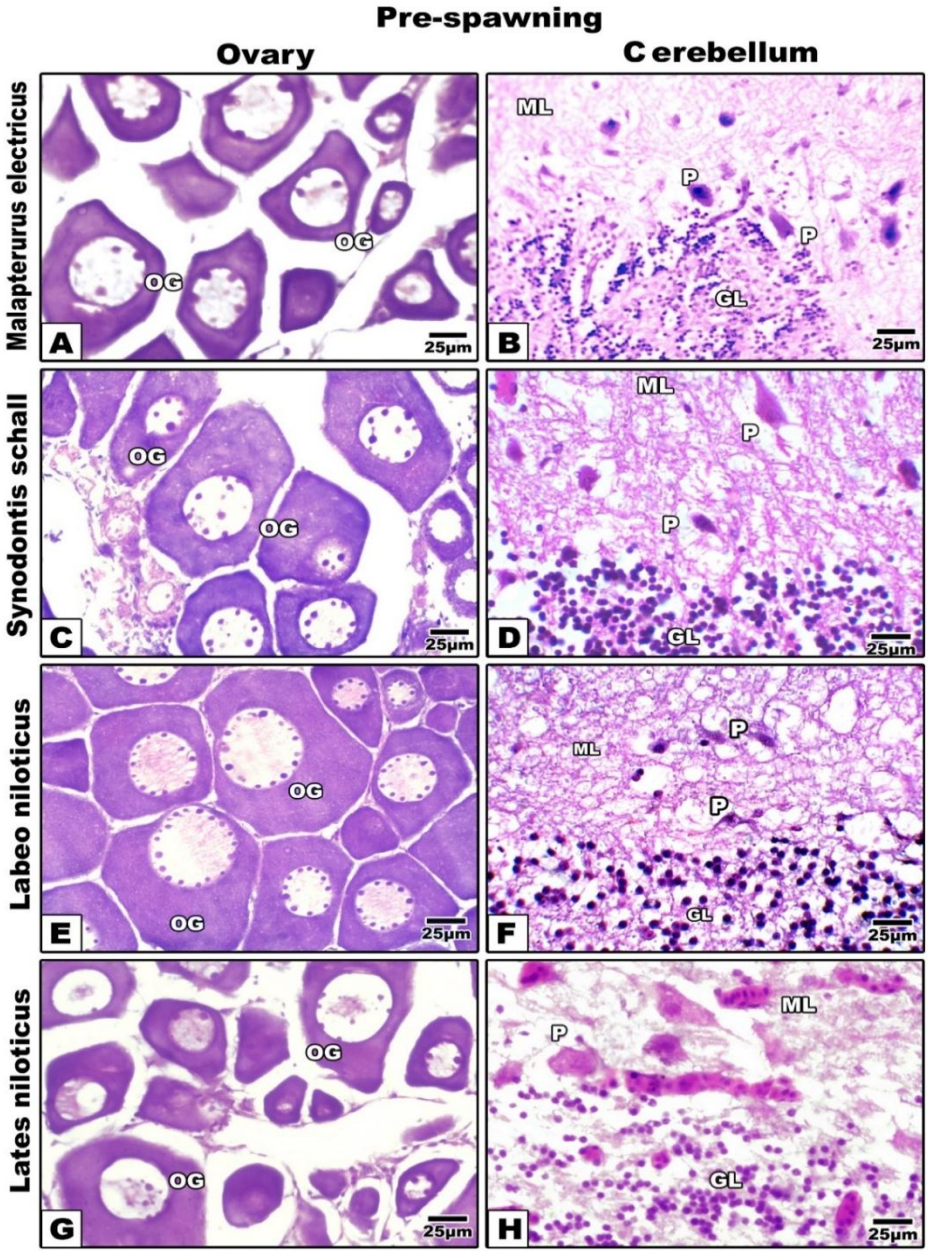

Fig. 2. Photomicrograph of transverse and sagittal sections of ovary and cerebellar cortex respectively of the studied fishes; *Malapterurus electricus*, *Synodontis schall*, *Labeo niloticus*, and *Lates niloticus* during prespawning season. (A, C, E, G) showing ovary with early developmental stage of oogonia. (B, D, F, H) showing cerebellar cortex characterized by the presence of characteristic molecular layer with nerve fibers, sparse distribution of Purkinje cells and granular layer formed of grouping granular cells. Note: the dense compacted granular cells in *Synodontis schall* (D) and the decreased number of purkinje cells in *Labeo niloticus* (F). **Abbreviation:** GL, granular layer; ML, molecular layer; OG, oogonia; P, Purkinje cell.

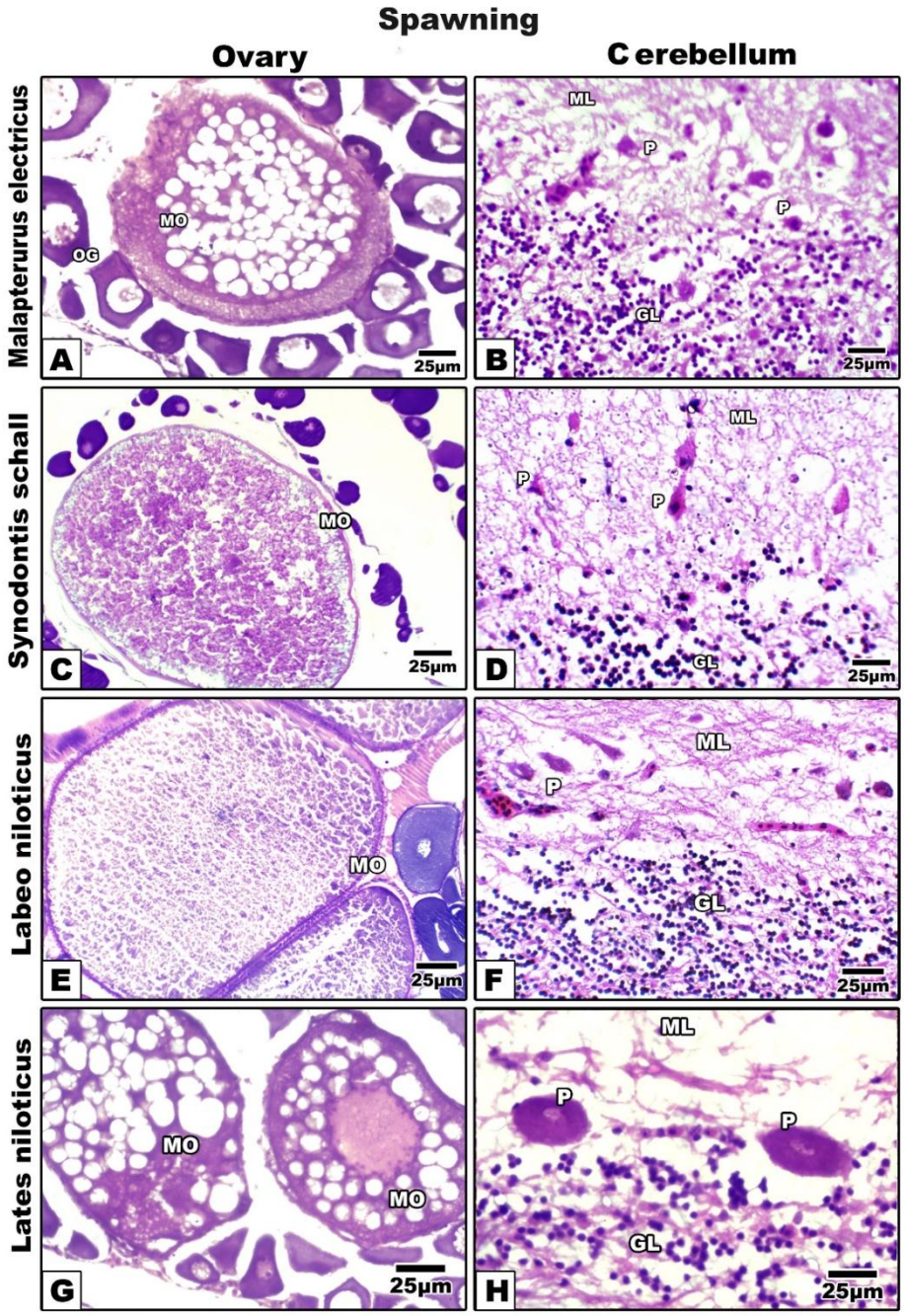

Fig. 3. Photomicrograph of transverse and sagittal sections of ovary and cerebellar cortex respectively of the studied fishes; *Malapterurus electricus*, *Synodontis schall*, *Labeo niloticus*, and *Lates niloticus* during spawning season. (A, C, E, G) showing ovary with fully mature ova in the studied species. (B, D, F, H) showing cerebellar cortex with fragile molecular layer in the studied species (B) showing molecular layer of *Malopterurus electricus* which exhibited a highly resistance to the stress of hormonal alterations. (D, F, H) showing fragile molecular layer in *Synodontis schall*, *Labeo niloticus*, and *Lates niloticus*. **Abbreviation:** GL, granular layer; ML, molecular layer; Mo, mature ova; P, Purkinje cell.

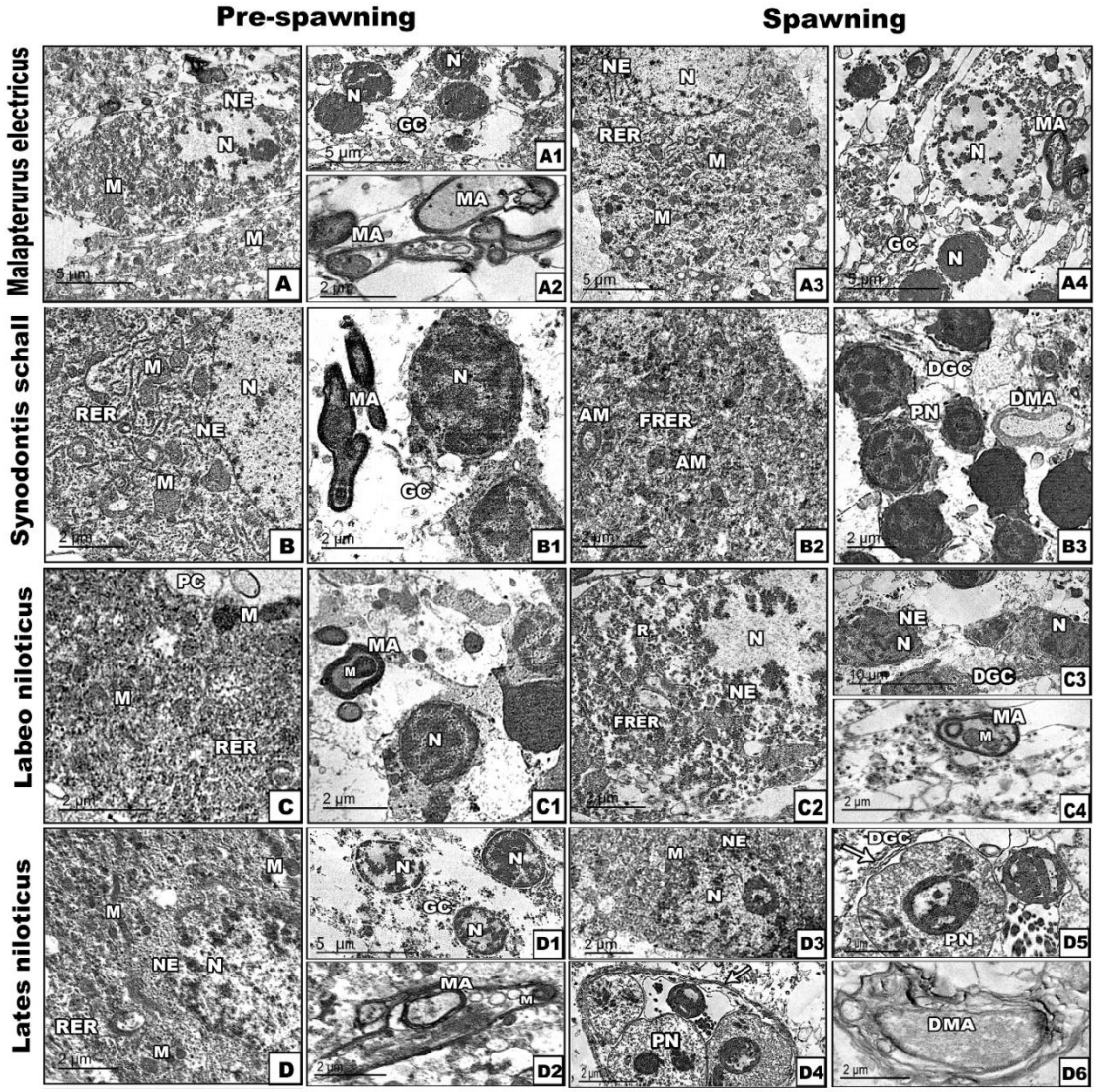

Fig. 4. Transmission electron micrographs of cerebellar cortex of studied fishes during prespawning and spawning seasons. During spawning season, *Malapterurus electricus* exhibited increased resistance to the hormonal changes (A3&A4). *Synodontis schall* showed demyelinated axons and electron-dense granule cells (B3). *Labeo niloticus* exhibited Fragmented rough endoplasmic reticulum (C2) and degenerated granule cells (C3). *Lates niloticus* showed swallowed granule cells (D3), pyknotic granule cells enclosed by nerve fibrous sheath (D4&D5) and demyelinated axons (D6). Arrows refer to nerve fibrous sheath. **Abbreviations:** AM, atrophied mitochondria; DGC, damaged granule cell; DMA, demyelinated axons; FRER, fragmented rough endoplasmic reticulum; GC, granular cell; M, mitochondria; MA, myelinated axon; N, nucleus; NE, nuclear envelope; PC, Purkinje cell; RER, rough endoplasmic reticulum; PN, pyknotic nucleus.

4. Immunohistochemistry of follicle stimulating hormone receptor (FSHr) and glial fibrillary acidic protein (GFAP)

Concerning follicle stimulating hormone receptor, the immunohistochemical reaction was highly detected in the cerebellum of the studied fishes during prespawning season compared to decreased expression in the spawning season. The immunohistochemical reaction was localized in the different cerebellar layers of Purkinje and granular cells and less intensified in the molecular layer (Fig. 5).

On the other hand, glial fibrillary acidic protein was expressed in the studied fishes. The immunohistochemical reaction was highly expressed during spawning season compared to prespawning season. In *Malapterurus electricus*, the immunohistochemical reaction was nearly similar in both prespawning and spawning season (Fig. 6).

Image analysis exhibited a significant decrease ($P< 0.05$) in the immunohistochemical reaction of FSH during spawning seasons while GFAP was significantly increased ($P< 0.05$) if compared with the prespawning season (Table 1).

Table 1. Average area percentage of both FSH and GFAP in the cerebellar cortex layers of the studied fishes during both prespawning and spawning seasons.

	Malopterurus electricus		***Synodontis schall***		***Labeo niloticus***		***Lates niloticus***	
	PS	**S**	**PS**	**S**	**PS**	**S**	**PS**	**S**
FSH (%)	**2.72 ±0.4**	**1.28 ±0.1***	**5.49±0.4**	**2.75±0.5***	**4.75±0.4**	**2.3 ±0.25***	**3.99±0.3**	**0.88 ±0.1***
GFAP (%)	**1.1± 0.13**	**1.37 ±0.22**	**1.29 ±0.12**	**1.69 ±0.2***	**0.74 ±0.1**	**1.94 ±0.32***	**0.19 ±0.05**	**1.24±0.14***

Each result represents the mean ± SD (n=7). Asterisk (*) means significant difference in comparison with prespawning season at p <0.05. **Abbreviations:** PS, prespawning; S,spawning.

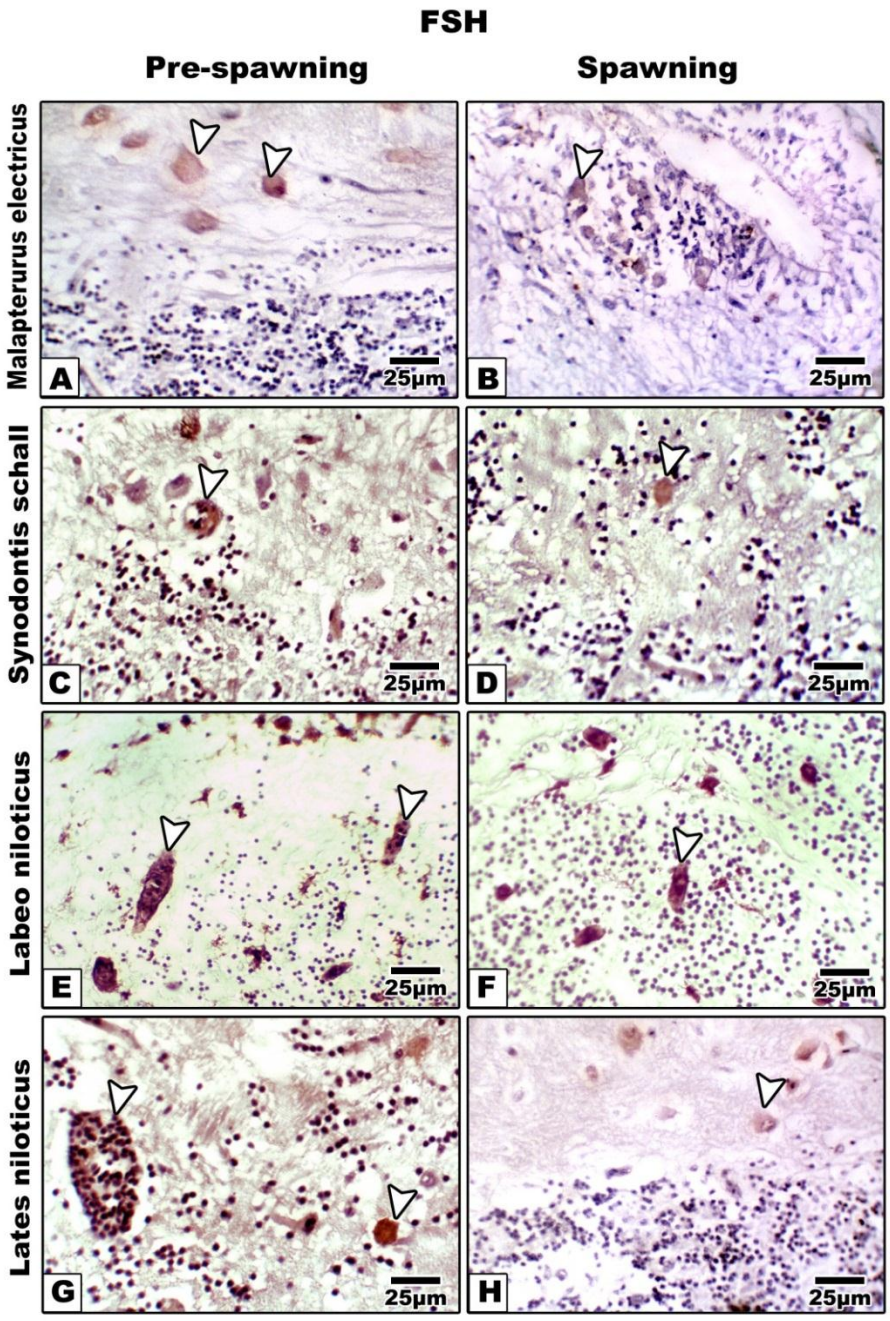

Fig. 5. Photomicrographs of sagittal histological sections of cerebellar cortex immunohistochemical stained with antibodies of FSH in the studied fishes; *Malapterurus electricus*, *Synodontis schall*, *Labeo niloticus*, and *Lates niloticus*. (A, C, E, G) showing dense immune reaction during prespawning season. (B, D, F, H) showing less dense immune reaction during spawning season.

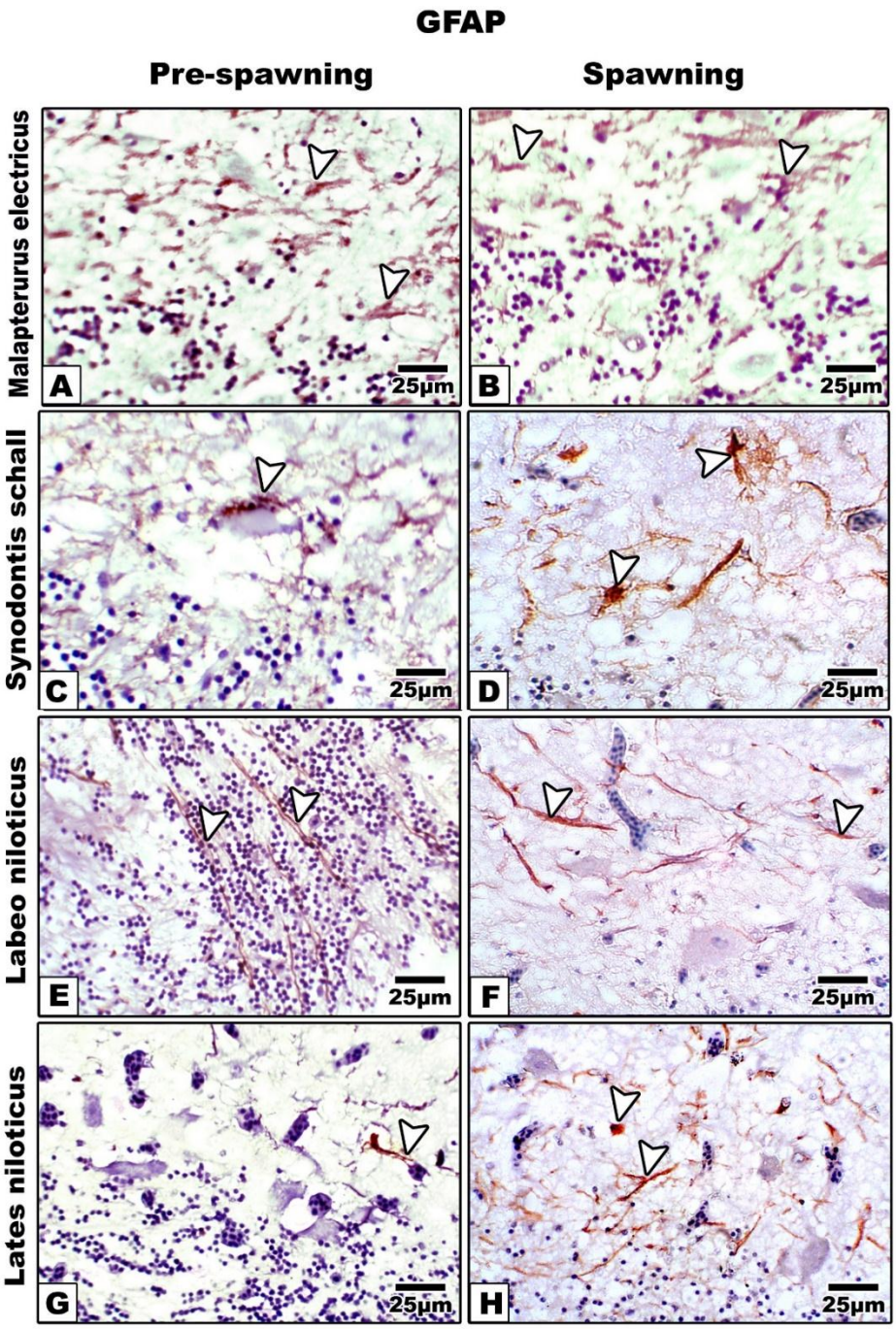

Fig. 6. Photomicrographs of sagittal histological sections of cerebellar cortex immunostained with the antibody of glial fibrillary acidic protein in the studied fishes; *Malapterurus electricus*, *Synodontis schall*, *Labeo niloticus*, and *Lates niloticus*. (A, B) showing similar expression of GFAP during both prespawning and spawning seasons in *Malapterurus electricus*. (C, E, G) showing weak expression of GFAP during prespawning season. (D, F, H) showing dense GFAP during spawning season of *Synodontis schall*, *Labeo niloticus*, and *Lates niloticus*.

5. Brain dopamine and serotonin level

The cerebellum dopamine and serotonin contents altered during the prespawning and spawning cycle. The cerebellum dopamine contents decreased in the prespawning season compared to significant (P< 0.05) increase in the spawning season. However, cerebellar serotonin contents markedly increased (P< 0.05) in the prespawning compared to the spawning period (Table 2).

Table 2. Brain dopamine and serotonin contents during both prespawning and spawning seasons of studied fishes

	Malopterurus selectricus		***Synodontis schall***		***Labeo niloticus***		***Lates niloticus***	
	PS	**S**	**PS**	**S**	**PS**	**S**	**PS**	**S**
Dopamine content (ng/mg)	**7.3 ±0.4**	**7.5 ±0.1**	**7.1 ±0.2**	**7.7±0.2***	**7.1 ±0.6**	**8.9 ±.0.5***	**7±0.2**	**7.41 ±0.1***
Serotonin content (ng/mg)	**173.6 ± 1**	**169.7 ±1.2***	**173.4 ±1**	**170.7 ±1.4***	**177.7 ±1**	**174.9 ±1***	**177.7 ±.0.8**	**175.76±1***

Each result represents the mean ± SD (n=7). Asterisk (*) refers to significant difference in comparison with prespawning season at p< 0.05. **Abbreviations:** PS, prespawning; S,spawning.

6. Brain ATPase, aromatase, LDH and G6PD activities:

From Table (3) the assayed ATPase, aromatase, lactic dehydrogenase and glucose-6-phosphate dehydrogenase activities were altered in the brain of the studied fishes during prespawning and spawning season. The brain activities of ATPase, aromatase and glucose 6-phosphate dehydrogenase were markedly increased (P< 0.05) in the spawning season in comparison with the prespawning season. However, the cerebellar lactic dehydrogenase activity was markedly (P< 0.05) increased during the prespawning season in comparison with the spawning season.

Table 3. ATPase, aromatase, LDH and G6PD **activities** in brain of studied fishes during both prespawning and spawning seasons.

	Malapterurus electricus		***Synodontis schall***		***Labeo niloticus***		***Lates niloticus***	
	PS	**S**	**PS**	**S**	**PS**	**S**	**PS**	**S**
ATPase (µmol/mg tissue)	**1.3 ±.03**	**1.4 ±0.04***	**1.3 ±0.1**	**1.5 ±0.07***	**1.4 ±0.04**	**1.5 ±0.04***	**1.6 ±0.02**	**1.68 ±.03***
Aromatase (µmol /mg tissue)	**0.7 ±.02**	**0.8 ±.03***	**0.85 ±0.02**	**0.9 ±0.02***	**0.8 ±0.01**	**0.82 ±0.01***	**0.9 ±0.05**	**1.1±.1***
LDH (µmol /mg tissue)	**0.81 ±.05**	**0.7 ±0.03***	**0.92 ±.03**	**0.81 ±0.04***	**0.9 ±0.03**	**0.83 ±0.02***	**0.9 ±0.04**	**0.81 ±0.03***
G6PD (µmol /mg tissue)	**1.07 ±0.02**	**1.15 ±0.03***	**1.2 ±.04**	**1.3 ±0.04***	**1.2 ±0.04**	**1.1 ±0.03***	**1.43 ±.07**	**1.58 ±0.06***

Each result represents the mean ± SD (n=7). Asterisk (*) refers to significant difference in comparison with prespawning season at p< 0.05. **Abbreviations:** µmol, micromole**;** PS, prespawning; S,spawning.

7. Brain fatty acid contents

Figures (7-10) illustrated the percentage of fatty acid contents of the studied freshwater teleost fishes during prespawning and spawning season. The studied fishes exhibited similarity of the brain contents of the total free fatty acids between prespawning and spawning season with some exceptions. In

Malopterurus selectricus, total omega 3 increased significantly (P< 0.05) in prespawning season while in *Labeo niloticus* it was significantly (P< 0.05) higher in spawning season. *Lates niloticus* exhibited markedly increased percentages of total saturated fatty acids (SFA) during spawning season and total omega-6 and total polyunsaturated fatty acids (PUFA) during prespawning season (P< 0.05).

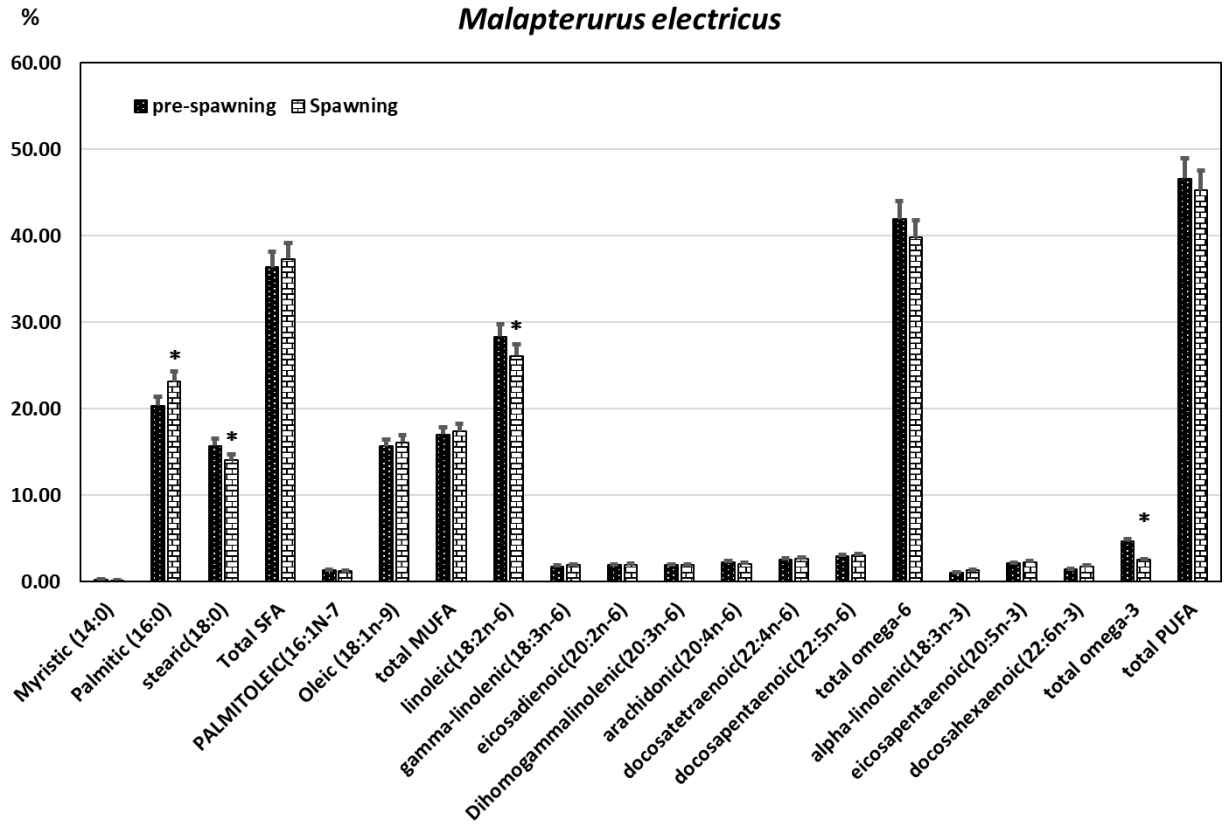

Fig. 7. Chart illustrating the percentages of brain fatty acids contents of *Malapterurus electricus* during prespawning and spawning period. Note, increased percentages of total omega 3 in prespawning season. Values are given as means ±SD (n=7). Asterisk (*) refers to significant difference in comparison with prespawning season at $p< 0.05$. **Abbreviations:** MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

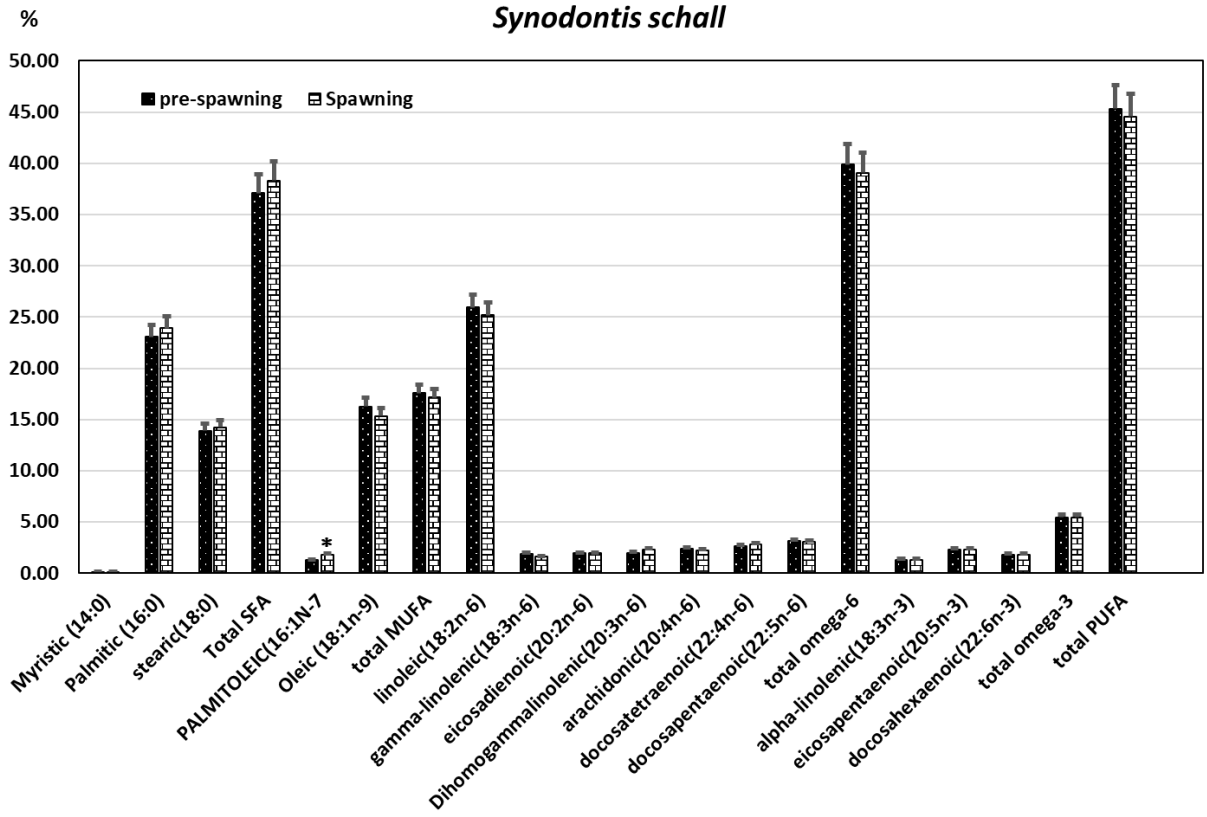

Fig. 8. Histogram illustrating the percentages of brain fatty acids contents of *Synodontis schall* during prespawning and spawning period. Note, similar contents of free fatty acids between both prespawning and spawning seasons. Values are given as means ±SD (n=7). Asterisk (*) refers to significant difference in comparison with prespawning season at $p < 0.05$. **Abbreviations:** MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

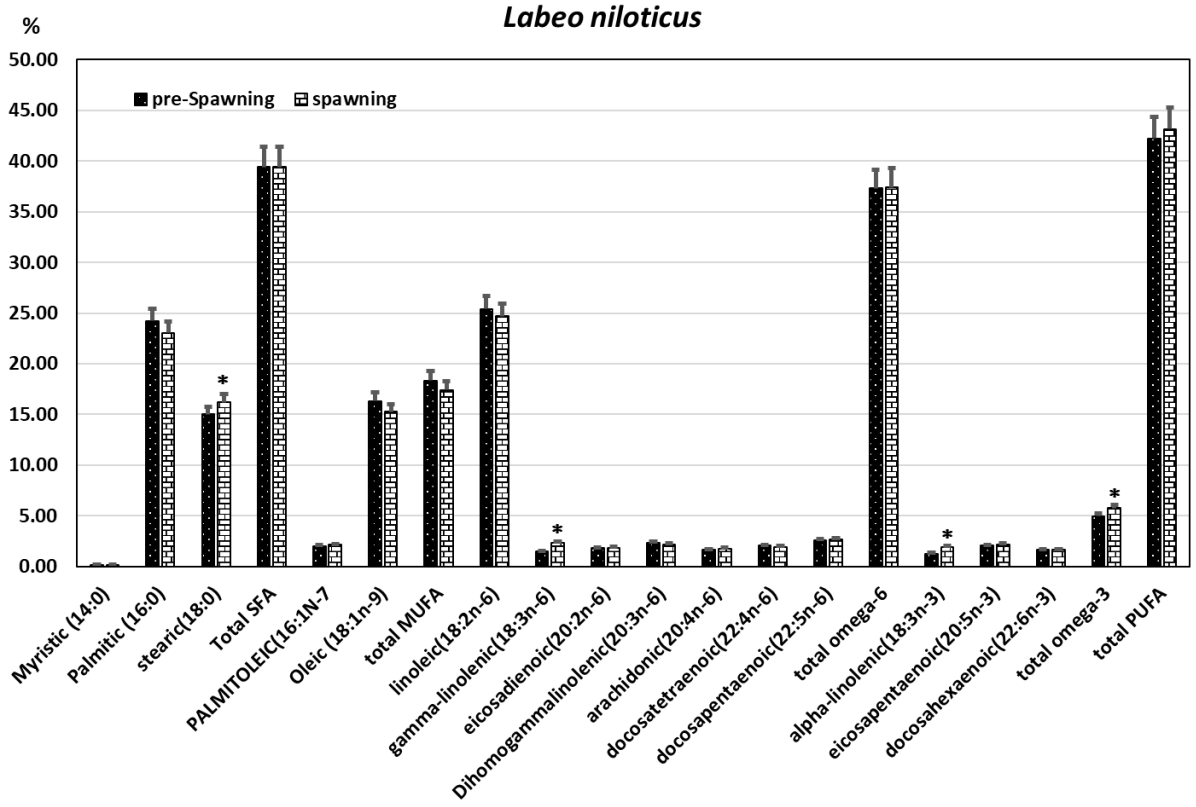

Fig. 9. Chart illustrating percentages of brain fatty acids contents of *Labeo niloticus* during prespawning and spawning season. Note, increased percentages of total omega 3 in spawning season. Values are given as means ±SD (n=7). Asterisk (*) refers to significant difference in comparison with prespawning season at $p< 0.05$. **Abbreviations:** MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

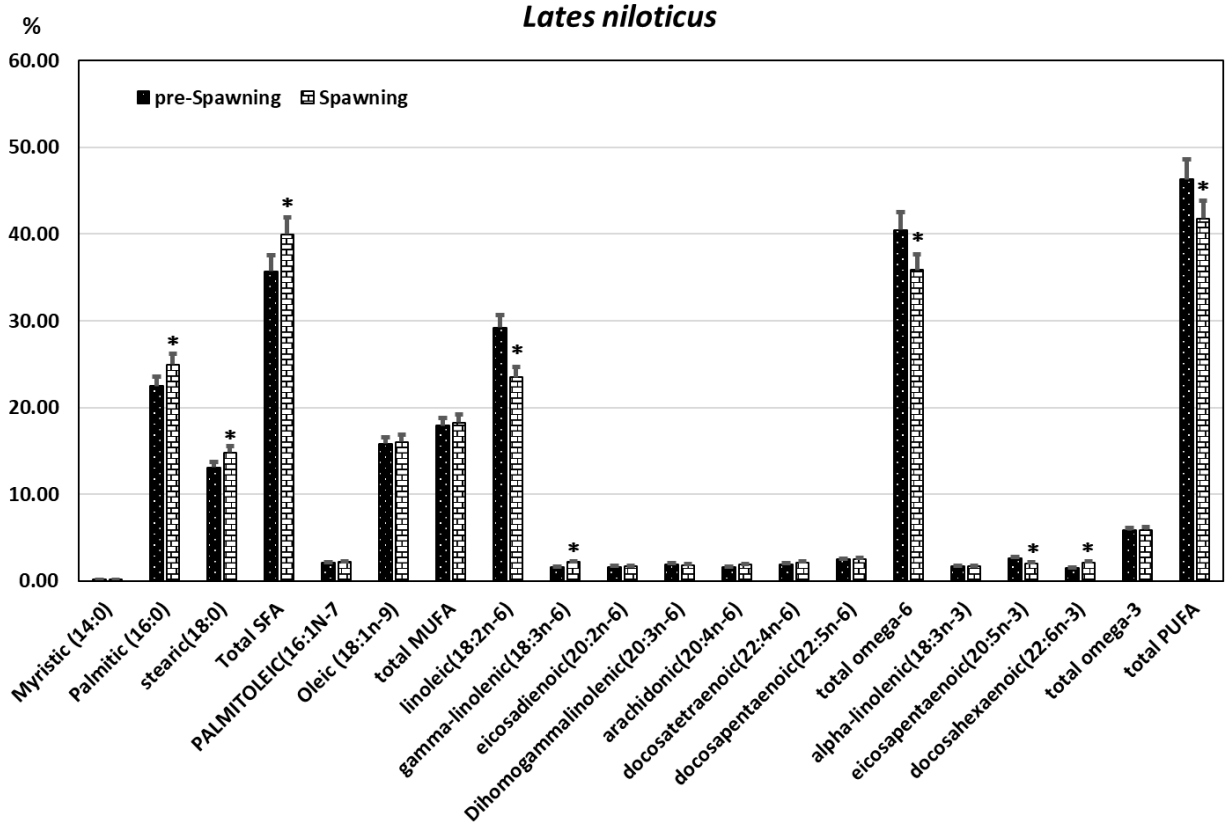

Fig. 10. Chart illustrating percentages of brain fatty acids contents of *Lates niloticus* during prespawning and spawning season. Note, increased percentages of total SFA during spawning season and total omega-6 and total PUFA during prespawning season. Values are given as means ±SD (n=7). Asterisk (*) refers to significant difference in comparison with prespawning season at p< 0.05. **Abbreviations:** MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

DISCUSSION

The brain of the studied fishes was dorsally composed of five main regions; olfactory lobes, cerebral hemisphere, optic lobes, cerebellum, and medulla oblongata, which are consistent with the report of **Hussein and Cao (2018)** who illustrated similar structures of the brain in teleost fishes. Macroscopic observation of dorsal brain region revealed varying structures. Electro-sensitive lateral line lobe was well detected in *Malapterurus electricus* and *Synodontis shall*. These finding consistent with the results of **Ito *et al.* (2007)** who reported that the crista cerebelli in electric fishes bulged and enlarged dorsolaterally forming the lateral line lobe, which receive the electrosensory and mechanosensory information. In nonelectrogenic catfish, the passive electrosense is used in biological activities such as orientation, social interaction, navigation, and prey detection **(Finger, 1986)**. The cerebellum of *Malapterurus electricus* and *Synodontis*

shall is enlarged and expanded over the optic lobes. Similar results were reported on some electric fishes; catfish (*Silurus asotus*), surgeonfish (*Prionurus scalprus*) **(Ito *et al*., 2007)** and African *Clarias gariepinus* **(Ching *et al*., 2015)**. The relative cerebellum size might be related to increased capability for movement and catching food **(Abrahão and Shibatta, 2015)**.

The olfactory lobe of *Synodontis schall* appeared stalked while in the other fishes is sessile and adjacent to the cerebral hemisphere. **Kasumyan (2004)** explained that in fishes with stalked olfactory lobe as in the wels catfish (*Silurus glanis*), the olfactory nerves passing from the olfactory organ to the olfactory lobe is short while the olfactory tracts, which connect between the lobe and the fore brain olfactory centers are markedly long.

Histologically, the cerebellar cortex of the studied fishes is composed of the outer molecular layer, middle Purkinje cells, and inner granular layer. This structure agrees with the report of **Ikenaga (2013)** on teleost fishes. However, during spawning season, the cerebellar cortex attained considerable fragility of molecular and granular layers especially in *labeo niloticus* and *Lates niloticus.* While, at the ultrastructural level during spawning season, fragmented rough endoplasmic reticulum, pyknotic granule cells, and demyelinated nerve axons were manifested especially in *Lates niloticus.* The cerebellar neuronal cells seemed to be less affected in *Malapterurus electricus* comparing with the other studied fishes. This damage of the cerebellar cortex layers during spawning season may happened as a result of the decline of sex hormones, which assessed by decreased expression of FSH during spawning season if compared to the prespawning one in the studied fishes. This result agrees with **Rime *et al*. (2004)** who reported the reduction of gonadotrophin during spawning season of rainbow trout. Also, **Kobayashi *et al*. (2002)** confirmed the drop of ovarian estrogen in goldfish (*Carassius auratus*) after vitellogenesis completion. In addition, **Slater *et al*. (1994)** reported that estradiol hormones level elevated strongly in female spring salmon during the period of egg production.

Tsutsui and Haraguchi (2020) explained that, progesterone enhances the growth of neuronal dendrites, synaptogenesis and spinogenesis through its effect on Purkinje cell receptors. In addition, progesterone synthesizes allopregnanolone in the cerebellum preventing Purkinje cell death. While, estrogen regulates brain structure and function **(Dieni *et al*., 2020). Larson (2018)** interpreted that sex steroids modulate synaptic plasticity, neural activity, growth factor function and expression, apoptosis, and cellular proliferation. It also modulates the impact of immune cells such as microglia and astroglia that facilitate the debris removal following axonal damage, demyelination and neuronal death in addition to neurogenesis. **Hoogenboom *et al*. (2012)** recorded higher oxidative damage in female trout during spawning period.

Reproduction process in vertebrates requires energy, causes an increase in individual metabolic rate **(Speakman *et al*., 2004)** and leads to oxidative stress **(Aras *et al*., 2009; Hoogenboom *et al*. 2012)**. The present study revealed increasing oxidative stress in the cerebellar cortex of the studies fishes during spawning season except in *Malopterus electricus*. This was assessed by the markedly elevated expression of GFAP. Several studies revealed that, declining estrogen levels by ovariectomy initiates neurodegeneration and damage to the brain **(Overk *et al*., 2012; Yao *et al*., 2012; Ding *et al*., 2013; Kireev *et al*., 2014),** while the return of estradiol to its normal levels leads to repair the brain damage and restore its normal structure (**Lu *et al*., 2018). Zárate *et al*. (2017)** explained that sex hormones specially estrogen act as an antioxidant that protect neurons. Therefore, its natural depletion is incorporated with synaptic decline, neuroinflammation, cognitive impairment and dysfunction. In addition, production of progesterone hormone is associated with the synthesis of superoxide dismutase that scavenges oxidative stress **(Behrman *et al*., 2001).** Furthermore, the cerebellar contents of dopamine and serotonin appeared varied during prespawning and spawning periods. Serotonin markedly increased during prespawning period than the spawning period while dopamine exhibits the opposite. This agrees with other researchers who reported the inhibitory effect of dopamine on the release of GnRH and reducing the number of receptors for GnRH **(Podhorec and Kouril, 2009)**, the secretion of LH **(Zohar *et al*., 2010)** and gamete production **(Rainis *et al*., 2003)**. On the other side, serotonin in teleosts manages the reproduction process. It initiates the secretion of GnRH from the hypothalamus of goldfish and seabream (*Sparus aurata*) **(Senthilkumaran *et al*., 2001)**. In addition, fish sex steroids control the level of serotonin in both brain and pituitary gland **(Hernandez-Rauda and aldegunde, 2002)**.

The observed brain tissue possessed increased aromatase activity in spawning season if compared to prespawning season. This result is consistent with the result of **Maruska *et al*. (2020)** who reported increased brain aromatase level in gravid female African cichlid fish (*Astatotilapia burtoni*). Aromatase in turn bind to its membrane-receptors in regions mediating neuroendocrine function **(Shaw, 2018; Vajaria and Vasudevan, 2018).** While **Marsh *et al*. (2006)** recorded increased level of brain aromatase in female bluehead fish (*Thalassoma bifasciatum*) during early vitellogenesis period then decreased during late vitellogenesis.

The present results revealed increasing activity of G6PD and ATPase of the studies species during spawning period. **Senturk *et al*. (2009)** reported that G6PD is the main enzyme that initiates the pentose phosphate pathway. Deregulation of G6PD leads to metabolic stress expressed as neurodegeneration **(Tiwari, 2017).** In addition, the increased activity of G6PD and ATPase may reflect the energetic activities of the neuronal cells. Also, the similarity of the total fatty acids content between prespawning and spawning seasons reflect its importance during these periods. The lipid metabolism

within the brain is tightly regulated to maintain neuronal structure and function **(Bruce *et al*., 2017).** Polyunsaturated fatty acids modulate neurotransmission, neuroinflammation, neuronal homeostasis, and cell survival **(Chouinard-Watkins *et al*., 2019).**

Oligodendrocytes can use lactate for the production of energy by its conversion to pyruvate. This process is catalyzed by Lactate dehydrogenase **(Rinholm and Bergersen, 2014)**. The energy produced is required by myelinated compartments of neurons for the formation of myelin **(Bergersen, 2015).** This may reflect the presence of demyelinated axons which coincides with lactate dehydrogenase depletion during spawning period. Also, lactate dehydrogenase maintains the functions of neurons including excitability, plasticity and memory consolidation **(Magistretti and Allama, 2018).**

CONCLUSION

The author concluded that, the brain of the studied fishes exhibited high sensitivity to the depletion of sex hormones during spawning season which confirmed by the decrease of follicle stimulating hormone and increase of GFAP. These alterations were assessed by decreasing of Purkinje cell number, appearing of fragile molecular layer and other several cellular damages in both granular and Purkinje cells. Also, functional changes were confirmed by the increase of dopamine, ATPase, G6PD and aromatase during spawning season if compared with prespawning season while LDH and serotonin exhibited the opposite. Decreasing oxidative stress and repairing of the cerebellar damage were confirmed during prespawning season of the studied fishes. *Malopterurus electricus* exhibited the highest resistance to the stress of hormonal alterations.

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