Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Physiology

Morphological Variation of the Pituitary-interrenal Tissue of the Mullet Fish (*Mugil cephalus*) Reared in Different Habitats.

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ABSTRACT

The current study was designed to illustrate the histological variations of pituitary gland and interrenal tissue of mullet fish (*Mugil cephalus*) reared in fresh and marine waters. In this study, the pituitary gland of *Mugil cephalus* consisted of adenohypophysis and neurohypophysis. Three regions were observed in adenohypophysis, rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). There was no adrenal gland in *Mugil cephalus* resemble mammals but interrenal tissue and chromaffin cells were located in the wall of posterior cardinal vein in the head kidney. Light microscope and statically studies showed an increase in the square surface area of RPD including the surface areas of the prolactin (PRL) cells adrenocorticotrophs (ACTH cells) in adenohypophysis and interrenal tissue in the head kidney of the freshwater fish than marine fish. These results indicate the important role of prolactin, adrenocorticotropin and cortisol hormones in osmoregulation in the freshwater *Mugil cephalus* and help in stress and habitat adaptation.

Key words: Morphological, Pituitary gland, Interrenal tissue, *Mugil cephalus*

INTRODUCTION

Aquaculture became one of the most important projects that any country can adopt to increase its animal wealth and provide protein sources. In addition to being consumed by humans, fish is a valuable source of animal and poultry feed since it is low cost and high in protein (Anderson and Mitchum, 1974).

One of the most important factors in aquaculture is salinity and each species has its optimal salinity levels for survival, growth and productivity (Ruscoe *et al.*, 2004). A few numbers of species can endure continuous exposure to water with salinities of 120%, and some can withstand greater salinities (Nordlie, 1985; Nordlie *et al.*, 1992 and Nordlie and Haney, 1998). Salinity frequently affects the growth of euryhaline species because the energy required for osmo-regulation is not available for growth. (Brett, 1979 and Wootton, 1990).

The fish species used in this study is a mullet fish (*Mugil cephalus)*, which is found in the coastal waters of the tropics and subtropics of all oceans (Robins and Ray, 1986). Too, it is exceeding-ly euryhaline, survive in a wide range of salinities from 0% in freshwater to hypersaline waters (Collins, 1985). Due to its high market value and easily cultivated by fish farmers, *Mugil cephalus* is a fish that have an important economic impact (Bahnasawy *et al.*, 2009). The global success of this fish species because of tolerance to wide range of salinities (Thomson, 1966 and De Silva, 1980). The interrenal tissue and pituitary gland play vital roles in salinity adaptation and osmoregulation in many teleosts (Ball, 1969a, b; Ball and Baker, 1969; Olivereau and Ball, 1970 and Schreibman *et al.*, 1973). In teleost, adrenocorticotropic hormone (ACTH) is released by the anterior pituitary has been considered as the main regulator of cortisol secretion from interrenal tissue through the hypothalamo–pituitary–interrenal (HPI) axis (Donaldson, 1981 and Wendelaar Bonga, 1997). The aim of this study: is to clarify morphological variation of pituitary-interrenal tissue of *Mugil cephalus* reared in different habitats.

MATERIALS AND METHODS

1. Fish

Total 40 adult *Mugil cephalus* fish of both sexes (0.9-1kg B.Ws.) were collected in a good condition from freshwater and marine water. Freshwater fish were obtained from fish farm in Toulumpat Barseq, Abu Hummus center, El-Beheira Gover-norate, while marine fish were obtained from Deeba hatchery, Damietta Governorate. The pituitary gland and head kidney were taken from both freshwater and marine fish. These specimens were fixed in neutral buffered formalin for light microscope study.

2. Histological procedures

The specimens were fixed in 10% neutral buffered formalin for 48 hours then dehydrated in a graded series of ethanol solutions and embedded in paraffin. Paraffin sections 5μm thickness were cut and mounted on slides. The selected sections were stained with the following stains and techniques: Harris hematoxylin and eosin (H&E), for general histological study; Masson's trichrome (MT) for detection of collagen fibers; Periodic acid Schiff (PAS) for neutral mucopolysaccharides; Peracetic acid -Alcian blue - Periodic acid Schiff - orange G (PAA-AB-PAS-OG) stain for differentiation of lactotrophs and lead hematoxylin for demonstration of adrenocorticotrophs. All the afor-ementioned stains and techniques were used outlined by Heath, (1965) and Bancroft and Gamble, (2002), then photomicrographs were taken by microscope with digital camera (Leica EC3. Photomorphometery was done using Fiji ImageJ software program. Statistical analysis was done with JMP (statistical software program). (Ethical approval number: VUSC-022-1-18).

RESULTS

1. The variation of the pituitary gland in freshwater & marine fish: -

The adenohypophysis was divided into three regions, rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). RPD was anterodorsal in position, covering approximately the half of the adenohypophysis surface area. It occupied large areas in freshwater fish than that of marine fish (Fig.1a&b). Statistical results showed that there was a significant difference in the square surface area of RPD between marine and freshwater fish (Fig.2). A historical study revealed that the RPD consists of two

types of cells, lactotrophs and adrenocorticotrophs.

1.a. Lactotrophs (PRL cells)

Lactotrophs had a high affinity to orange G stain and appeared orange in color. They occupied a larger surface area of freshwater fish than marine fish (Fig.3a & b). Statistical results showed that there were significant differences in the square surface area of lactotrophs between marine and freshwater fish (Figure 4).

2.b- Adrenocorticotrophs (ACTH cells)

They were basophilic cells that had high affinity to lead hematoxylin stain and appeared dark blue in color. These cells occupied a larger surface area in freshwater fish than marine fish (Fig.5a & b). Statistical results showed that there was a significant differences in the square surface area of ACTH cells between marine and freshwater fish (Fig. 6).

1- The variation of the head kidney in freshwater & marine fish: -

The head kidney of Mugil cephalus was composed of hematopoietic cells, and melano-macrophage centers. The melano-macrophage centers were nodular structures and consisted of primarily of macrophages and surrounded by hematopoietic cells (Fig. 7a&b). In *Mugil cephalus*, there were interrenal and chromaffin cells instead of adrenal gland. They were located on both sides of posterior cardinal vein in the head kidney. In marine *Mugil cephalus*, the interrenal cells were polymorphic in shapes arranged around blood vessels. The cytoplasm was eosinophilic with ovoid or oval nuclei. The chromaffin cells were visible between the interrenal cells. They were pale-staining cells with eccentric spherical nuclei (Fig. 8a&b). Interrenal cells reacted positively with PAS while chromaffin cells were PAS-negative (Fig. 9a). The connective tissue of the interrenal tissue was mainly collagen fibers were visible in the wall of posterior cardinal vein and between interrenal cells (Fig. 9b). Interrenal cells in freshwater fish were polymorphic in shape with ovoid or elongated nuclei. Normal hypertrophy of nuclei was observed (Fig. 10). In freshwater *Mugil cephalus*, a larger area of interrenal tissue were observed in the wall of posterior cardinal vein than that of marine fish (Fig. 11a&b). Statistical results showed that there were significant differences in the square surface area of interrenal cells between marine and freshwater fish (Fig. 12).

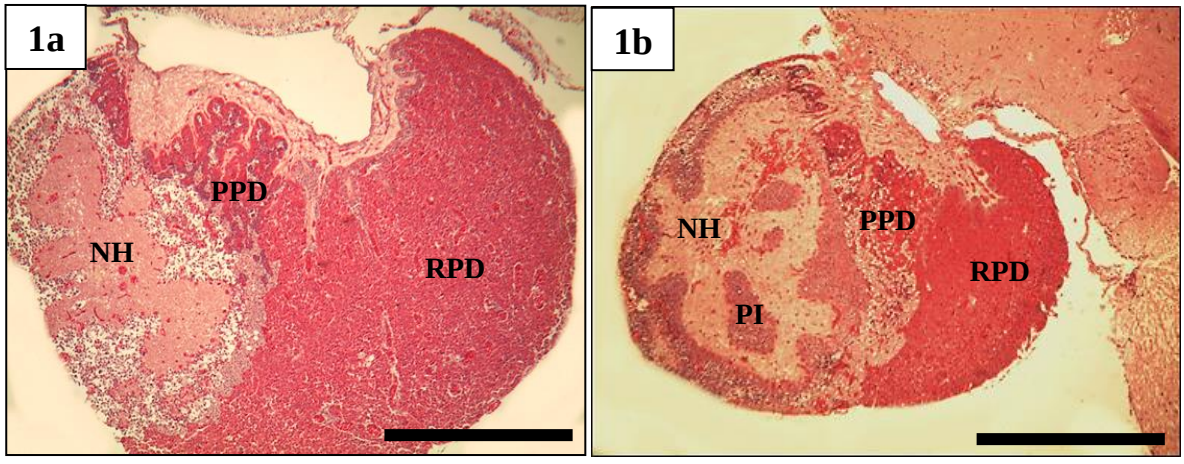

Fig. 1: Photomicrograph of the pituitary gland of *Mugil cephalus* showing larger surface area of rostral pars distalis (RPD) in freshwater fish (**Fig. 1a**) than marine fish (**Fig. 1b**). PPD (proximal pars distalis, PI (pars intermedia and NH (neurohypophysis. (H&E, bar 500µm).

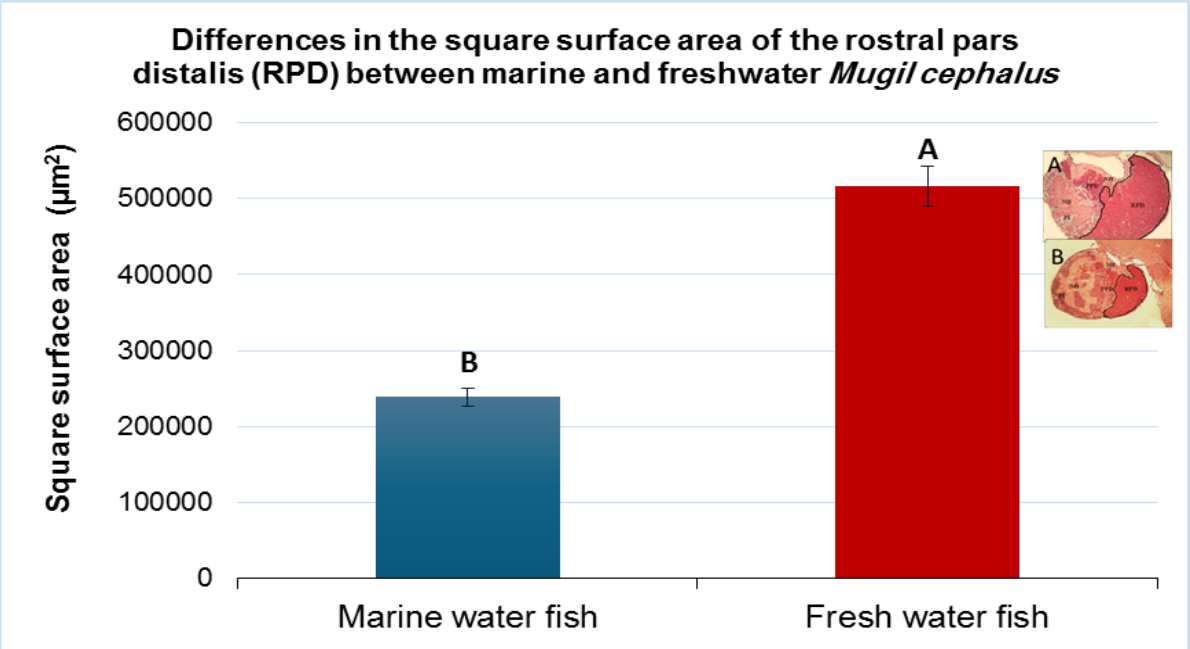

Fig. 2: Diagram presenting the differences in the square surface area of RPD between marine and freshwater *Mugil cephalus.* Different letters mean statistically significant differences ($p < 0.001$).

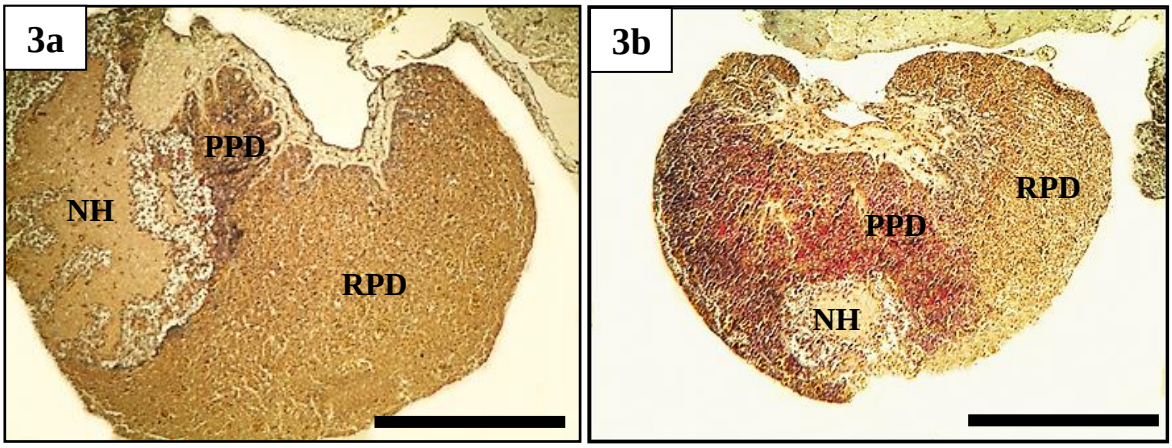

Fig. 3: Photomicrograph of pituitary gland of *Mugil cephalus* showing a larger surface area of the PRL cells in freshwater fish (**Fig. 3a**), than marine fish (**Fig. 3b**). (PAA-AB-PAS-OG, bar 500µm).

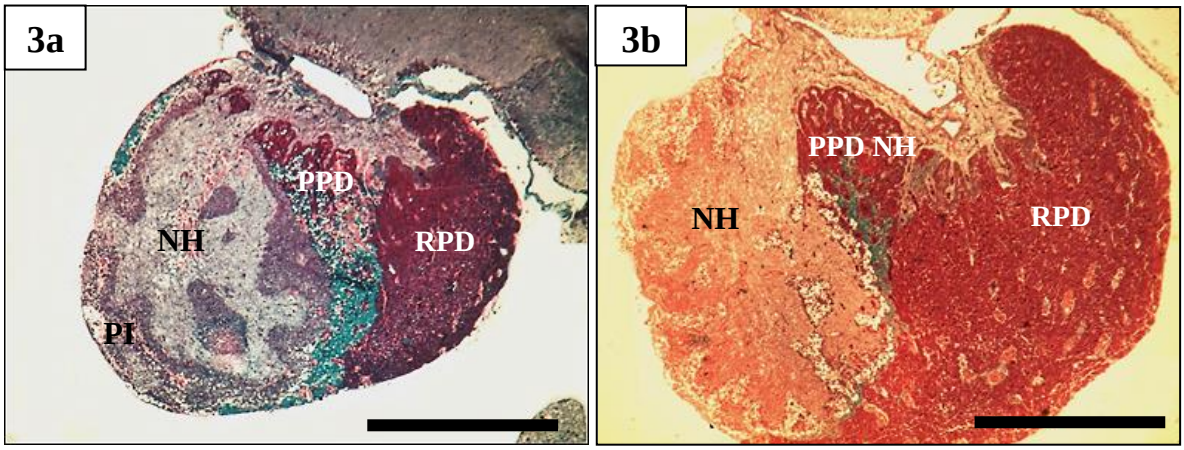

Fig. 3: Photomicrograph of pituitary gland of *Mugil cephalus* showing rostral distalis (RPD), proximal pars distalis (PPD), neurohypophysis (NH) and pars intermedia (PI), (**Fig.3a**): marine fish; (**Fig. 3b**): in freshwater fish. (MT, bar 500 µm).

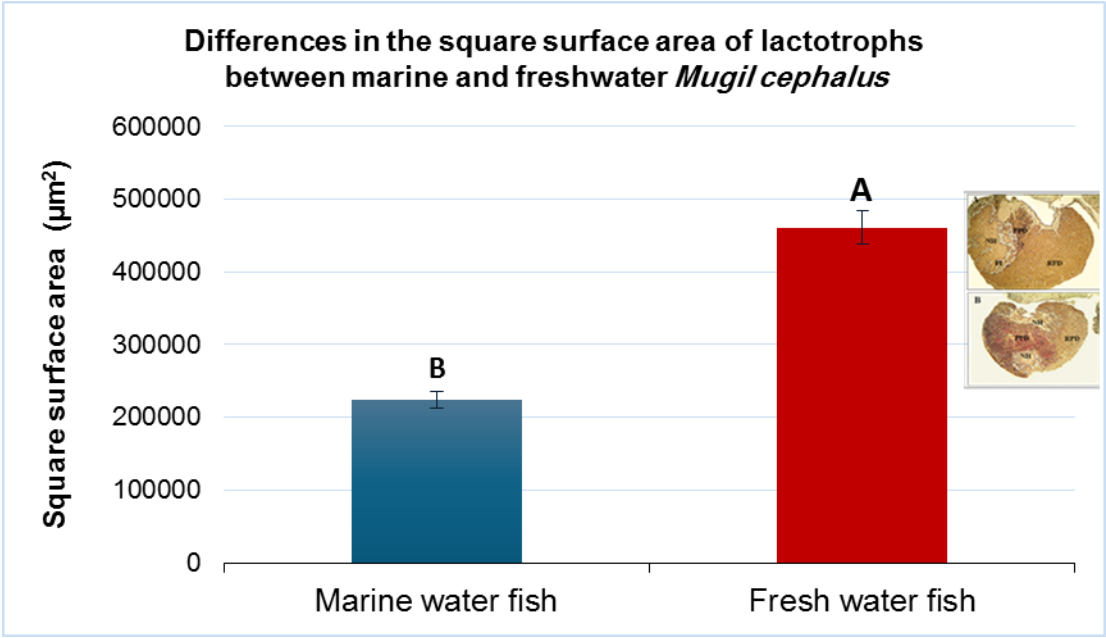

Fig. 4: Diagram presenting the differences in the square surface area of PRL cells of pituitary gland between marine and freshwater *Mugil cephalus*. Different letters mean statistically significant differences ($p < 0.001$).

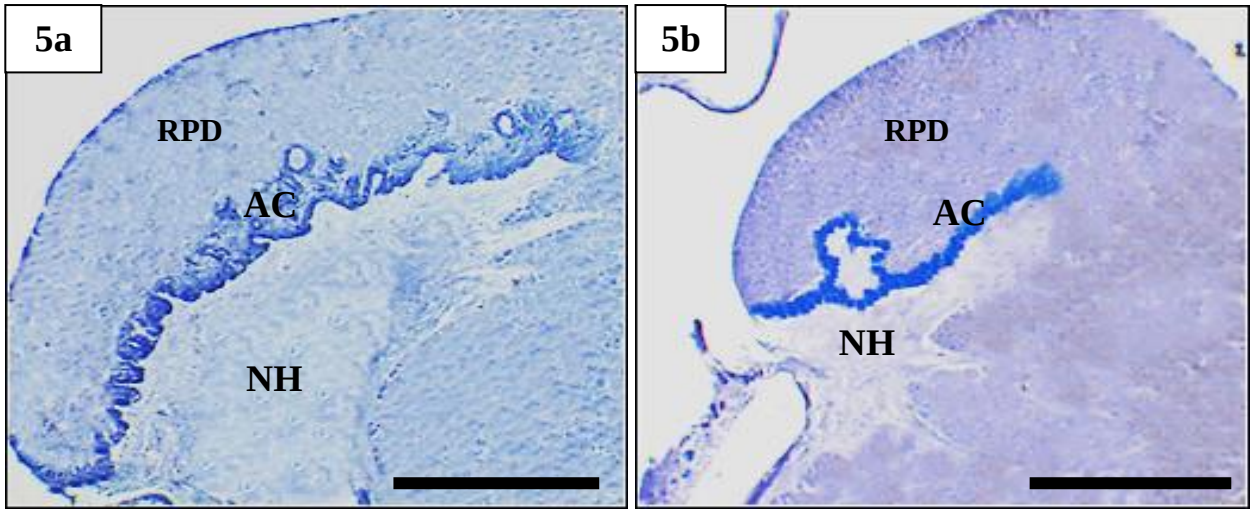

Fig. 5: Photomicrograph of pituitary gland of *Mugil cephalus* showing a larger surface area of the ACTH cells (AC) in RPD of freshwater fish (**Fig. 5a**) than marine fish (**Fig. 5b**), Neurohypophysis (NH). (Lead hematoxylin, bar 500µm).

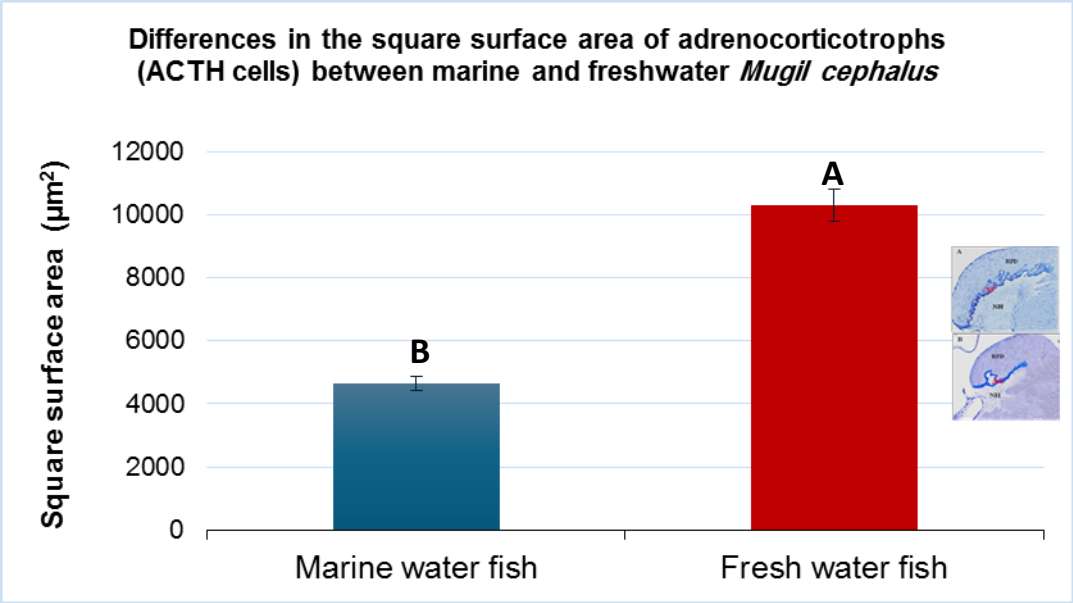

Fig. 6: Diagram presenting the differences in the square surface area of ACTH cells of pituitary gland between marine and freshwater *Mugil cephalus*. Different letters mean statistically significant differences ($p < 0.001$).

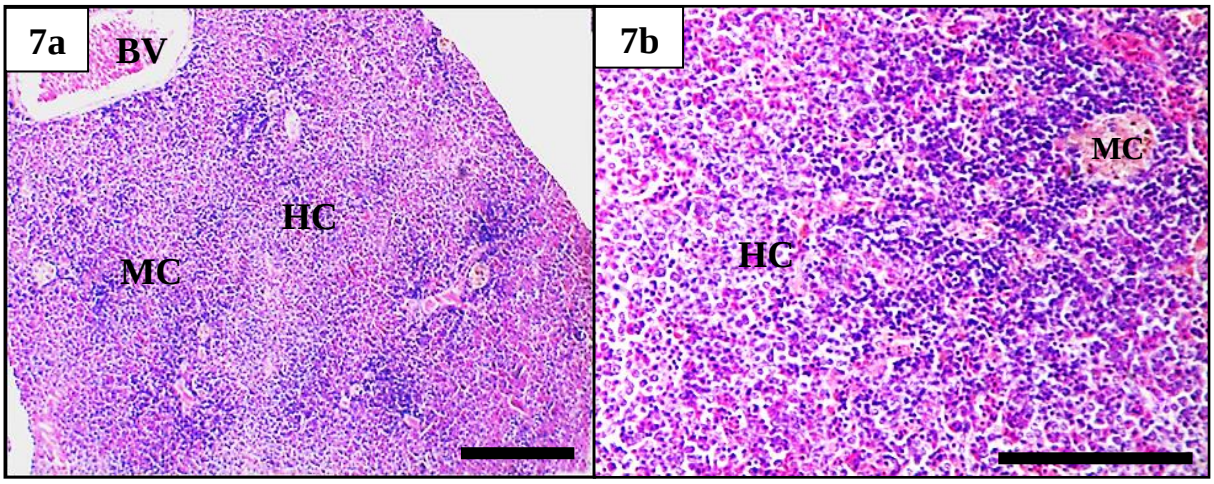

Fig. 7: Photomicrograph of head kidney of *Mugil cephalus* showing hematopoietic cells (HC), melano-macrophage center (MC), blood vessel (BV) (**Fig. 7a**, H&E; bar 250µm). A higher magnification (**Fig. 7b**, H&E; bar100 µm).

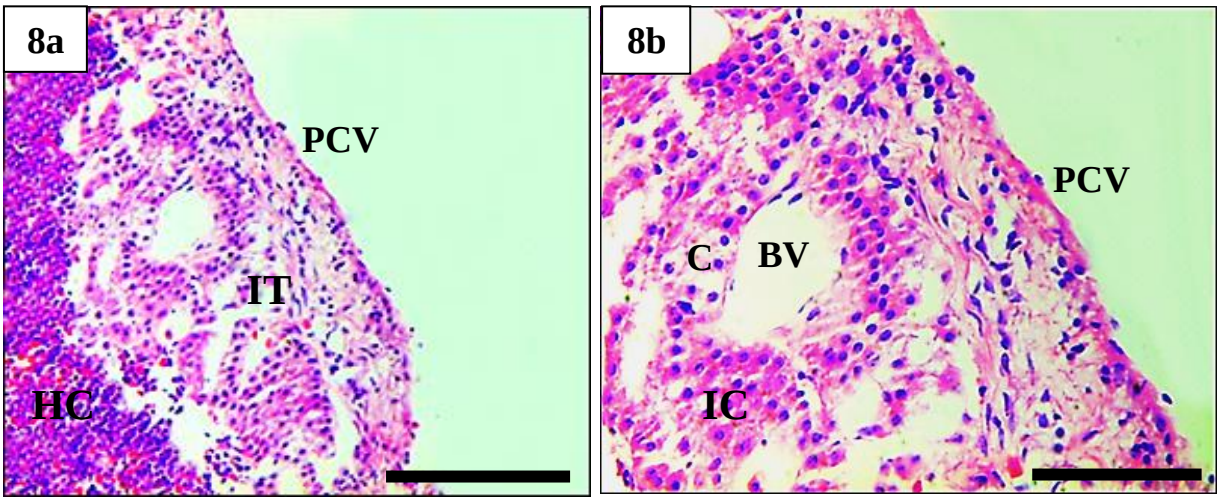

Fig.8: Photomicrograph of head kidney of marine *Mugil cephalus* showing hematopoietic cells (HC), interrenal tissue (IT) and posterior cardinal vein (PCV) (**Fig. 8a**). A higher magnification of showing polymorphic interrenal cells (IC) and pale staining chromaffin cells (C) arranged around blood vessel (BV). (**Fig. 8b**, H&E; bar 50µm).

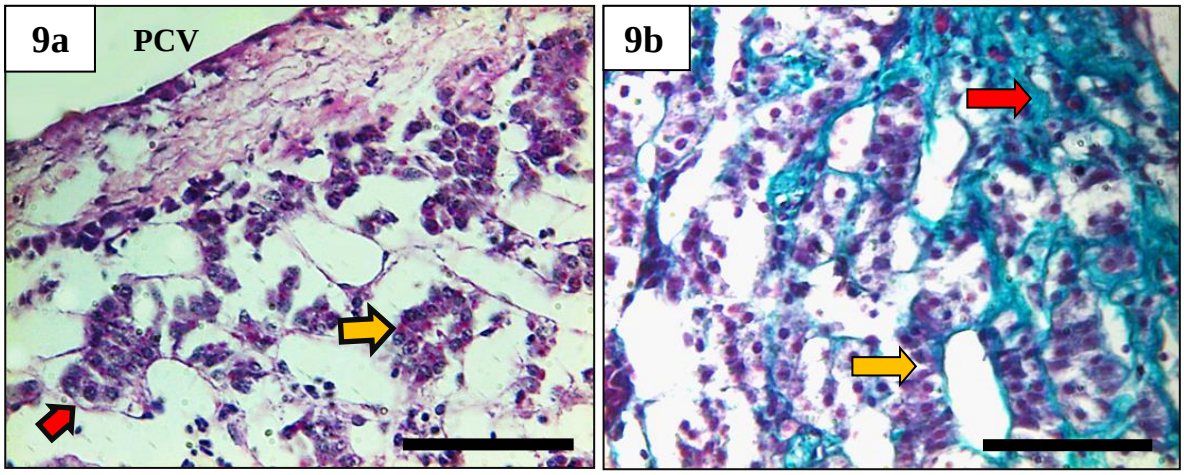

Fig. 9: Photomicrograph of the head kidney of *Mugil cephalus* showing PCV, PAS-positive interrenal cells (yellow arrow) and PAS-negative chromaffin cells (red arrow), (**Fig. 9a**, PAS; bar 50µm). (**Fig. 9b)** showing the collagen fibers (red arrow) around interrenal cells (yellow arrow) (MT, bar 50µm).

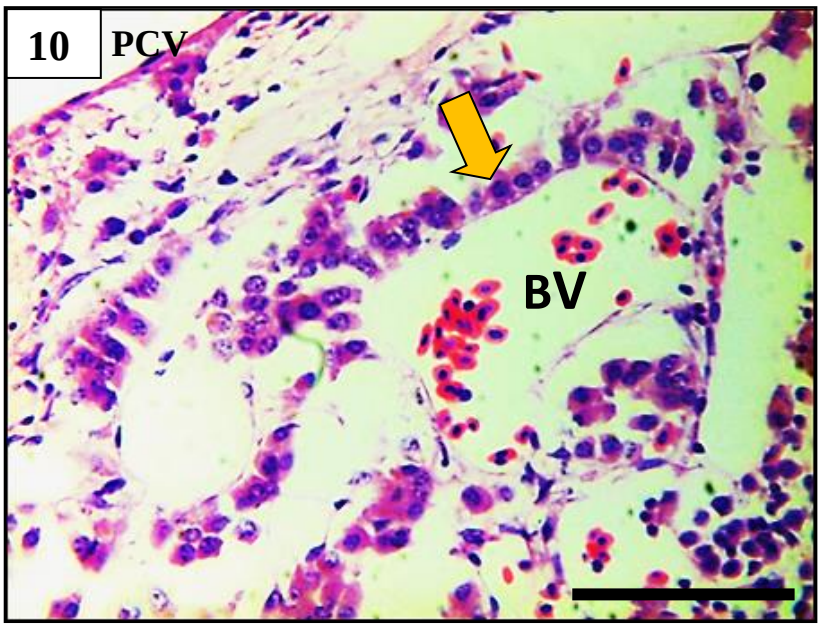

Fig.10: Photomicrograph of head kidney of freshwater *Mugil cephalus* showing posterior cardinal vein (PCV), blood vessel (BV) and interrenal cells (yellow arrow). (H&E, bar 50µm).

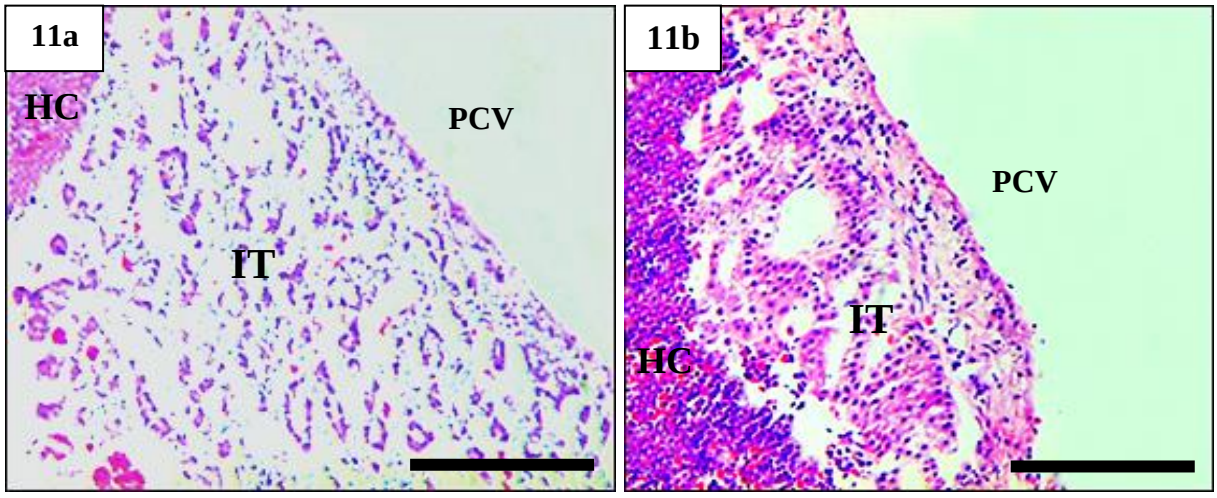

Fig.11: Photomicrograph of head kidney of *Mugil cephalus* showing a larger surface area of the interrenal tissue (IT) of freshwater fish (**Fig. 11a**) than marine fish (**Fig. 11b**). (H&E, bar 200µm).

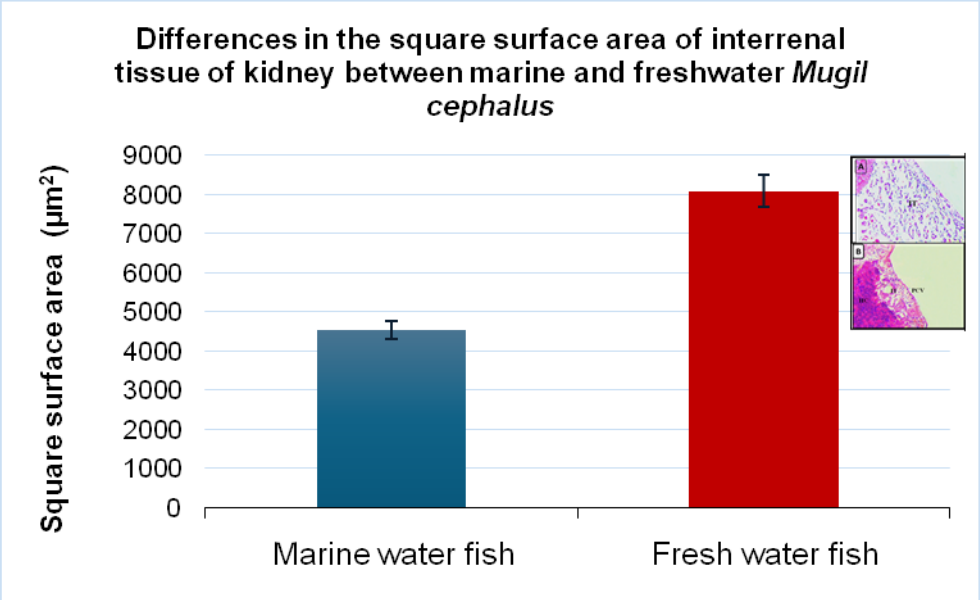

Fig. 12: Diagram presenting the differences in the square surface area of interrenal tissue of head kidney between marine and freshwater *Mugil cephalus*. Different letters mean statistically significant differences ($p < 0.001$).

DISCUSSION

In the current study, the RPD of the pituitary gland in freshwater *Mugil cephalus* occupied a larger surface area than that of marine fish, the same result was obtained by Blanc-Livni and Abraham, (1970) in *Mugil cephalus* and Nagahama *et al.*, (1973) in *Gillichthys mirabilis*. Also, an increase in the square surface area of lactotrophs (PRL cells) was observed in the pituitary gland of the *Mugil cephalus* freshwater fish than marine fish. Leatherland, (1970) mentioned that hormone release of prolactin cells is high in freshwater, the heavily granulated cells indicate that

hormone synthesis is equally high. Manzon, (2002) reported that freshwater exposure, prolactin gene expression, synthesis, secretion, and plasma levels all increase.

Increase square surface area of ACTH was observed in the pituitary gland of freshwater *Mugil cephalus* than that of marine fish. Chester Jones *et al.*, (1969) mentioned that both adrenocorticotropic and prolactin hormones act together to maintain water and electrolyte balance of the freshwater fish. Maetz, (1970) analyses the possible effects of cortisol and prolactin hormones on the cellular level in the gills of euryhaline marine fish and suggested that cortisol controls the synthesis of the transport carrier or enzymes associated with Na transport such Na-K-ATPase, while prolactin controls the synthesis of the inhibitor of the transport carrier.

In euryhaline fish, prolactin generally decreases NaCl and water absorption in gastrointestinal tract through reducing the permeability of the epithelium, although there is species variability (Manzon, 2002). On the other hand, cortisol increases ion and water permeability as well as active uptake of ions, especially chloride transport, that increases the osmotic uptake of water (Loretz, 1995). Cortisol is also suggested to help in regulation of ion and water movement across the intestinal epithelium in freshwater fish (Hirano *et al.*, 1975).

Prolactin has appeared to influence chloride cells, both by repressing the improvement of sea water chloride cells (Herndon *et al.*, 1991) and by promoting ion-absorbing cell morphology (Pisam *et al.*, 1993). Cortisol also plays a role in promoting the uptake of ions and freshwater-type chloride cells in various teleost (Perry and Goss, 1994). Although an interaction between prolactin and cortisol has been suggested in the control of acclimatization to freshwater (McCormick, 2001).

In this study, the head kidney of *Mugil cephalus* was made up of hematopoietic cells, interrenal tissue, and chromaffin cells. The same results were mentioned by Yaron, (1970) in *Acanthobrama terrae-sanctae*; Abdel-Aziz *et al.*, (2010) in *Epinephilus tauvina*; Morandini *et al.*, (2014) in *Cichlasoma dimerus* and Senarat *et al.*, (2016) in short mackerel.

According to Nandi, (1962); Hathaway & Epple, (1990); Civinini *et al.*, (2001) and Sampour, (2008), the posterior cardinal veins that puncture the head kidney are assumed to be completely in contact with the adult teleost's adrenal homology, which contains both chromaffin cells and interrenal tissue. This was similar to that was found in *Mugil cephalus* in the present study.

The present study revealed that interrenal cells are polymorphic in shapes arranged around blood vessels with eosinophilic cytoplasm with basophilic nuclei. The same result was mentioned by Silva and Martinez, (2007) in *Astyanax altiparanae* and kaptaner, (2017) in pearl mullet.

Our study showed that the chromaffin cells are located between interrenal cells in the wall of posterior cardinal vein. While the chromaffin cells of *Sciena equila* and *Anguilla anguilla* are in touch not only with the posterior cardinal vein but also with the anterior cardinal veins and the ductus Cuvier, according to Chester Jones and Mosley, (1980).

The interrenal cells positively reacted with PAS, on the other hand, Yoakim & Grizzle, (1980) in fathead minnow (*Pimephales promelas*); Misra, (1991) in *Anabas scandens*; Rocha *et al.*, (2001) in *matrinxã* (*Bryon cephalus*) and Abdel-Aziz *et al.*, (2010) in *Epinephilus tauvina* mentioned that the interrenal cells are negatively reacted with PAS.

In this study, the interrenal tissue occupied a larger square surface area in head kidney of freshwater fish than that of marine fish. Also, interrenal cells showed larger nuclei in freshwater fish.

Johnson, (1972) in *Mugil cephalus* mentioned that the interrenal tissue of freshwater mullet is consistently more active than that of marine fish. In freshwater, they show larger nuclei.

Some studies have concentrated on the impacts of osmotic stress, pollution, and fish reproductive, which increases the secretory activity of interrenal cells (Gallo *et al.,* 1997 and Civinini *et al.*, 2001).

To date, no clear evidence that teleost fishes produce aldosterone (Chester Jones *et al.,* 1969; Balm *et al.,* 1989; and Sangalang *et al.,* 1994). Because of this, it is believed that cortisol functions in teleost not only as a glucocorticoid but also as a mineralocorticoid and is essential for osmoregulation, especially during the adaptation period to both freshwater and marine environments (McCormick, 2001).

Many euryhaline marine teleost, including mullet (*Mugil cephalus*), seabass (*Dicentrachus labrax*), gilthead sea bream (*Sparus aurata*), and starry flounder (*Platichthys stellatus*), have shown similar increases in plasma cortisol levels after transferring to habitats with lower salinities (Takei *et al.,* 2014).

Cortisol within the gills of salmonids, eels, killifish, and tilapia, elevated excretion of excess ions through increasing the differentiation of ionocytes into sea water-type and elevates the activity and transcription of key transporters in sea water-type ionocytes such as Na+-K+-ATPase and Na+-K+-2Cl co-transporter (McCormick, 2001; Takahashi and Sakamoto, 2013 and Takei, *et al.,* 2014).

Cortisol plays an important role in sea water and freshwater adaptation in most euryhaline fish through glucocorticoid receptor. In salmonids, eels, killifish and tilapia, cortisol enhances the differentiation of freshwater-type ionocytes in the gills and increases the branchial influx of Na＋ and Cl- in freshwater (Takahashi and Sakamoto, 2013).

According to Zhou *et al.,* (2003), cortisol and prolactin together had a stronger influence on transepithelial resistance and the potential for in vitro branchial cell preparation than either hormone alone.

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