HISTOLOGICAL STUDY OF THE NERVOUS SYSTEM OF *RUTILUS FRISII KUTUM* KAMENSKY, 1901 FINGERLINGS

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ABSTRACT

The nervous system and its development play a crucial role in fish survival, locomotion and adaptation. *Rutilus frisii kutum* is one of the ecologically and economically important fish species of the Caspian Sea which is annually cultured and released in the Caspian Sea as millions of fingerlings. In order to investigate the nervous system structure of the Caspian Kutum, *Rutilus frisii kutum*, fingerlings were studied through histology technique. Results showed that the nervous system of the fingerlings is well developed, and composed of central (brain and spinal cord) and peripheral (nerves and ganglia) nervous tissue. The brain showed the normal structure of a ray-finned bony fish as in other teleosts, which shows that the fingerlings have the complete nervous system for facing and adapting to the new environment after release to the sea.

RÉSUMÉ: Étude histologique du système nerveus de *Rutilus frisii kutum* Kamensky, 1901 doigts.

Le système nerveux et son développement jouent un rôle crucial dans la survie, la locomotion et l'adaptation des poissons. *Rutilus frisii kutum* est l'une des espèces de poissons écologiquement et économiquement importantes de la mer Caspienne qui est cultivée et relâchée chaque année dans la mer Caspienne sous forme de millions d'alevins. Afin d'étudier la structure du système nerveux du Kutum caspien, *Rutilus frisii kutum*, des alevins ont été étudiés par la technique histologique. Les résultats ont montré que le système nerveux des alevins est bien développé, composé de tissu nerveux central (cerveau et moelle épinière) et périphérique (nerfs et ganglions). Brain a montré la structure normale d'un poisson osseux à nageoires rayonnées comme les autres téléostéens, ce qui montre que les alevins disposent du système nerveux complet pour faire face et s'adapter au nouvel environnement après leur libération à la mer.

REZUMAT: Studiu histologic al sistemului nervos la alevinii de *Rutilus frisii kutum* Kamensky, 1901.

Sistemul nervos și dezvoltarea lui joacă un rol crucial în supraviețuirea, locomoția și adaptarea peștilor. *Rutilus frisii kutum* este una dintre speciile de pești importante din punct de vedere ecologic și economic din Marea Caspică, care este cultivată și eliberată anual în Marea Caspică ca milioane de pui. Pentru a investiga structura sistemului nervos al speciei *Rutilus frisii kutum*, puieții au fost studiati prin tehnica histologică. Rezultatele au arătat că sistemul nervos al alevinilor este bine dezvoltat, compus din țesut nervos central (creier și măduva spinării) și periferic (nervi și ganglioni). Creierul a arătat structura normală a unui pește osos cu aripioare raze ca și alți teleostei, ceea ce arată că puieții au sistemul nervos complet pentru a se confrunta și a se adapta la noul mediu după eliberarea în mare.

INTRODUCTION

The nervous system is considered as the basis of the structural and functional mechanisms of living beings in response to environmental and internal stimuli, for this reason, the extent of the nervous system is in all parts of the body (Macdonald and Montgomery, 2003). The nervous system in fish is generally divided into two parts: the central and the peripheral nervous systems. The brain is the most specific location of the community of nerve cells in fish, and the control of different parts of the body is considered its main and primary function (Abdelnaeim Hussein and Cao, 2018).

The fish brain is relatively simple compared to the brains of mammals, but it is still capable of processing sensory information, controlling movement, and regulating basic bodily functions. The brain of a fish is divided into different regions, each responsible for specific functions such as processing visual information, regulating behavior, and controlling motor movements (Abrahão et al., 2015).

Fish also have a network of nerves that extend from the brain and spinal cord to the rest of the body, forming the peripheral nervous system. This network of nerves allows fish to sense their environment, respond to stimuli, and coordinate movements (Butler, 2011).

Water is in general under the permanent high impact of numerous stressors all over the world (Bănăduc et al., 2022, 2023).

The Caspian Sea is one of the largest enclosed seawater bodies in the world, located in the northern region of Iran. The Caspian Sea ecosystem is home to many ecologically and economically important fish species such as the Caspian Kutum, *Rutilus frisii kutum* (Khoshnood et al., 2013).

For maintenance and protection of the fish population in such an endangered ecosystem, millions of fingerlings are produced and released annually into the sea by the Iranian Fisheries Organization, These fingerings face different environmental factors and need to be prepared and capable of coping with them, therefore, having a complete and functional nervous system would be one of the key factors for such situation (Khoshnood, 2017). In order to know the optimum age for releasing the fingerlings into the sea for maximum survival, this study focused on the structure of the nervous system of *R. frisii kutum* at the age of release.

MATERIAL AND METHODS

Caspian Kutum, *Rutilus frisii kutum*, larvae and fingerlings were obtained from Shahid Ansari Fish Proliferation and Culture Center, Rasht, Iran. The mean total length and mean body weight of fingerlings were 3.5 cm and 2.6 g respectively. For histological studies, fish were euthanased in 100 mg/l of MS222 and 100 mg/l of sodium bicarbonate and immediately immersed into Bouin’s fixative for 24 hours, washed and dehydrated in an ascending series of ethanol for embedding in Paraffin (Merck). Following embedding in Paraffin (Merck), transversal and longitudinal sections of 6 µm were cut on a Leica RM2255 microtome, collected on glass slides, and stained with Haematoxylin and Eosin and finally observed by light microscopy (Khoshnood et al., 2015; Khoshnood, 2015).

RESULTS AND DISCUSSION

The brain, which is the prominent part of the beginning of the spinal cord, is composed of three main parts: the anterior brain or Prosencephalon, the middle brain or Mesencephalon, and the posterior brain or Rhombencephalon. The forebrain itself is divided into two parts, telencephalon and diencephalon. Therefore, the five sub-sections of the brain structure are respectively: telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon.

The cerebrum is composed of attached neurones; the diencephalon or midbrain is devided into two regions: dorsal epithalamus which contains pineal gland, habenular ganglion and thalamus itself, and the hypothalamus at ventral region. The hypothalamus consists of infundibulum and two inferior lobes which is the strict feature of the diencephalon and its role is the regulation of pituitary gland.

The mesencephalon is large and covered by optic tectum layers and the tegmentum is located at its inferior part. The optic tectum is devided into two optic lobes (Fig. 1).

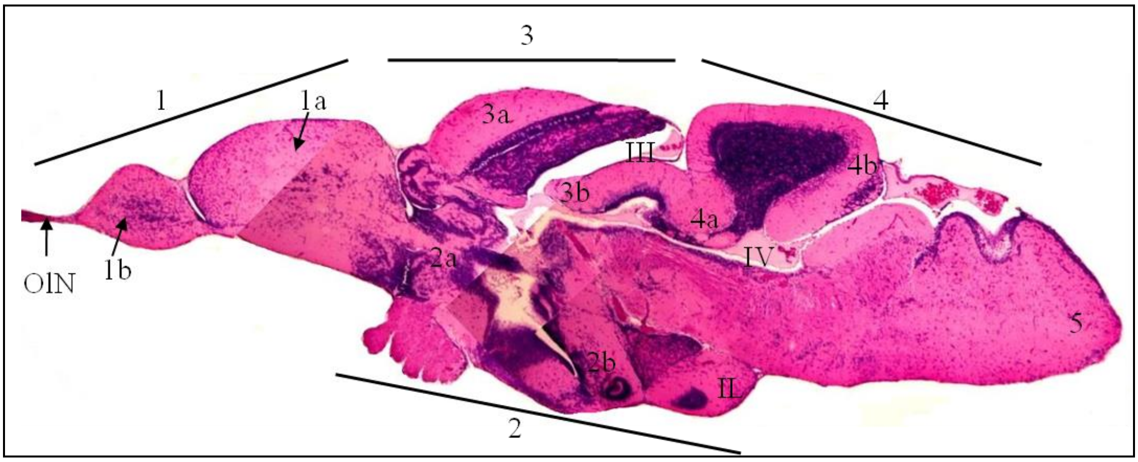

Figure 1: Histological micrograph of the brain in *R. frisii kutum* fry (H&E). The general structure of the brain (Fig. 1) is illustrated from the snout part (left) to the caudal part (right). The brain is devided into 5 distinct section: 1) Telencephalon or cerebrum is at the anterior part of the brain and is composed of two cerebral hemispheres (1a) and a pair of olfactory lobes (1b); 2) Diencephalon, which is composed of epithalamus and one thalamus at the dorsal part (2a) and one hypothalamus at the ventral part (2b) and some other parts such as pineal and pituitary glands; 3) Mesencephalon composed of optic lobes at the dorsal part (3a: optic tectum) and tegmentum (3b) at the ventral part; 4) Metencephalon composed of cerebellum with valvula cerebelli (4a) and corpus cerebelli (4b); 5) Medulla oblongata is the final part of the brain and is continued to the spinal cord. III: third ventricle; IV: fourth ventricle; OlN: Olfactory Nerve; IL: Inferior Lobe.

The cerebellum is the most important part of the dorsal metencephalon. The role of this part is to regulate coordination between motor responses as seen in other vertebrates. The cerebellum is well developed in Caspian kutum fingerlings (Fig. 1). Medulla oblongata is the main part of the hindbrain, from the caudal portion of it, the spinal cord is formed. This part contains nuclei of some cerebral nerves (5 to 10) and is the formation center for Mauthnerian system which is a well-developed nervous-muscular system in teleost fish. Stimulation of mauthnerian cells causes rapid and strong movements of the fish tail during stress and flight (Fig. 1).

The brain and the spinal cord consist of neurones and neuroglial cells. The myelinated axons of the neurons are making the white matter of the brain and spinal cord while the gray matter is made up of neuron cell bodies (Fig. 2).

Along the length of the spinal cord, some aggregations of the neurons are visible, named spinal ganglions (Fig. 2). Ganglial neurons are large and the ganglia are covered with connective tissue.

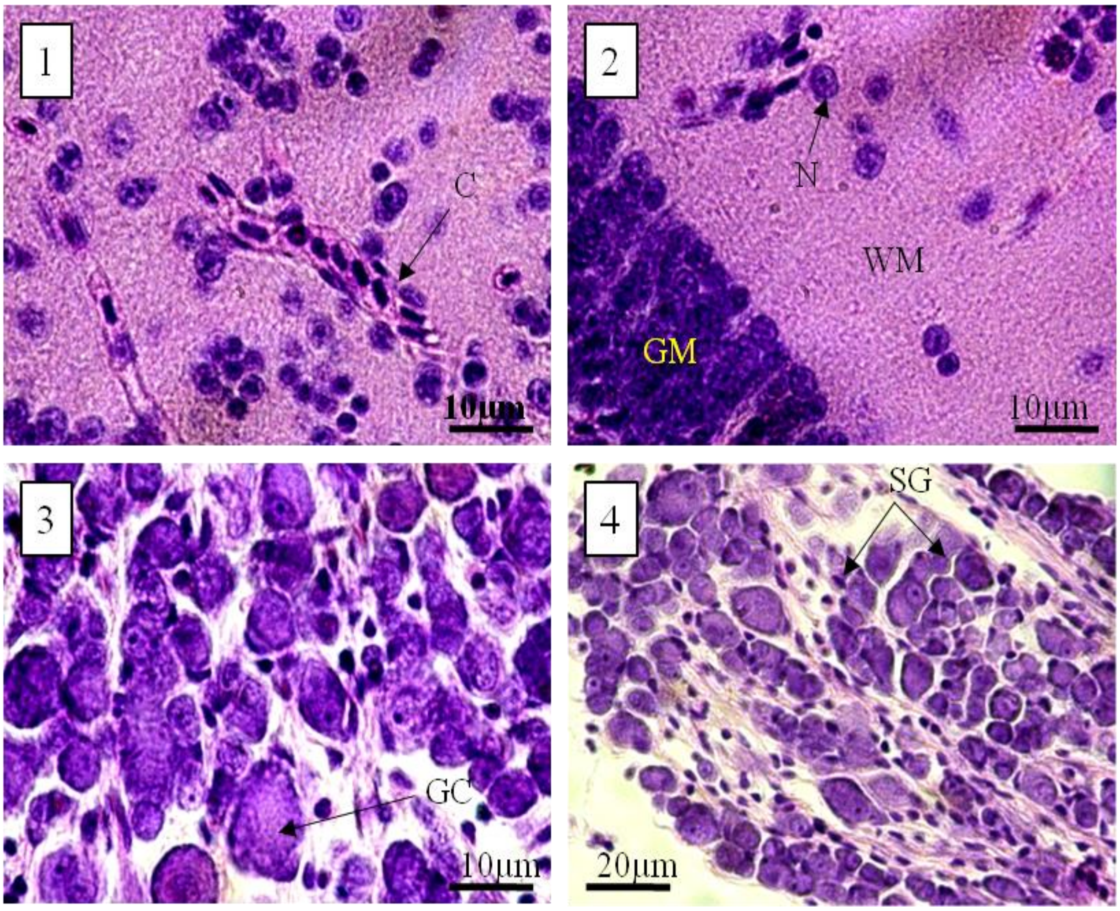

Figure 2: Histological micrograph of the nervous tissue in *R. frisii kutum* fry (H&E). The brain tissue consists of two parts: white matter (consisting of myelinated nerve fibers) and gray matter (consisting of the cell bodies of neurones) (2); In the nervous tissue, delicate capillaries are seen, in which there are nucleated red blood cells (1); Spinal ganglion is seen on the side of the spinal cord (4) which consists of neurones with large cell bodies that have large basal nuclei (3). C: Capillary; N: Nucleus; WM: White Matter; GM: Grey Matter; GC: Ganglionic Cell; SG: Spinal Ganglion.

The brain in Caspian Kutum fingerlings is composed of forebrain or Prosencephalon, midbrain or Mesencephalon and hindbrain or Rombencephalon, the forebrain consisting of telencephalon and diencephalon. Therefore five parts are distinguishable as follows: telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon.

The telencephalon consists of olfactory lobes and cerebral hemispheres; diencephalon consists of epithalamus, thalamus and hypothalamus; mesencephalon consists of optic lobes and tegmentum; metencephalon consists of cerebellum; and myelencephalon consists of medulla oblongata. Medulla oblongata becomes wider at the end, followed by the spinal cord. In the middle of the spinal cord a fine canal is visible which is connected to the cerebral ventricles. The telencephalon is reduced compared to the same area in mammals and consists of olfactory lobes and cerebral hemispheres (cerebrum). Cerebral ventricles are partially visible in this part. The diencephalon or midbrain consists of dorsal epithalamus (including pineal gland, habenular ganglion and thalamus) and ventral hypothalamus. Mesencephalon is large and covered by optic tectum layers with the tegmentum at the inferior part. Optic tectum is divided into two optic lobes and is made up of layers. The cerebellum is the most important part of the dorsal metencephalon. The role of this part is to regulate coordination between motor responses, and as is seen in other vertebrates, the cerebellum is well developed.

The medulla oblongata is the main part of the hindbrain, and the spinal cord is formed in its caudal part. In the spinal cord, the grey matter is different from what is seen in higher vertebrates, in this part the horns of this matter are very close to each other in such a way that white matter can hardly be seen between them, and the gray matter is seen as an Y letter.

Neurons and neuroglia cells form the cellular part of the brain and spinal cord. Lateral ventricles are either absent or surrounded by nervous tissue. In 1893, Gage stated that the unusual arrangement of the forebrain is due to the fact that its lateral walls fold outward during development, while in other vertebrates, these parts curve inward. In 1896, Studnička also confirmed this idea based on the folding of the side walls of the forebrain, and finally this idea was known as the inversion theory and was studied in diverse fish species (Butler, 2000).

Newly, it has been shown that there are two regions in the dorsal and temporal parts of the telencephalon in teleost fishes: the dorsal inverted region and the ventral region, which grew laterally and was not involved in inversion. Thus, the inversion theory can be ended in the form of local temporal inversion (Mueller and Wullimann, 2009; Wullimann and Mueller, 2004; Wullimann and Rink, 2002; Wullimann, 2009). Most of the studies on the brain and nervous system of fishes are in the field of studying the details of diverse parts of the brain, and few studies have been done in the field of comparing the brains of different fish species with each other (Nieuwenhuys, 2011; Ferreiro-Galve et al., 2008; Wullimann and Rink, 2002).

There are differences between the fish brains that live in different ecosystems, which depends on the neurological needs of each fish in its own environment. There are differences between the relative size of the telencephalon, and the relative size and external morphology of the cerebral hemispheres between sharks and teleost fishes (Lisney and Collin, 2006).

The reason why these regions are more widespread in the brain of sharks compared to teleost fishes is not well known, because many of the functions of these parts in the brains of both groups are still unknown (New, 2001; Smeets, 1998; Meek and Nieuwenhuys, 1998).

In both groups of sharks and teleost fishes, pelagic species have relatively smaller sizes compared to coastal species or related to coral reefs (Northcutt, 1978; Huber et al., 1997).

This shows the role of the brain in fish that live in complex milieus with multiple structures. Still, the brain in pelagic fish such as *Carcharhinus falciformis* shark and *Coryphaena hippurus* teleost fish is visibly large and significant, this can be referred to the life behavior of them; for example, it has been determined that sharks of the Carcharhinidae family are social animals and form herds of ten to more than thousands of individuals and have very complex social and reproductive behaviors, including reproductive dances and social hierarchy (Ritter and Godknecht, 2000; Gruber and Myrberg, 1977; Johnson and Nelson, 1973).

Terrain size may not be related to habitat complexity, but instead to complex social behaviors and intra- and inter-species interactions, especially seen in coral reef fishes (Kotrschal et al., 1998). It can be proportional and instead, it should match the complex social behaviors and intra- and inter-species interactions that are especially seen in coral reef fishes. For example, the relative size of *C. falciformis* and *C. hippurus* can be credited to their complex social behavior in their community (Kotrschal et al., 1998; Bshary et al., 2002).

For a long time, the size of the cerebellum has been attributed to the complexity of the movement behaviors of fish (New, 2001; Bauchot et al., 1977).

But despite little information about this part of the fish brain, it is generally accepted that this part of the brain obviously plays a very important role in neuro-motor behaviors (New, 2001; Meek and Nieuwenhuys, 1998; Nieuwenhuys et al., 1998; Smeets, 1998).

For example, the size of the cerebellum and the amount of its folds are very significant in *Alopias superciliosus* and *C. falciformis* sharks, and these species have high swimming power (Compagno, 1984). Brain growth has an inverse allometric relationship with body growth (Northcutt et al., 1978), because, both ontogenically and phylogenically, smaller fish have relatively larger brains (and vice versa) (Brandstaètter and Kotrschal, 1990; Ridet and Bauchot, 1990a; Bauchot et al., 1982).

The brains of fish show the typical arrangement of the brain of most vertebrates. The existence of a small number of exit routes from the brain has caused a large part of the motor system in fishes to be controlled by the spinal cord (Davis and Northcutt, 1983; Northcutt and Davis, 1983; Nieuwenhuys et al., 1998).

Mattner's system, which is seen for sudden escapes in the brain, is an exception to this. Sensory signals received from different parts of the body reach the brain through cranial nerves, these nerves include the trigeminal (V), facial (VII), vagal (X) and three lateral line nerves (two front and one posterior). In the front part, the spinal cord is connected to the brain stem and the cerebellum. The cerebellum protrudes from the upper front part of the spinal cord and a pair of visual lobes cover the cerebellum. The cephalopod brain is made up of a pair of brain hemispheres, in front of which there are olfactory lobes. The brain stem is the main center of all bodily sensory structures except smell and vision (Webb and Northcutt, 1997; Northcutt, 1996; Ariens Kappers et al., 1967; Herrick, 1906; Johnston, 1901; Allis, 1897).

The roof of the fourth ventricle is also covered by the choroid network, which shows different degrees of differentiation in different fishes (Weiger, et al., 1988).

CONCLUSIONS

Results of the present study showed that the nervous system of the Caspian Kutum, *Rutilus frisii kutum*, fingerlings at the size and age of releasing to the sea is fully developed and potentially capable of performing normal nervous functions to cope with the environmental challenges and survival behaviors.

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