THE EVOLUTION OF FISH BRAINS

The anatomical differences in brains of baby reef shark and kingfish, specific to olfaction

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June 15th - July 31st 2017

Introduction

Evolution is the study of changing characteristics of a species over a period of time. These changes can be structural and/or genetic. It is the result of biodiversity, to the extent that evolution within a population can give rise to a new species, and allows species to adapt and survive with the changing environment. As an entity, evolutionary studies help understanding the past and predicting the future. As a result, studies in evolution help understand the functioning of different organisms on earth and solve potential biological situations that impact our daily lives that range from medicine to technology.

Evolution in marine ecosystem is a field of research that is not studied to a great extent though is equally important as studies on land. One reason is the economic dependency that humans have on the ocean, which in turn depends on the balance of aquatic food chains and ecosystems. Additionally, being a rather unexplored field, there is a lot of curiosity regarding aspects of the ocean. And lastly, I have always been interested in all things marine. Being a deep-sea diver, I have grown up around sharks and corals and am engaged in experiencing aspects other than what we see on the surface.

This report lays emphasis on the evolution, specific to the sense of olfaction. Fish mainly rely on olfaction as their primary sense to carry out daily functions. These functions range from feeding, to navigating and protecting themselves from predators. As a result, olfaction is the most variable in sense in terms of the way its used, the extent to which it is used and its importance to different species of fish. Furthermore, olfaction has distinct structures in the brain, which can be analyzed clearly to draw correlations between its roles in the lives of different species. The report also focuses on the shark and kingfish species as they represent two distinct sub-classes in the phylum chordate and class Pisces (fish). The results found could thus, to an extent, be generalized to most cartilaginous fish and bony fish in the ocean, covering a large array of the total fish species and achieving a greater understanding of olfaction across species.

Literature Survey

Classification

Fish are ectothermic (cold-blooded) vertebrates who depend on water for their survival. They form a part of the kingdom Animal, the phylum Chordata, therefore, they have a backbone. They are divided further into 3 dominant classes: Agantha (jawless fish), Chondrichthyes (cartilaginous fish), and Osteichthyes (bony fish). These groups are paraphyletic, which means they have given rise to successive groups. For example, Chondrichthyes have given rise to Agantha, the class from which the ancestors of Osteichthyes (Acanthodiians) has developed.

[1]The shark, scientific name *Selachimorpha* , is part of the class Chondrichthyes and sub-class Elasmobranchii. Elasmobranchii is further divided into two super-orders, the extinct Cladoselachimorpha and the Euselachii, which is made up of 12 Orders, though only 9 currently exist. The baby reef shark is classified into the order Carcharhiniformes (ground sharks), characteristic of sharp-edged teeth, wide mouths, 5 pairs of gills, and moveable membranes over their eyes to protect them when feeding.

[2]The kingfish, scientific name *Scomberomorus cavalla*, belongs to the class Osteichthyes, sub-class Actinopterygii (ray-finned fish), and order Perciformes. They are also part of the family Scombridae. Characteristic of elongated and fusiform bodies, pointed snouts, retractable fins and a highly stream lined body. The latter 2 characteristics make the fish fast swimmers, with high activity.

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[1] *The Shark Trust - Shark Taxonomy*, www.sharktrust.org/en/shark_taxonomy.
[2] *Marine Species Identification Portal : Tunas - Family Scombridae*, species-identification.org/species.php?species_group=fnam&id=686&menuentry=groepen.

Basic Anatomy

Fish are one of the most diverse organisms with over 32,000 species in total[3]. However, despite this diversification, the basic anatomy of most fish is fairly similar. The most common shape is presented in Figure 1.

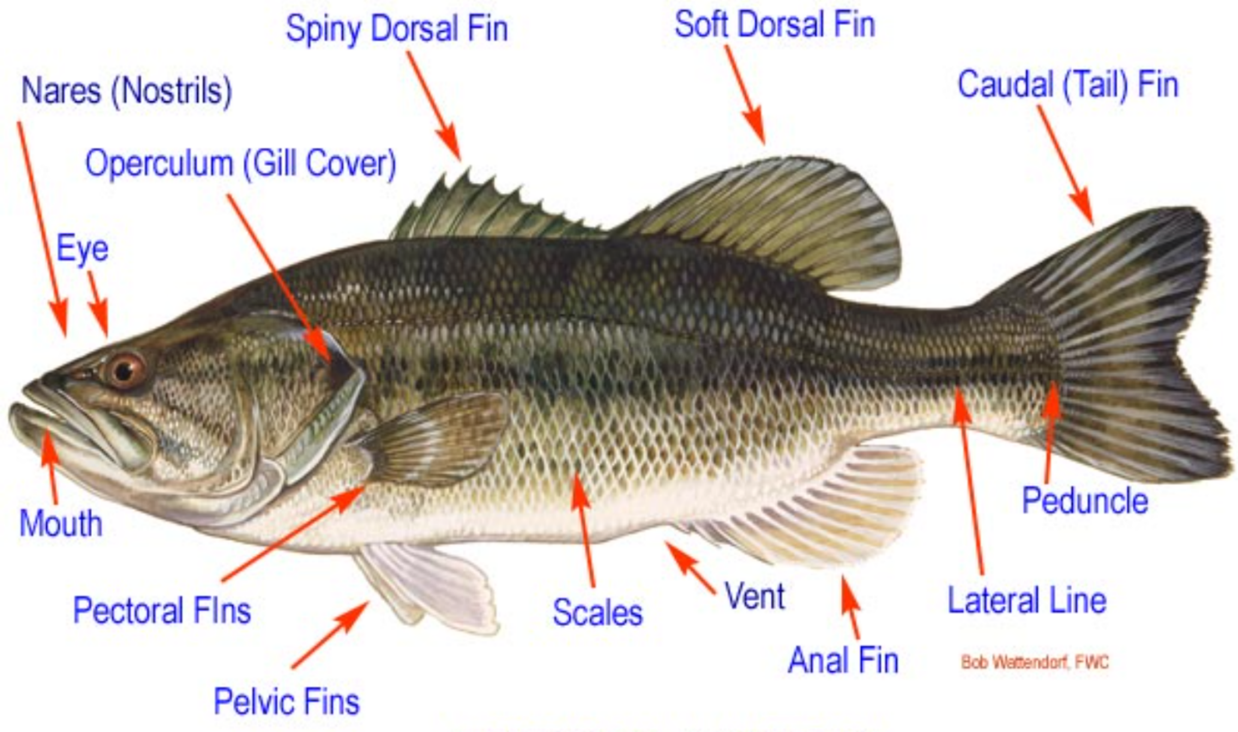

Figure 1: The External Anatomy of a common fish with its different body parts labeled.

[4]Fish use appendages called fins to locomote and maintain its position. They have many types of fins located at different positions on the fish, responsible for various functions involving movement. The Spiny Dorsal Fin, also known as the First Dorsal Fin, is located on the back of the fish and helps it maintain an upright position. As suggested by its name, spines called rays support the fins. The Soft Dorsal Fin and the Anal Fin, located behind the Spiny Dorsal Fin on the underside of the fish respectively, lend stability whilst the fish swims. The Caudal (Tail) Fin acts as a propeller and helps the fish change directions in the water. Lastly, while the pair of Pelvic Fins controls the vertical movement of the fish, the pair of pectoral fins control the horizontal movement. Movement of this fish is also facilitated by the lateral line, which detects water pressure, currents and movement.

The body of the fish is protected by a layer of scales and slime. The types of scales, however, differ from species to species[5]. For example, whilst sharks are covered in Placoid scales, most bony fish have (smooth edges) and Ctenoid scales (jagged edges). Scales can be used to determine the age of the fish. The mucus layer helps prevent infections. Though the eyes of a fish are always open, as they have no eyelids, they rely on their sense of smell to find food. Their eyes can detect colour and are more round due to the refractive index of water. On the other hand, fish have a pair of nostrils to detect odours in the water. The food they catch is ingested through a small mouth, which may or may not contain a set of teeth. The shape of the

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[3] Scottish Government, St. Andrew's House, Regent Road, Edinburgh EH1 3DG Tel:0131 556 8400 ceu@scotland.gsi.gov.uk. “Scottish Government.” *How Many? Oldest? Smallest? Biggest?*, Scottish Government, St. Andrew's House, Regent Road, Edinburgh EH1 3DG Tel:0131 556 8400 Ceu@Scotland.gsi.gov.uk, 8 Mar. 2012, www.gov.scot/Topics/marine/education/faq/howmany.

[4] S.L.Wong, Alan. “Parts of a Fish and Their FunctionsMouseover the Picture to Learn More.” *Anatomy of a Bony Fish*, www.vtaide.com/png/fish.htm.Use the "Insert Citation" button to add citations to this document.

[5] “Australian Museum.” *Fish Scales - Australian Museum*, australianmuseum.net.au/fish-scales.

mouth and presence or absence of teeth can serve as good indications to the diet of the particular fish. For example, carnivores will have sharp canines, while herbivores may not necessarily teeth at all. The olfactory and optical senses are stronger and weaker in different species. Lastly, fish have gills, which they use as a breathing apparatus underwater. They are protected by a cover called the operculum. When fish gulp in water through their mouth, the surrounding blood vessels take absorb dissolved oxygen and the deoxygenated water is expelled through the operculum.

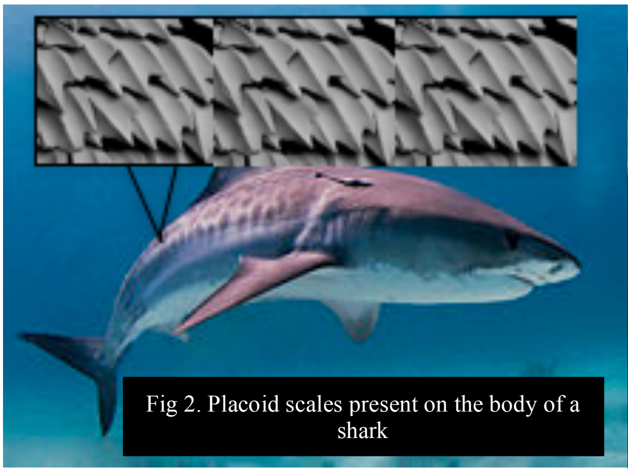

Fig 2. Placoid scales present on the body of a shark

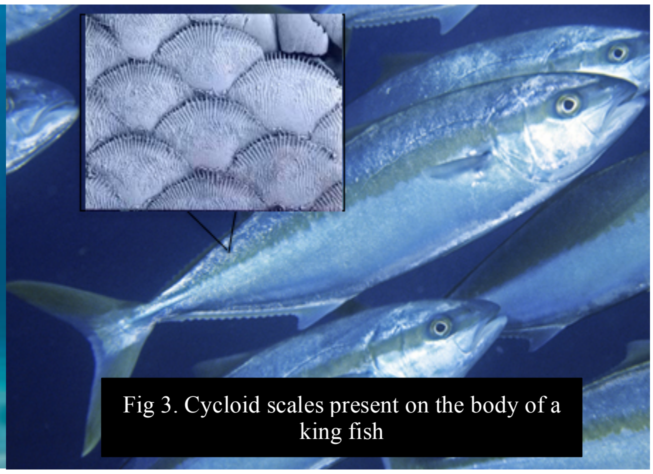

Fig 3. Cycloid scales present on the body of a king fish

The Brain

The brains of vertebrates, in general, begin developing from the embryo, which initially forms the basic structures. In the third or fourth week, a groove begins to form, which develops into the neural tube. At the end of the tube, the three main divisions, the forebrain, midbrain, and hindbrain, form. The primitive brain then expands up to triple its initial size and the different parts are organized in to the three broad divisions.

[6]Like in the brain of most species, the fish brain is further divided into 5 different regions based on discrete functions: diencephalon (forebrain), mesencephalon (midbrain), myelencephalon (hindbrain), and telencephalon (forebrain). [7]The diencephalon is made up of the epithalamus, thalamus and hypothalamus and its primary function is process information related to homeostasis. The mesencephalon consists of the dorsal optic lobes and the ventral tegmentum and is responsilbe for processing visual information in relation to its motor function and those of all senses. The metencephalon is compised of the cerebellum and integrates information of the lateral line that from the inner ear and muscles. It is thus essential to locomotion, muscle tone, orientation and balance. The myelencephalon is composed of mainly the medulla oblangata, which is responsible for all involuntary bodily functions. Mauthner cells, responsible for relaying sensory information for motor coordination, is located at the base of the medulla. These include processes like respiration and digestion. Lastly, the telencephalon is formed by the olfactory and telencephalic lobes. As a result, its main function is olfactory (smell). This paper will focus on the function of olfaction.

Olfaction

Olfaction is a chemoreception that forms the sense of smell. In many animals, olfaction is the leading sense and is used to carry out many functions. These include detection of hazards, pheremones, and food. The vertebrate olfactory system that originated in marine organisms as the first olfactory receptor genes detect water-borne substances. The olfactory systems of different species of fish have been known to differ in many aspects. This paper will discuss the differences in cartilaginous and bony fish.

The olfaction systems differ in bony and cartilaginous fish in many aspects.

[6] "Teleosts." *EAFP Necropsy Manual*, necropsymanual.net/en/teleosts-anatomy/nervous-system-and-the-sensory-organs/.
[7] "Parts of Hind Brain, Midbrain, and Forebrain and Functions." *Its All about Zoology , Botany and Biology*, biologyboom.com/write-notes-on-parts-of-hind-brain-midbrain-and-forebrain-what-functions-does-each-part-perform/.

Firstly, the olfactory organ in sharks is located on the ventral surface of the head, whilst in king fish it is located on the dorsal head. Both organs contain nostrils for ventilation; however, the shapes and functions of the nostrils differ in the two fish. In kingfish, the nostrils are shaped like long or short pipes. The anterior pipes are responsible for inflow and the posterior pipes for outflow. In other bony fish, the pipes are separated and have special valves from which functions are carries out. In sharks, on the other hand, the nostrils don't catch the air, rather the olfactory cavity does. The figure below displays the difference in positions of the nostrils in the 2 fish.

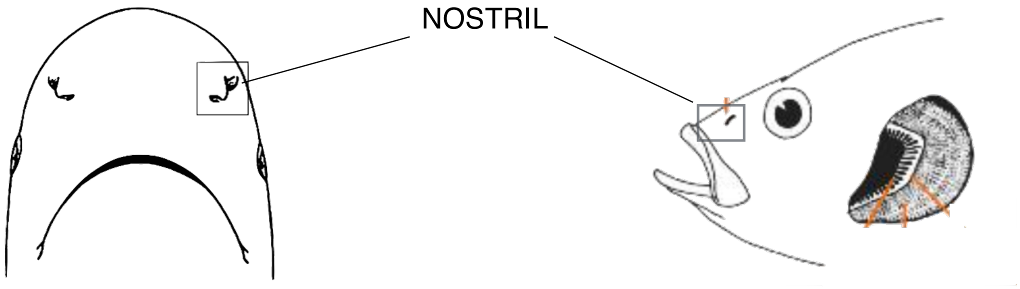

Figure 4: The venral side of a shark, as displayed on left, with a pair of nostrils on the sides of the head. The dorsal side of a kingfish, as displayed on the right, with one nostril the left hand side of the fish.

[8]Another component of the olfactory system, in most fish, is the nasal bridge. These bridges act like funnels and direct water into nasal cavity and are not present in the cartilaginous species like sharks. To substitute for the nasal bridge, these fish have well developed skin outgrowths (lamallae). These are so large that in some species, like shark, they cover a special nasal-oral groove, forming a link between the nasal and oral cavities. These outgrowths are motile in some sharks. The fish that do have nasal bridges, including bony fish like kingfish, that are grown in artificial environments, are not susceptible to the pathogenic bacteria of their environment, resulting in the damage of the nasal bridge. The percentage of artificially raised sturgeon juveniles without a nasal bridge may reach several dozen due to malformations. This activity is mostly attributed to pollution and low water quality.

[9]As mentioned before, fish possess olfactory lamallae whose main function is to increase the perception of chemical stimuli by increasing the area of the olfactory epithelium. The epithelium is the site for olfactory receptors, therefore, increasing its area consequently increases the receptor number and potential. These lamallae help the fish localize food, avoid predators, protection from chemicals in general, and in chemosensory communication with conspecifics. In the bony fish species, primary and secondary lamallae are present, whereas in cartilaginous species, a tertiary lamallae can exist as well. Tertiary lamallae folding increases the surface area of the olfactory epithelium. Though direct correlations based on the sensitivity to chemical stimuli have not been confirmed, it has been inferred by many researcher that the additional lamallae in shark facilitates more docile chemical detection.

The olfactory systems of bony and cartilaginous fish also differ in relation to the olfactory lobes. Bony fish, like most fish, possess seccile lobes that are located adjacent to the forebrain and has a significantly long nerve fiber attached to it. Cartilaginous fish have stalked lobes that are isolated from the forebrain and next to the olfactory organ (nostrils). Though the nerve fiber attached to it is quite short, the olfactory tracts, which connect the lobes to the center of the forebrain, are relatively long. The size of the lobes is comparatively larger as well. In fact, [10]two-thirds of a shark's body weight is comprised of its olfactory lobes. Larger lobes result in more sensory cells; therefore larger lobes have been correlated to a more acute sense of smell. It has been hypothesized that this evolution is due to the dependency of shark on their sense of smell, in terms of localizing their prey.

Lastly, sharks have a highly complex mechanism of '*orientation*', which facilitates their daily functions involving olfaction. Orientation is the comparison of the attracting stimulus in left and right olfactory organs

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[8] Kotrschal, Kurt. "Fish Brains: Evolution and Environmental Relationships Fish Barins : Evolution and Environmental Relationships." March 2015 (1998)

[9] Kotrschal, Kurt. "Fish Brains: Evolution and Environmental Relationships Fish Barins : Evolution and Environmental Relationships." March 2015 (1998)
[10] www.sharktrust.org/en/404.

in stalled waters. In other words, they can detect from which direction a particular smell is coming from or has come from. Research on this characteristic was conducted by Johnsen and Teeter (1985). Their experiment included a shark in a tank who was attached to a device that placed varying volumes of crab extract (10-0.5ml) in the left or right or both nostrils and in the tank. The area where it was released in the tank was the scent zone. Results showed that the shark swam in circles but always reached the scent zone, even with a very low concentration of crab extract. When the stimulus was released to only one nostril, it was observed that the shark swam in the direction where the stimulus was released, demonstrating the sensitivty of their olfaction and how they use it as a guide. The study concluded that sharks have the ability to determine the direction of a given scent based on the timing of scent detection in each nostril. This is very similar to the method used by mammals to judge the direction of sounds. Though bony fish, like cartilaginous fish, can detect the amount of stimulus and identify it, they have not been proven to possess the orientation mechanism that sharks have.

However, an alternative perspective is proposed by researchers in relation to the olfaction abilities of teloest (bony) and elasmobranch (cartilaginous) fish. Contrary to popular science media, certain science literature and journals, the olfaction of elasmonranch fish are not more sensitive than that of teloest fish, but is very similar. Research conducted by Hansen and Zielinski (2005), Hara (1994), Yamamoto (1982) on secondary lamallae proves this. Although the secondary folding of lamallae, characteristic of elasmobranches, increases the surface are of the olfactory epithelium, the organs are not more sensitive to *amino acids* as compared to the organs of small teloests, which do not posses secondary lamallaer folding. It was thus concluded that, although elasmobranches are purported to have a more sensitive olfactory system, they display comparable amino acid thresholds with teloests, thus demonstrating the parallels in olfactory systems between the two groups.

Another common characteristic of olfaction between bony and cartilaginous fish is their repellent behaviour towards the Red Sea Moses sole *Pardachirus marmoratus*. When these shore-line fish are frightened, they secrete a milky, odoured, coloured liquid, which has a repellent effect on predators, especially shark (cartilaginous) and eels (bony). The repellent behaviour is observed in natural and artificial conditions.

Lastly, it has been theorized that the environment plays a large role in the olfactory sensitivity of fish. For example, Caprio (1982) suggested that if the concentrations of a specific amino acid is very high, then the fish's olfactory system would adapt to that concentration, increasing the olfactory threshold for that amino acid. A similar behaviour would be observed if the concentration is to low. In context of sharks, they can detect blood at a concentration of 1ppm because their mode of feeding has made this characteristic a necessity. As a result, it has evolved in the species so all have this level of sensitivity to blood for survival. Furthermore, Bruch and Rulli (1988) and Cagan and Zeiger (1978) conducted research on the binding affinity of olfactory receptor molecules. Some molecules have a higher affinity of binding with certain odours and these receptors are different in different species. The binding affinity again depends on various environmental factors like concentration of the chemical, frequency of binding etc. As a result, it is challenging to make comparisons on the olfactory system of fish as a whole.

Material Methods

Overview

In this experiment, the brains of baby shark (cartilaginous) and kingfish (bony) have been anatomically compared, largely focusing on their olfactory systems. Through dissections of the heads of the fish, the different olfactory organs were recognized and compared in terms of appearance, length, and width. Using this data correlations were drawn and then compared to literature results.

Apparatus And Reagents

2 pointed forceps
1 blunt forceps
1 angular scissor
Trays
40% Formaldehyde Solution
2 Heads of kingfish
5 Heads of baby sharks
Water

Safety Measures

[11]Formaldehyde is a toxic chemical, which is used as a preservative in this experiment. However, one must be careful while using this chemical as it can lead to severe burns and allergic reaction when in contact with skin, eyes and mouth. As a result, gloves, lab coats, and safety goggles should be used while handling the chemical and substances that have been submerged in formaldehyde. It is also extremely toxic to inhale and thus must be used in a well-ventilated room or outdoors while wearing a mask to avoid inhalation. Formaldehyde is also carcinogenic; to avoid any possible genetic defects due to formaldehyde, the above mentioned safety measures must be carried out whilst conducting this experiment. Furthermore, it is a water pollutant as it is harmful to underwater organisms and can cause a shift in pH levels of water bodies. Therefore, before disposing the chemical, it must be severely diluted and any debris submerged in it should be washed before throwing. Lastly, it is advisable to keep this chemical away from gas sources, as it is highly flammable.

Preparation of the fish

The kingfish heads were bought from a local fish-market in Mumbai. They were wrapped in ice and newspaper and stored overnight in a freezer. The heads of the fish were then cut off using a knife and submerged in the formaldehyde solution. The shark heads were already present in the Sophia College laboratory, submerged in formaldehyde. Since sharks are an endangered species, they were not bought from the market but the heads, disposed by the venders, were taken, as they were no longer of use to the venders. Before dissection, the heads were removed from formaldehyde using large forceps and were placed under trickling water for at least half an hour. The heads were then placed in trays filled with water, ready for dissection.

Dissection of the Kingfish

The fish was dissected to obtain a clear view of the brain only so the surrounding body parts were disposed off. The fish was rinsed in freshwater and placed in a tray. The first step was to remove the skin of the fish using forceps. Then, the position of the spinal chord was identified, which was first observed as a ring of cartilage. The underlying fat on either side of the spinal chord was then removed using pointed forceps. Since the skin was rather soft, forceps were the primary instrument used. Any connecting parts that prevented the fat from being removed were cut off using angular scissors. For example, the tissue connecting the eyes to the head were cut of in order to remove the eye. The parts removed were placed in a tray filled

[11] "Railroad Intelligence." *Scientific American*, vol. 1, no. 25, May 1846, pp. 2–2., doi:10.1038/scientificamerican03051846-2c. /.latest_citation_text

with water. Using hands, the jaw of the fish was opened and the surrounding fat was removed. After the head was cleaned off all the fat, the spinal chord and brain, covered with the cranium, remained. Using pointed forceps, 2 openings were made on either side of the cranium. The forceps were placed at an angle horizontal to that of the surface being removed to prevent penetration of the forceps and thus damaging the brain. The openings are used to further break the cranium and expose the brain. Once the top is removed, the brain is scooped out from the cranium and the spinal chord is cut off. The brain is now intact and clear for observation. 2 dissections of the kingfish were done in total. In the first dissection, the cerebellum was damaged due to forceps but in the second it was intact.

Dissection of the Shark

The shark was dissected in a very similar way, however, due to the tough skin, angular scissors were used more frequently. The scissors were used to remove most of skin and fat pieces and the forceps were used nearer to the brain. Though the procedure near the cranium was overall the same, dissecting the shark was trickier as the protruding olfactory lobes were extremely delicate and connected to the brain by a single fiber. As a result, the lobes kept getting detached from the brain and took 4 dissections before obtaining a perfect brain. In the first dissection both lobes became detached; in the second and fourth one lobe became detached and the cerebellum was damaged; in the third one lobe was detached. In the fifth, both lobes and cerebellum were intact.

Results

In total, 2 dissections of the kingfish and 4 dissections of the shark were done and their physical structures were observed and compared. Pictures of one dissection of each fish have been attached below.

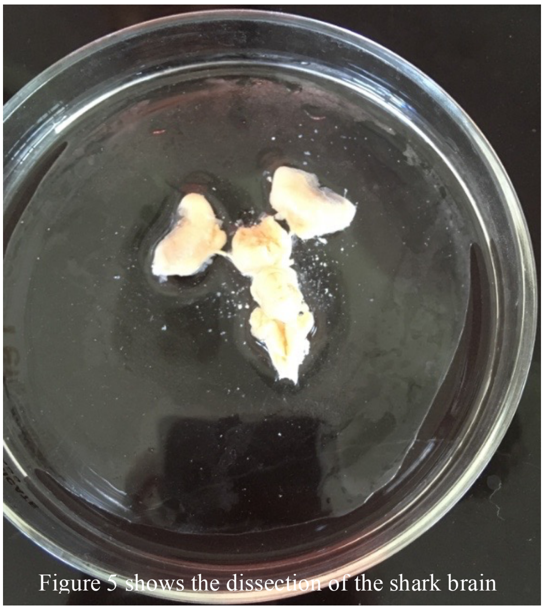

Figure 5 shows the dissection of the shark brain

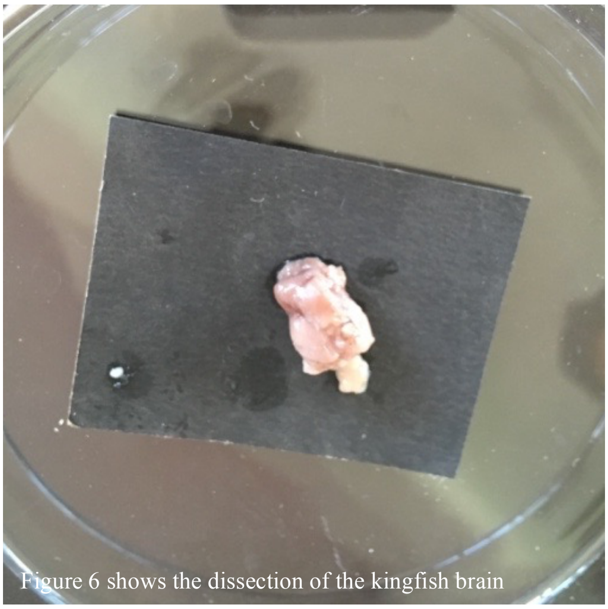

Figure 6 shows the dissection of the kingfish brain

As seen in the images, the olfactory lobes of the shark are much bigger than those of the kingfish, as mentioned in the literature survey as well. Additionally, the olfactory lobes in shark are at a significant distance from the cerebellum, connected by thin nerve fibers, whereas in kingfish, the lobes are attached on the same structure. Due to the thin connection in shark, it was harder to dissect out the brain without damaging the fiber and detaching the lobes and thus required more dissections. As observed, the matter in the shark's brain is more spread out as compared to kingfish, where most of the matter is concentrated in the middle region of the brain.

The differing brain structures can be correlated to feeding habits and habitat conditions of each fish. However, to completely validate these correlations solely observing the outer structure will not suffice. As a result, for a deeper understanding, analysis about the specific genes involved in olfaction of each fish, their natural environment and other biological processes would have be paid attention to.

Conclusion

From the results it can be concluded that the olfactory structures differ across fish species, using kingfish and sharks as an example. The difference in structure ranges from the size, shape, and position of the olfactory components in the brain. Specifically, the olfactory lobes in sharks are bigger, branched and away from the brain, whereas, in kingfish they are smaller, circular, and attached to the brain. As mentioned before, correlations can be drawn between these structures and the daily lives of each fish. For example, larger lobes could signify a sharper sense of smell due to the preferred diet of shark. However, these correlations can't be assumed to be true because contrary research exists. As a result, the sensitivity levels of the olfactory systems in both species of fish are up for questioning.

Further Scope

After this stage, many questions remain unanswered but worth dwelling in to. For example, how do biological differences between different species of fish affect their olfaction and other sensory systems? Biological differences refer to the genomic information, anatomy, and processes in the bodies of the species. Furthermore, by analyzing these biological differences in relation to the surrounding environment, concrete correlations can be drawn between the two fields that will most likely explain why these species evolved in the way did. Lastly, it is worthy to draw comparisons between salt water and fresh water fish in respect to these aspects as pollution and chemicals seem to have an effect on basic functions like olfaction. Additionally, this comparison will make the research conducted in this topic, more generalizable to many fish species in different habitats.