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HISTOLOGICAL APPROACH ON THE LATERAL LINE ORGAN OF JACK MACKEREL (*Trachurus japonicus*) FOR MECHANICAL SENSING IN SWIMMING BEHAVIOR

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Abstract: The function of lateral line organ system of jack mackerel (*Trachurus japonicus*) was studied through histological observations, for identifying the structure and distribution of lateral line organs both for the head part and body trunk, with the identification of the histological details of pores and hair cells. Histological observations were conducted with a binocular microscope, with three different approaches as haematoxylin, methylene blue and di-4-ASP dye for identifying the structure and distribution of lateral line organs, and then to identify the histological details of pores and hair cells by preparing the sampled tissue for photo-microscopic observations. The results showed that seven canal systems were identified in the head part; such as supra temporal, postoptic, optic, supra orbital, infra orbital, operculum and mandibular canals, with the width ranging 0.9-1.5 mm. Higher density of pore distribution was examined on the nasal and dorsal areas of the head part, which is 7-8 and 5 pores/mm^2 respectively. In the other areas of the head part, the density of pores was ranged as 1-2 pores/mm^2. Concerning the body trunk, 29 pores of 12-13 µm diameters were identified along the main lateral line. The functions of lateral line organ of jack mackerel are discussed in relation to the swimming behaviour and performance.

Keywords: Jack mackerel, lateral line organ system, hair cell, histological observation, marine, *Trachurus japonicus*.

Introduction

The studies on fish behavior and functions of the sensory system are of great benefit to improve fishing gear design and the strategic operation (Uyan *et al*., 2006). The sensory organs of fish are of primary importance to understand the behavioral response of fish during the capture process. Histological examination is one of the useful methods for comprehensive understanding on the functions of a sensory organ system.

The lateral line system is a mechanosensory organ of fish. The aquatic vertebrate's lateral organs system is a mechano-receptor, which is sensitive to movement in the water (Diaz *et al*., 2003). Therefore, it is believed to affect the several aspects of fish behavior, such as swimming, feeding behavior and individual interaction in a school. Observations on the Eurasian ruffe *Gymnocephalus cernuus* have given evidence that the lateral line can be used to substitute for visual organs. Janssen (1997) reported that, the ruffe has greater distance to detect the daphnia magna than yellow perch *Perca flavescens*, and swim faster while searching for pray in dark condition monitored by the infrared camera, Thus, the structure of the mechanosensory lateral line organ affects the level of sensitivity of a fish on the environment.

Almost all of the mackerels are a pelagic species, which has schools during the migration period. In case for the Spanish mackerel *Scomberus niphonius,* the schooling behavior starts to develop from 17 to 19 days old. During this period, the separation angle and nearest neighbor distance decrease significantly, as well as adapting the characteristic of aggregating in parallel orientation (Masuda *et al*., 2003). Presumably, the lateral line of pelagic fish has started to grow up and develop in that age. Thus, they can be orientated in school for swimming. A study on the morphology, distribution and structure of the lateral line organ system are required for initiating appropriate to know characteristic of swimming behavior of the fishes. Hence, this study will discuss the structure and function of lateral line for mechanical sensing in swimming and schooling behavior of jack mackerel.

Materials and Methods

This study started in 2007, and accomplished in 2013. In 2007, we just used two methods for visualizing the lateral canal organ system, and during followed the Program Academic Recharging at Fish Behaviour Laboratory of Tokyo University of Marine Science and technology in 2013, the research continued by using di-4-ASP method as a new method for visualizing and observing the clumping neuromast in lateral organ system. Twenty-nine adult jack mackerels of total length 22.20±1.50 cm (Mean ± STDEV) were used for the study. The fish were obtained from the farmer in Numazu, Suruga Bay, Japan. They were kept at 21.5°C water temperature in the fiberglass tank, and were given the fish meal pellet every day. Eight jack mackerels were used to observe the distributions and morphometrics of the lateral line organ in both the head and body trunk. A solution of hematoxylin was injected into the lateral line canals by a small syringe (22 G x 1" (0.70x25 mm), Terumo, Japan) (Uyan *et al*., 2006). Six jack mackerels were dyed in di-4-ASP solution in 120 minutes for checking distribution of clumping neuromast in the canal organs. Whole head and body of the photograph were used to create computer aid for tracking the photo object. Another five jack mackerels were used to observe the distribution of pores over a lateral line organ. The fishes were decapitated, fixed into the bouin solution, and sectioned at respective lateral line organ part. For quantitative analysis, the number of pores was examined from photograph under microscopic observation. Estimation of the number and density of pores was calculated by the equation $Np = (Pn/ Wa)x100$. Where as, Pn is number of pores and Wa is the width of photograph.

Ten individual mackerels were finally used for histological analysis of hair cells (Siregar, 1994). The lateral line organ parts were enucleated and fixed in formalin 10% or 20% more than 2 days. The tissues were transferred by 75% ethanol overnight, dehydrated in a graded series of ethanol (80%, 85%, 90%, 95%, and 100%, 3 x 30 min), cleared in xylene (3 x 30 min), embedded in paraffin, cut into 2 - 8 µm thick cross-sections, and stained with hematoxylen-eosin for histological examination under a photomicroscope. The morphometric of neuromast observed and recorded by microscopic observation.

Results

The lateral line organs were identified and drawn in Figure 1. The highest density of lateral line organs identified in the head. The lateral line organ were interconnected, while the ventral trunk line were not interconnected with main and dorsal trunk line. Seven lateral line organs identified in the head part, namely supratemporal, postotic, otic, supraorbital, infraorbital, preoperculum, and mandibular canal with widths ranging 0.9-

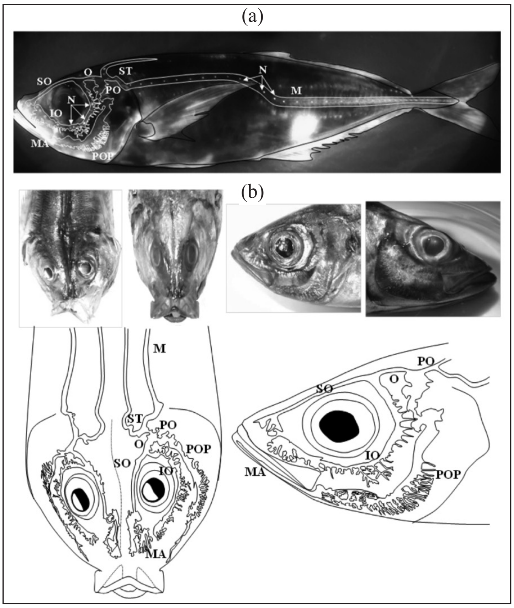

Figure 1: Diagram of the lateral organ systems on the head and trunk body of an adult jack mackerel. IO, infraorbital canal; MA, mandibular canal; M, main lateral line canal; O, otic canal; PO, postotic canal; POP, preoperculum canal; SO, supraorbital canal; ST, supratemporal canal and N, neuromast

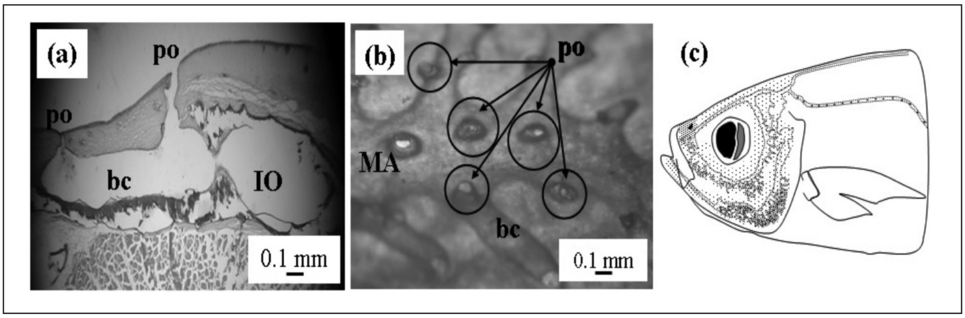

Figure 2: Photomicroscopic and photograph image of (a) transverse section of infraorbital canal. Pores (po); branch canal (bc); infraorbital canal (IO). (b) the horizontal section of mandibular canal. Mandibular canal (MA). (c) Distribution of the pores on the body part

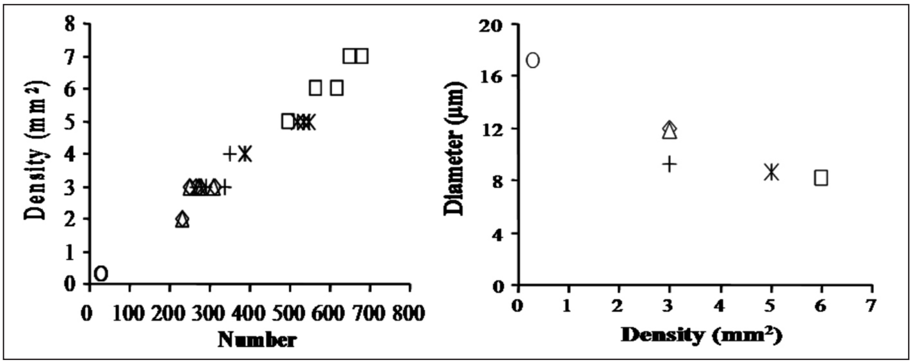

Figure 3: (a) Relationship between number and density of pores at body parts; (b) Relationship between density and diameter at respectively body part. Dorsal surface of the head (◊), infraorbital (Δ), mandibular ($_*$), nasal (□), preoperculum (+) and main trunk line (○)

1.5 mm (Figures 1a and b). The supraorbital canal extends along the dorsal surface of the skull and turns ventrally to be connected with the infraorbital canal. The preoperculum canal identified along operculum, and interconnected with the mandibular canal. Its lower portion is almost parallel to the infraorbital canal. The infraorbital and mandibular canal identified densely branched (Figure 1b). Supratemporal canal were not interconnected between the right and left lateral line organs system on the top of the head.

Three lateral line canals in the trunk body were identified as main trunk canal with widths ranging from 0.9-1.0 mm (Figure 1a). The supratemporal canal interconnected with the main and dorsal lateral line canal. The main lateral line canal runs the overall length of the body and deflects above the tip of pectoral fin.

Figure 2b shows that pores of jack mackerel examined to the lateral line canal organ system and tip of branch canal. The branch canals were interconnected with the lateral line canal organ. Figure 3a shows that the highest density and number of pores was indentified at the nasal and mandibular region as 6 pores/mm² (602 pores) and 5 pores/mm² (475 pores) in average respectively. It was lower as 3 pores/mm² (305 pores) at the preoperculum, and was 3 (268 pores) at the infraorbital and dorsal surface of the head (Figure 3a). At the main lateral line canal, the pores number were identified as 29-30 pores, which is located just above the line canal.

Clumping of neuromast cells was found in the otic, postotic, supraorbital, infraorbital, and preoperculum canals as yellow spots in figure 1a, which is embedded on the base of canals between the dermal and hypodermal layers as showed at

Figure 4a. Neuromasts consisted of several rows of hair cells (Figure 4b), which is cylindrical in shape and occupies the upper half of the sensory epithelia (Figure 4c). Its dimensions were 1.43 µm in height and 0.48 µm in width (Figure 4c). A spherical nucleus was centrally located in the hair cell, which was identified as 0.34 µm in diameter and was surrounded by mitochondria. The upper side of hair cell was identified as the cupula (Figure 4b), which contained kinocilium and stereocilia. Supporting cells were lined up alongside the basal membrane connected to hair cells by a nerve ending efferent (Figure 4b and c).

Discussion

Jack mackerel is one of a number of species to form huge schools when migrating. Schooling behavior is particularly predominant in pelagic fish. The major function of schooling behavior is supposed by many to be predator avoidance, the early detection of, and confusion of the attack from predators (Masuda *et al*., 2003). The maintenance and structure of the schools formation are aided by mechano-sensory organs. It is believed that the mechano-sensory organs function as a sensory system initiating appropriate behavioral response for environmental stimuli. Avoidance of obstacles while swimming, schooling behavior, prey capture at, and below the surface and predator avoidance are all believed to be directly influenced by the function of the mechano-sensory organs (Popper & Platt, 1993). The lateral line organ system is important for the anchovies *Engraulis japonicus* and maintenance of the school in the dark. The lateral line sense is also known to be important in the schooling of tuna *Euthynus affinis*, saith *Pollachius virens*, red sea bream *Pagrus major* and bluegill *Lepomis macrochirus* (Uyan *et al*., 2006). From observation, the lateral organ system is well developed in the jack mackerel. The many canal systems present are very important for the transmission and consequent registry of movement in the ambient water. The inside of lateral canal can be found with some clumping of the neuromasts, which play an important role to detect and transform water movement and vibrations to nerve impulses for interpretation by the fish's brain. Sensitivity of the fish is to detect objects and water motions

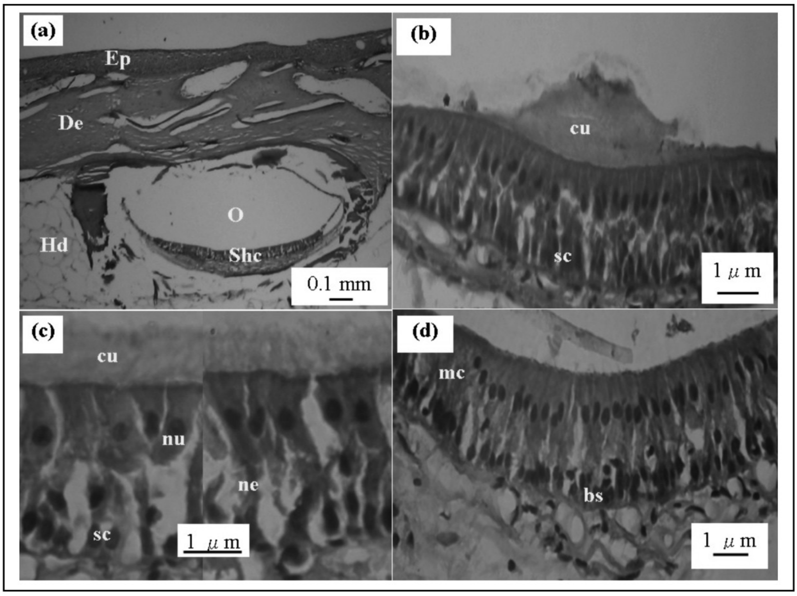

Figure 4: (a) Structure and position of the clumping neuromast in otic canal. Epidermis (Ep); dermis (De); otic canal (O); sensory hair cell (Shc); Hypodermis (Hd). (b) Neuromast canal. Cupula (cu); mitocondria(mc); basal membrane (bm); (c) hair cell; nucleus (nu); supporting cell (sc); nerve ending efferent (ne)

which depends on the morphology and distribution of lateral organs system in the body. One likely functional consequence of a wider canal is greater sensitivity to low frequency signals (Janssen *et al*., 1999). There is, however, a greater susceptibility to interference from low frequency noise (Denton & Gray, 1988; 1989; Janssen, 1996). The fish's movement in the water, especially when schooling will produce both water movement and vibration. Reception by the lateral line canal system and subsequent interpretation by the brain and central nervous system help the fish maintain distance from each other. It is believed that, the canal organs systems help jack mackerel to maintain and determine the direction of migration when schooling. Furthermore, it is used to detect a potential predator's movement as well as any potential item of prey. Some species of fish can detect the prey by using the lateral line organ. For example, the *Batrachocottus baicalensis* can detect their prey, such as amphipods moving to a position parallel to their body (Janssen *et al*., 1999).

The morphology and distribution of jack mackerel's lateral organ system present in the head displays similarities with the rainbow trout *Oncorhynchus mykiss* (Siregar, 1994) and anchovy *Engraulis japonicus,* (Uyan *et al*., 2006), except in regard to the trunk line canal. In jack mackerel, present were the dorsal, main, and ventral trunk line canals. Identity is confirmed by the appearance of initial gnathostomes and teleost fishes (Northcutt, 1989; Northcutt *et al*., 2000). The morphology of trunk lateral line is affected by the pectoral fin position, size, and shape. The dorsal deflection of the anterior portion of trunk line in several fishes avoids water disturbance caused by movement of the pectoral fin (Dijkgraaf, 1963). The main trunk lateral line of jack mackerel has distinct deflections which circumvent the tip of pectoral fin. Dissimilarity exists here in comparison with the anchovy and rainbow trout, as they have a straight main trunk lateral line due to a shorter pectoral fin, as well as the position of the pectoral fin also lower than jack mackerel.

The pores play a role to support the function of the lateral line organ system. It has functions as a receiver channel for both the water motion and vibration from outside into the lateral canal organ. Water carries sound vibrations through small pores in a fish's skin and into the lateral line, an inner fluid-filled canal. Moving through the canal, the vibrations stimulate the hair of sensory organs. As Figure 2a and b show that all of the pores connect to the lateral line organ system. It brings through the water motion and vibrations to the clumping of neuromast in the lateral organ system. So, the relationship between the number of pores and density of pores is very important to consider for estimating the sensitive area of jack mackerel's body regarding the detections of water movement.

The highest density and number of pores on the jack mackerel's body were identified on the head, especially in the nasal and mandibular regions. The sensitive area in the frontal zone of the fish receives oncoming water motion and vibration as they move through the water. The pores concentrated on the preoperculum and infraorbital function to detect the water motion and vibrations from the side. The pores on the dorsal surface of the head function as a receiver of water motion and vibration coming from above.

In the lateral canal organs system of jack mackerel is the same distribution of canal neuromasts. The canal neuromast, however, are efficient at detecting hydrodynamic stimuli even in the constant presence of background water flow, because of the filter properties of lateral line canals (Engelmann *et al*., 2000). It is composed of rows of hair cells, supporting and mantle cells. They have a special function of translating sound into electric signals that nerves can convey to the brain. For detecting water displacement, sound at the frequency higher than 500 Hz, magnetic fields and location of obstacles (Germana *et al*., 2002).

The sensory tissue is important for the mechanosensory function of lateral line organs. The shape and size of the cupula vary considerably, depending upon the location of the neuromast on the body and depending upon species (Evan, 1993). As Figure 4a, b and c show that the hair cells are cylindrically shaped and have stereocilia at the top of the cell and a nucleus at the bottom. Alfons *et al.* (1989) stated that the sensory hair cells are specialized epithelial cells, which function as mechano-electrical transducers in the acousticolateralis organ of vertebrates. Thus, hair cell as electrical transducer in the lateral line organ helps the jack mackerel to detect water motions and low frequency sounds when swimming.

Behavior studies showed that, whereas most fishes can detect to sound up to 1-3 kHz, several species of the genus Alosa (*Cluepeiformes*, i.e. herring and their relative) can detect sounds up to 180 kHz (over even higher) (Popper, 2000). It is suggested that this capability evolved in these fishes, so they can detect water motion and low frequency, and obstacle, which included on the jack mackerel. In the present study, based on distribution and structure canal organ system of the jack mackerel have many small branches arranged as those of adult clupeoid fishes that form large schools. Presumably, the dense branching of the lateral line canals on the head enhances the sensitivity of the cephalic lateral line and might be a characteristic of schooling pelagic fish (Masuda *et al*., 2003).

Visual and lateral line organ play an important role on fish to pass through the mesh and avoid the fishing gear. Underwater observations show that the fish could be passing at the mesh center without touching twine in case of the voluntary swimming, and rather conscious to upward for taking a space with upper twine and touching with lower twine. In capture process of the set net, the schooling of fish could detect the leader net of set net from 100 meters distances (Arimoto & Anraku, personal communication, November 27, 2007). The visual organ of fish could not see the object for that distance. Probably, fish was detected by lateral line organs function, which is friction between water and net by current potentially to deliver water motion and vibrations. Thus, fish can detect it. Therefore, further research will be concerned on the schooling behavior of jack mackerel against the net panel, which is related on a role of the lateral line organ in swimming and schooling.

Conclusion

According to the distribution of lateral organ canal system and pores; the sensitive area to detect object and water motions for jack mackerel is on the head. Then the left and right side of the head that is possible to avoid and arrange distance from the side position. This is most important to arrange distance while swimming in schooling, as well as fish can detect the object direction near the fish body. The sensitive area to detect the object was in frontal side and up of the dorsal surface of head, through the hypothesis that highest density of pores can be the index of near field sensor.

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