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Olfactory Organs of Two Pelagic Teleost Fish—Opah (*Lampris guttatus*) and Dolphin fish (*Coryphaena hippurus*)

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Abstract

Olfactory organs of two pelagic teleost species—opah (*Lampris guttatus*) and dolphin fish (*Coryphaena hippurus*) were investigated with scanning electron microscope. Gross morphological observation showed that in both fish the paired olfactory organ is situated on the snout. Anterior and posterior openings are present in both fish. Numerous number of lamellae radiate around a short raphe. Olfactory ventilation sac is present in both fish but is more developed in opah. Olfactory sensory epithelium is found intermingled as islets or patches within the nonsensory epithelium. Ciliated olfactory receptor neuron and microvillous olfactory receptor neuron are observed in both fish with the former being more abundant. The population of receptor neurons is estimated to be ~3.0 and ~7.7 million in opah and dolphin fish respectively. Ciliated nonsensory cell is rare or absent in all lamellae examined while goblet cells are observed in both sensory and nonsensory epithelia. Epidermal cells forming microridge of finger-print like patterns are the primary cells forming the nonsensory epithelium.

Keywords: *Coryphaena hippurus*, *Lampris guttatus*, olfactory organ, pelagic fish

Introduction

Vision and chemoreception are probably the most important sensory systems used in oceanic fish in search of food in vast pelagic environment. Olfaction in particular has shown to induce prey-searching behaviors and feeding responses in little tuna (*Euthynnus affinis*) and yellowfin tuna (*Thunnus albacares*) (VAN WEEL, 1952). ATEMA *et al*. (1980) demonstrated that the yellowfin tuna can form chemical (olfactory) search image in procurement of food as a convenient system that enables the fish to switch to a major food source while ignoring less abundant food source. As a means to delay dilution of potent cues in open ocean, prey odors and other chemical cues are being entrained in lipid components of liposomes so as to provide persistent arousal and search cues for tunas (WILLIAMS *et al*., 1992) and other pelagic critters. Recently, similar chemosensory information carriers are found in land animals (LAZAR *et al*., 2001).

Olfactory cues are detected by the olfactory organ and relevant behaviors are released in any given organism. Literature showed that structures of olfactory organ of Genus *Thunnus* were studied especially its relevance to Scombridae taxonomy (IWAI and NAKAMURA, 1964). GOODING (1963) revealed that the skipjack (*Katsuwonus pelamis*) has a well-developed olfactory organ and showed that the olfactory ventilation sac may function

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as a pumping device to draw in water into the olfactory chamber during swimming. By scanning electron microscopy YAMAMOTO and UEDA (1979) first studied the olfactory organs of bluefin tuna (*Thunnus thynnus*) and other small pelagic fish. MANA *et al*. (1998) also revealed that the olfactory organs of some large pelagic species possess two types of olfactory receptor neurons—ciliated and microvillous olfactory receptor neurons on the lamellar surface, both of which are comparable to the receptor neurons found in red sea bream (*Pagrus major*) (MANA, 2001). Further the olfactory system in bigeye tuna (*Thunnus obesus*) and striped marlin (*Tetrapturus audax*) not only possess an olfactory ventilation sac but the density of olfactory neurons ranged from 40 000–68 000/mm^2 (MANA, 2000).

To reveal the diversity of olfactory systems in pelagic fish, the olfactory organs of opah (*Lampris guttatus*) and dolphin fish (*Coryphaena hippurus*) were investigated with scanning electron microscopy. Results indicated that opah has a well-developed olfactory ventilation sac with ~3.0 million olfactory receptor neurons in one rosette while dolphin fish has ~7.7 million olfactory receptor neurons per rosette. Adaptive morphological features of the olfactory systems of pelagic fish are discussed in relation to pelagic mode of life.

Materials and methods

Source of Materials

Specimens used in this study were caught by tuna longline on board Kagoshima University training vessel, Keiten Maru during ocean cruise at Northern Pacific and South of Okinawa in 1996–1997. Table 1 showed the localities where fish were sampled, standard body length and number of lamellae per olfactory organ.

Ultrastructures

For scanning electron microscopic (SEM) observation, specimen was sacrificed by decapitation. Immediately each nasal sac was flooded with 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) through anterior nasal opening for ~10 min as suggested by MORAN *et al*. (1992). Then the rosettes were surgically removed and fixed in the same fixative for 12 hr. The lamellae were post-fixed in 1% OsO_4 for 2 hr. After dehydrated through a gradient series of ethanol, the lamellae were dried in liquid CO_2 critical-point apparatus Hitachi HCP-2, coated with platinum-palladium in a Hitachi E-1030 ion sputter and viewed with a Hitachi S-430 scanning electron microscope.

In density analysis of olfactory receptor neurons (ORNs) we estimated the number of ORNs based on the SEM micrographs that included both nonsensory and sensory regions to minimize the effect of the unique sensory pattern in both fish. Micrographs were taken randomly on lamellar surface at a magnification of 2000 depicting an area of 750 μm^2. The counts were then converted to density/mm^2. Lamellar areas were determined by cutting and weighing of the well-preserved lamellae. A total of 16–24 micrographs from the lamellae of 3–5 rosettes in each fish species were examined.

Results

Gross Morphology of Olfactory Organ

The opah and dolphin fish possess a pair of olfactory organs situated on the dorsolateral side of the head just anterior to the eye (Fig. 1 A, B). The olfactory chambers are not

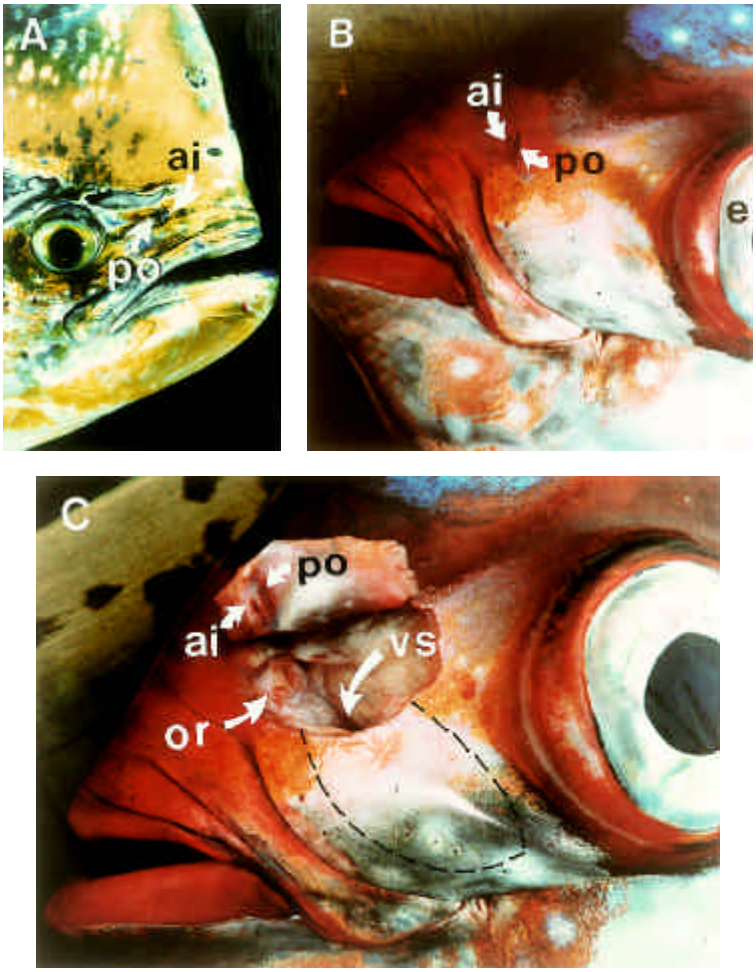

Fig. 1 Head region showing the position of olfactory nostrils and olfactory organ in (A) dolphin fish and (B) opah. (C) Olfactory chamber of opah exposing the olfactory rosette (or) and an opening leading to a ventilation sac (vs) as shown by the hatched lines. (ai) anterior opening, (po) posterior opening, (e) eye.

connected to the respiratory system in both species. When a pelagic fish swims in open seas/oceans the water containing odorants enters into the olfactory chamber via an anterior inlet and exits via a posterior outlet. In both species, the inlet and outlet are separated by a nasal bridge of epidermal tissue which is ~1–2 mm wide in both species and the inlet is smaller (~1 mm diameter) than the outlet (~2 mm diameter). In the dolphin fish an

upstanding nasal flap around the inlet probably serves to catch the water into the nasal cavity from the faster moving layers not immediately in contact with the body when the fish is swimming. Ventilation sac is present in both species and well-developed in the opah (Fig. 1c) but it was not thoroughly investigated in the dolphin fish. The ventilation sac in the opah connects to the olfactory chamber by an opening and is situated beneath the lachrymal bone. There are no muscles attached to the ventilation sac. It was demonstrated that as the mouth closed the ventilation sac is compressed and expanded when the mouth is opened. Olfactory rosette, an outgrowth of the floor of the olfactory chamber comprised numerous lamellae radiating from a short midline raphe (Fig. 2) and it (olfactory rosette) is seen

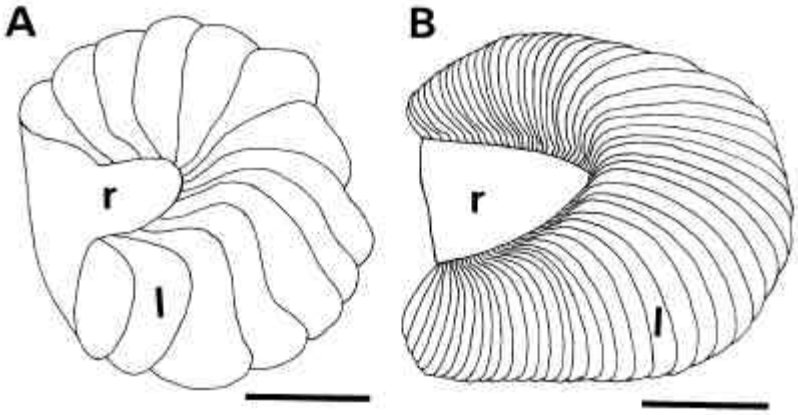

Fig. 2 Semischematic diagrams of olfactory rosettes of (A) opah and (B) dolphin fish. (r) midline raphe, (l) lamellae. Scale bar = 3 mm.

directly through the anterior opening in both species. The olfactory lamellae contain the olfactory mucosa. The number of lamellae varies between fish in each species and between the pair of organs in the same specimen. There are 14–16 and 61–64 lamellae in the opah and dolphin fish respectively (Table 1).

Table 1. Sampling area and localities, standard length and number of olfactory lamallae of two pelagic fish

Species name (Common name) [Japanese name]	Area of fish sampling	Localities of fish sampling		Standard length in cm	Number of lamellae	
					in right rosette	in left rosette
Lampris guttatus (Opah) [Aka mambou]	Northern Pacific South of Okinawa	18°34' N 22-25°N	132°57' E 127-131°E	101.1 104.9	14	16
Coryphaena hippurus (Dolphin fish) [Shiira]	Northern Pacific South of Okinawa " "	27°43' N 22-25°N "	130°59' E 127-131°E "	104.8 101.5 106.6	61	64

Fine Structures of Olfactory Epithelium

In the opah and dolphin fish the lamellar faces were lined with sensory and nonsensory epithelia. The sensory epithelium was found separated into patches or intermingled as islets within the nonsensory epithelia in all lamellae examined (Fig. 3). Both species sensory

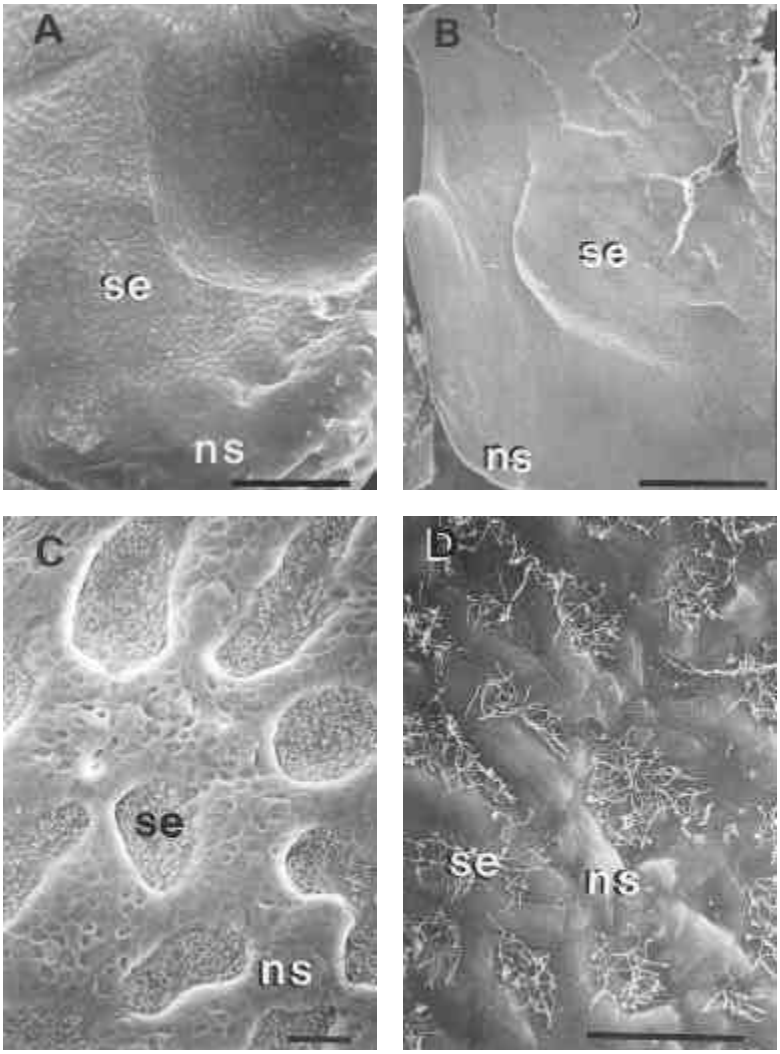

Fig. 3 Topographic distribution of sensory epithelium in (A) opah and (B) dolphin fish. In both species sensory region (se) covers the lamellar face except for the lamellar margins which consist mainly of nonsensory epithelium (ns). Sensory epithelia which are thrown into islets or patches are surrounded by nonsensory epithelium in (C) opah and (D) dolphin fish. Scale bar = 0.6 mm for A and B, 30 μm for C and D.

epithelia were composed of olfactory receptor neurons (ORNs), sustentacular and goblet cells. Ciliated olfactory receptor neuron (cORN) and microvillous olfactory receptor neuron

(mORN) which send their axons directly to the sessile type of olfactory bulb were observed in both species. cORN and mORN were confirmed to possess axon-like processes in a marine species (MANA, 2001). The dendrites of cORN are consisted of a protruding knob (1.0–1.3 μm in diameter) which bears 3–8 cilia radiating around it. These apical dendritic knobs tend to extend further to the surface of the adjacent epithelium in opah than the ones observed in dolphin fish (Figs. 4–5). This suggests of species specificity since all ORNs

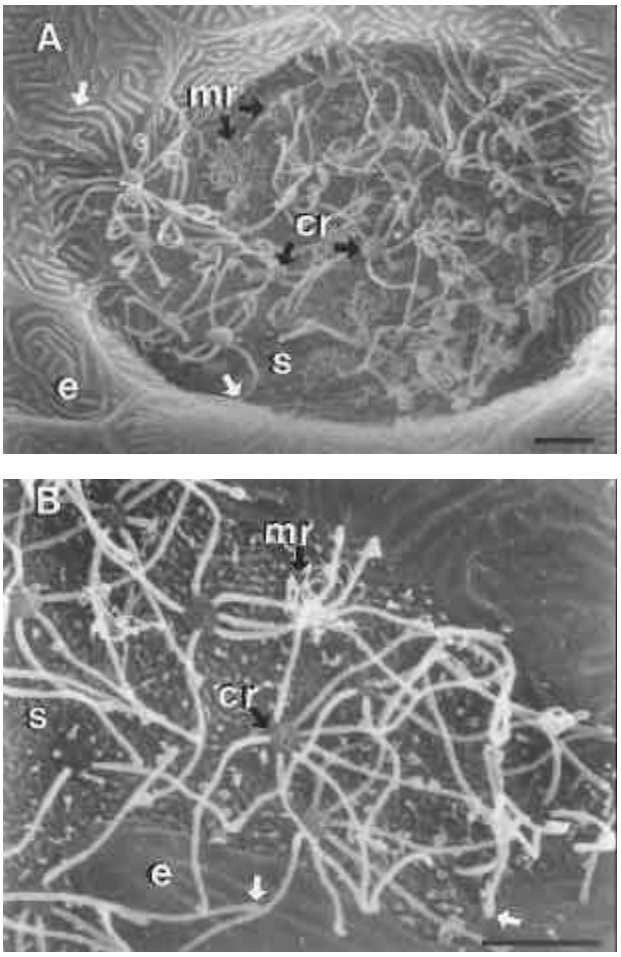

Fig. 4 Sensory epithelium of (A) opah and (B) dolphin fish which bear the ciliated olfactory receptor receptor neuron (cr) and microvillous olfactory receptor neuron (mr). (s), sustentacular cell, (e) epidermal cell. White arrows indicate tapering cilia of cr in (A) which contrast to bloated apical processes of the same type of neuron in (B). Scale bar = 3.0 μm.

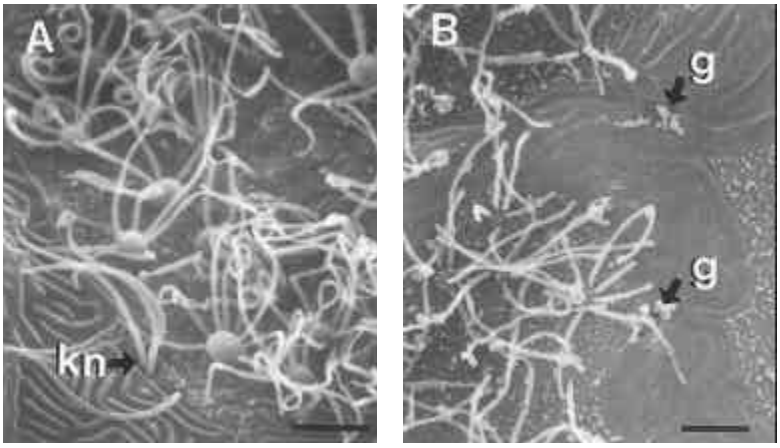

Fig. 5 Ciliated nonsensory cell (kn) in (A) opa and goblet cell (g) in (B) dolphin fish. Note the apical knobs of ciliated receptor neuron are more extended to the neighboring epithelial surface in (A) than in (B). Scale bar = 3.0 μm.

observed displayed the similar dendritic knob form. Those cilia were much longer in opah (5.6 μm) with bulbous apical processes than the tapering cilia in dolphin fish (5.0 μm) (Fig. 4). The second type of ORN comprised 20–80 microvilli projected from an olfactory knob which is usually buried in the sensory epithelium in both species. Opah possesses more number of microvilli per olfactory knob than dolphin fish. Microvilli of mORN are 1.4 μm long and 0.1 μm in diameter. There is no evidence so far to suggest motility in the cilia and microvilli of the ORNs. Another cell type found in the sensory epithelium is the sustentacular cell. They have microvilli-like protrusions on their most apical surface (Figs. 4–5). Ciliated nonsensory cell is rare in both species (Fig. 5). Goblet cells were observed in both sensory and nonsensory epithelia (Fig. 5) of both species. Nonsensory epithelium consists mainly of epidermal cell with a finger-print like pattern of microridges (Figs. 4–5).

Density and Population of Olfactory Receptor Neurons

The mean density of ORNs in opah and dolphin fish are 55 000/mm^2 and 32 000/mm^2 respectively. cORNs are the most abundant in both species while mORNs are occasionally observed especially in dolphin fish. Calculation of lamellar area showed that the exact sensory area in opah is 1.82±0.49 mm^2 (mean±SD, n = 4) while the nonsensory area is 2.36±0.50 mm^2 (n = 4). In dolphin fish the sensory and nonsensory areas are 1.92±0.37 mm^2 (mean±SD, n = 6) and 2.49±0.46 mm^2 (n = 6) respectively. Therefore the number/-lamellae in both species are much higher than density/mm^2 (Fig. 6). ORNs found in patches or islets in dolphin fish tend to be more scattered on lamellar surface than in opah as indicated from high standard deviation in mean density/lamellar area (Fig. 6). Opah with 15 lamellae would yield ~3.0 million ORNs in one olfactory rosette. Similarly dolphin fish with 63 lamellae would yield ~7.7 million ORNs in one olfactory rosette.

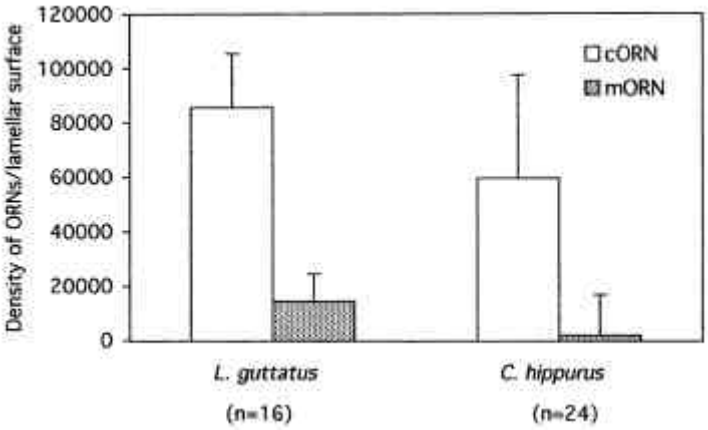

Fig. 6 Density of olfactory receptor neurons (ORNs) in two pelagic fish. Bar represents means ± SD of microvillous (mORN) and ciliated (cORN) receptor neurons on a lamellar surface.

Discussion

This report describes the macromorphology and microstructures of the olfactory organ of two pelagic species—opah and dolphin fish. Both fish possess an olfactory ventilation sac into which the seawater containing odorants is drawn into the olfactory chamber via an anterior inlet when fish opens its jaws and subsequently, the incurrent olfactory water leaves the olfactory chamber via the posterior outlet when the fish closes its mouth. The same ventilation system is observed in skipjack (GOODING, 1964), bigeye tuna and striped marlin (MANA, 2000), indicating that even at lower cruising speed the olfactory organ is continuously sampling olfactory water in the fast-swimming pelagic teleosts. At higher swimming speed it is most likely that water current through olfactory chamber is produced by the forward motion of the fish. Further almost all pelagic species studied so far tend to possess a round rosette or similar form with numerous lamellae radiating around a short midline raphe (GOODING, 1964; IWAI and NAKAMURA, 1964; YAMAMOTO and UEDA, 1979; MANA *et al.*, 1998; MANA, 2000) and the most rostral part of the rosette reach the anterior opening. These intrinsic features of olfactory organs display not only an ideal arrangement for fish that inhabit vast deserts of open oceans but also a central design in pelagic forms. Another common feature in pelagic fish insofar, is the distribution pattern of the sensory epithelium on lamellar surface—sensory epithelium is found intermingled as islets or patches within the nonsensory epithelium which may be regarded as a manifestation in fast-swimming pelagic species. Other types of lamellar topography in YAMAMOTO and UEDA's (1979) classification would not be an ideal form for fast-swimming fish especially the scombroids (Families Scombridae, Istiophoridae and Xiphiidae) because the olfactory seawater entering the anterior inlet is of high pressure and any shearing force acting upon the delicate olfactory mucosa is perhaps reduced by the unique structural pattern of the sensory

epithelium on the lamellar surface to ensure strictly laminar flow 0.2<Re<2.0 (ATEMA, 1988) over and in between the olfactory lamellae; a smooth water flow over the olfactory mucosa is prerequisite for the olfactory system to detect and encode biologically relevant cues while avoiding adaptation effect on receptors (HARA, and LAW, 1972). The nonsensory epithelium is comprised mainly of epidermal cells which form a characteristic microridge of finger-print like pattern and these cells are thought to play a role in supporting tissues exposed to abrasive forces (UEHARA *et al*., 1991). Adaptational forces to pelagic way of life have rendered the kinocilia of nonsensory ciliated cells redundant in pelagic forms. This cell has a motility function (SLEIGH, 1989) and it is thought to draw in water into the olfactory cavity and propel olfactory water/mucus over the lamellar surface in fish that possess nonsensory ciliated cells in high density such as red sea bream (MANA and KAWAMURA, 2002). In planktonic life-form of pelagic fish larvae, kinocilia might be present to aid in larval olfaction and these kinocilia are becoming redundant as the fish grows. This could explain the rarity or absence of these cells in all pelagic fish studied so far.

In fish two olfactory receptor neurons are commonly present—cORN and mORN. Both of these receptor neurons are found in opah and dolphin. However, cORN is more dominant in both species (Fig. 6). Similarly, cORN is also dominant in bigeye tuna and striped marlin (MANA, 2000) and in other scombroid fish—yellowfin tuna and albacore tuna (*Thunnus alalunga*) (MANA, unpublished). The reason as to why one receptor neuron type is dominant over another is poorly understood in pelagic fish olfaction. However since most scombroids and other pelagic species are highly migratory, abundant cORN might be related to migratory behavior in fish, as postulated by PYATKINA (1976). In non pelagic forms, recent biomolecular studies in goldfish have revealed that mORN express amino acids receptors (CAO *et al*., 1998; SPECA *et al*., 1999). This evidence perhaps strengthened THOMMESEN's (1982) suggestion in salmonids that, cORN is more specific to bile salts while amino acids are detected by mORN. On the contrary, ZIELINSKI and HARA (1988) showed that only cORN was present in developing rainbow trout and responded to amino acid stimulation. Further, the sea lamprey (*Petromyzon marinus*) possesses only cORN and it's amino acid receptors are not only restricted to arginine (LI and SORENSEN, 1992) but the fish can also detect bile acids at a threshold concentration of 10^{-13} M (LI and SORENSEN, 1993). In light of the current evidences on olfactory receptors specificity in fish it can be said that the responsiveness of olfactory receptor neurons is species-specific. Although little is known about the olfactory sensitivity in many pelagic fish, yellowfin tuna can detect free amino acids at threshold 1×10^{-11} M (ATEMA *et al*., 1980). This threshold concentration is similar to free amino acids present in open seas (GARRASI *et al*., 1979). Although the population of olfactory receptor neurons in the opah (~3.0 million) and dolphin fish (~7.7 million) are much lesser than the adult red sea bream (~13.3 million) (MANA, 2001), it would be grossly unrealistic to make any kind of comparison between a macrosomatic fish and the pelagic fish at this stage. Moreover, THOMMESEN (1983) found that adult salmonids (arctic char *Salmo alpinus*) possess less density of receptor neurons (24 000/mm^2) and a fewer number (~12) of olfactory lamellae which yielded 0.5–1.0 million receptor neurons per olfactory organ nonetheless, all current evidences point to olfaction as the major sensory

system that guides those long distance migratory salmonid fish to their natal waters to spawn. Thus, all we can say is the pelagic fish have evolved to inhabit the vast oceanic environment. Evolution of olfactory system in pelagic forms not only has overcome hydrodynamic constrains in time but this distance chemosensory system is perfectly designed and tuned to the survival and success of those organisms.

In conclusion, macromorphology and micromorphology of olfactory organs of the opah and dolphin fish were studied. The results indicated that both species have functional olfactory systems best evolved for pelagic way of life. Further studies on other pelagic species olfaction will make us understand the central design of the olfactory system and it's function in pelagic fish behavior.

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