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Studies on *Tursiops truncatus* and *Stenella coeruleoalba* Dolphin Species: from Retinal Cell Morphological Comparisons Towards its Surrounding environment.

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Abstract

In this current study, the retinal cell morphology of two dolphin species, *Tursiops truncatus* and *Stenella coeruleoalba* was compared, and supplemented with a miniature review of how it relates to surrounding environment. Retinal cell morphology involved sectioning and retino-separation of eyes, morphometric analysis of retinal cell layers and its corresponding neurons, followed by stratigraphy of both retina and area/density of ganglion neuron cell bodies. A qualification criteria was developed to describe both thickness and visibility. To relate with surrounding environment of studied species, we searched relevant synthesized literature combining such key words as 'dolphin', '*Tursiops truncatus*', '*Stenella coeruleoalba*', 'eye', 'vision', 'ecology' and 'environment'. Retinal cell morphology comparisons showed that the thickness of outer nuclear layer had upper (37.8 – 38.5 µm) whereas outer plexiform layer had lower (7.8 – 8.7 µm) range values, with some differences between individual retinal layers ($p<0.05$) but specific to some cases. Area of ganglion cell layer of multipolar neurons of retina of both studied species could surpass the 800 μm^2 mark, which suggests the presence of 'giant' size cell types. Plausibly, the retino-morphological comparisons of studied dolphin species depict the context of micro-view, and able to relate with a macro-view with respect to its surrounding environment.

Keywords: Dolphin species; Retina Layers; Ganglion neurons; Cell Stratigraphy

1.0 Introduction

Understanding the behavioral and orientation mechanisms of cetacean species seems to be an area of increasing research interest (Mass and Supin, 1997, 1999, 2007). In the past years researchers have improved information concerning morphological and optical adaptations of cetaceans increasing the knowledge about their visual system (Li *et al.* 1983; Mass and Supin, 1999). For example, cetacean eyes are well-known to appear hemispherical shape with its axial length smaller compared to its diameter, and appears equi-distant at head region (Mass and Supin, 2007). In particular, previous comparative studies have revealed some interesting features in the organization of dolphin retina (Mass and Supin, 2012). While investigating dolphin's retina, earlier researchers have largely focused on density of ganglion cells, particularly the vision and its associated features (Dral 1983; Mass and Supin, 1986). The ganglion cells of aquatic mammal retina, identified due to the presence of well-stained Nissl substance and clearly defined nucleolus (Stone, 1965; Hughes 1975; Provis, 1979; Mass *et al.,* 1986), have been observed through variables like density, distribution as well as size, which characterized the ganglion cell distribution or multipolar neurons (Bjerager *et al.,* 1983; Mass and Supin, 2007). The structural organization of ganglion cells of retina directly relate with visual mechanisms and resultant perception(s) (Mass and Supin, 1986). Moreover, it seems that among some cetacean species, the concentration of ganglion cells in retina might be highly present in some areas compared with some others (Mass and Supin, 2007). These developments are largely due to advances in microscopy that contributed in making available information about retina cell structure with respect to constituent layers, ganglion/bipolar cells and photoreceptors (Dral and Beumer, 1974; Waller, 1982; Li *et al.,* 1983) as well as retino-topography of some marine and river species such as bottlenose dolphin (Mass and Supin, 1992), Dall's porpoise (Murayama *et al.,* 1995), and Pacific white-sided dolphin (Murayama *et al.,* 1998), respectively.

The organization of visual system of cetaceans can vary based on their respective environment (Li *et al.* 1983; Murayama and Somiya, 1998; Mass and Supin, 1999). Moreover, morphological characteristics of the cetaceans retina are correlated with visual behaviors (Wursig et al., 1990; Murayama and Somiya, 1998). Visual system, in fact, plays a fundamental role during social and predator-prey interactions, foraging strategies, migration and orientation as well as in the well-developed visual communication (Herman, 1990; Mobley and Helweg, 1990; Wursig et al., 1990; Murayama and Somiya, 1998). In particular, a parameter generally used to estimate the ability of eye to localize object is the retinal resolving power (visual acuity) (Mobley and Helweg, 1990; Wursig et al., 1990; Murayama and Somiya, 1998). Visual acuity allows for a rapid detection of moving targets (Dawson *et al*., 1980) that together with acoustic system may permit to gather food from fishing gear (Murayama and Somiya, 1998). Although dolphins found in Mediterranean waters are believed to bring about depredation of fishing gears (Brotons *et al.,* 2008; Bearzi *et al.,* 2011), there is increasing need to preserve the cetacean (and related organisms) and its ecological/surrounding domain, which may well justify the rationale behind the (abovementioned) investigatory efforts into the retina structure and its function/role for vision.

Given that additional studies is needful/warranted to supplement existing information, the specific objective of current study was to investigate the retinal cell morphology comparisons of *Tursiops truncatus* and *Stenella coeruleoalba* dolphin species, supplemented with a miniature review about how it can relate to its surrounding environment.

2.0 Materials And Method

2.1 Overview of study

This study specifically sought to compare the retinal cell morphology between two dolphin species, namely *Tursiops truncatus* and *Stenella coeruleoalba*, supplemented with a miniature review conducted about how it can relate to its surrounding environment. The schematic outlay of the conducted experimental activity is shown in Figure 1. The specimens were collected from already dead samples during previous field expeditions. Hence no ethical approval was required to further examine the retina. In brief, the eye of both species, that constituted four specimens in total was fixed, processed and sectioned to obtain the retina, followed by staining procedures, and thereafter retino-sectional analyses. Microscopy allowed for the overall layer stratigraphy of constituent layers in terms of visibility and thickness. A qualification criterion was developed to further help to describe retinal layers by thickness and visibility (Table 1). Subsequently and based on body cells, the quantification of overall and individual thickness as well as area/density of ganglion neurons was also performed. All chemical employed in this study were of reagent grade.

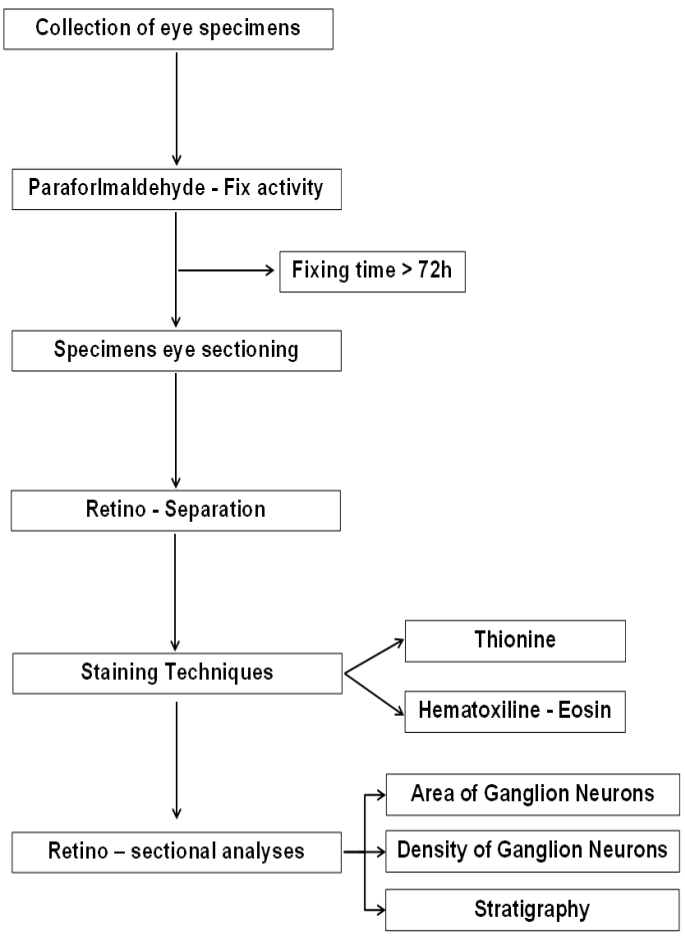


Figure 1. Schematic overview of the study design starting from the collection of eye specimens up to different retino-sectional analyses.

2.2 Specimen collection, sectioning and staining procedures

Complete eye samples were obtained from two dolphin species, *T. truncatus* (identified as specimens ID 55 and ID 107) and *S. coeruleoalba* (identified as specimens ID 170 and ID 218) courtesy of Tissue Bank for Marine Mammals Department/Unit of University of Padua, Italy. In particular, eye specimens were extracted from dead dolphins for not more than 12 h due to natural causes and without pathologies that may affect the eye structures. Further, eye specimens were first fixed in 4% buffered paraforlmaldehyde (pH 7.4) solution for not less than 72 h and then were processed by paraffin inclusion and sectioned horizontally consistent with the method described by Mass and Supin (1997). The rotary microtome (Microm HM 350, Microm International GmbH, Walldorf, Germany) was used to prepare the eye sagittal sections (5-10 µm thick). Subsequently, classical histological staining techniques of hematoxylin-eosin and thionine were used in order to identify the nucleus, ribosome and the Nissl substance as well as cytoplasm, respectively (Rosati *et al.,* 2006).

. In all situations, the sections were deparaffinized in xylene for time period(s) between 30 min and 1 h. Thereafter, the sections were made to pass through gradual hydration using descending series of alcohols (3 min in 100%, 2 min in 95%, 2 min in 90%, 2 min in 80%, 2 min in 70%, 2 min in 50%) followed by application of distilled water (~ 2 min). For the hematoxylin method, the inspected tissue was stained with hematoxylin for 20 min followed by differentiation in tap water (20 min), and thereafter by eosin for 30 sec thereafter washing using distilled water. Dehydration is next, subsequently followed by increasing series of alcohols (2 min in 50%, 2 min in 75%, 2 min in 80%, 2 min in 90%, 2 min in 95% and 2 min in 100%), and thereafter, cleared in xylene, then microscopy using Entellan (Merk, Darmstaldt, Germany). For the thionine method, the inspected tissue was stained with thionine (0.125% thionine) for 30 min, thereafter washed with distilled water and thereafter dehydration using ascending series of alcohols (50, 75, 80, 90, 95 and 100%). Tissues were kept briefly in xylene prior to microscopy using Entellan (Merk, Darmstaldt, Germany).

2.3 Microscopy observations

Per inspected specimen(s), the various sections have been microscopically observed. In particular, both microscopy observations of section already stained with either thionine or hematoxylin-eosin using Zeiss Axioplan instrument (Carl Zeiss, Oberkochen, Germany), were subsequently followed by morphometric analysis of layers and corresponding neurons using AxioVision Rel Software (Version 4.8.2). This software allowed for further determination of cell area (measurement of body cell area, unit in μm^2) and density (without unit) of ganglion neuron body cells as well as retinal stratigraphy (measurement of retinal length and layer thickness, unit in µm). Specifically, the number of neurons present per sagittal retinal section helped to define the multipolar neurons density. The images were digitally captured using DMC Polaroid (Polaroid Corporation, Cambridge, MA, USA) digital camera and its software (DMC2) and resultant images thereafter processed using Adobe Photoshop software (Adobe System 10, San Jose, CA, USA).

2.4 Data analysis

When required, the Minitab Express™ software (version 1.3.0 for Windows, Minitab Ltd., Coventry CV3 2TE, UK) was used to process the quantifiable data. One-way analysis of variance (ANOVA) by *post-hoc* was performed to determine the overall differences across retinal layer thicknesses of sectioned dolphin species specimens. In addition, T-test was performed to establish differences between retinal cell layer thickness per dolphin species specimens. For differences to be statistically significant, the level of probability must be $p<0.05$. Resultant data was expressed as mean value of different measurements ± standard deviation (SD).

2.5 Towards surrounding environment

To decipher how retina of the studied dolphin species can relate to its surrounding environment, a miniature/terse review was performed using key words 'Dolphin', '*Tursiops truncatus*', '*Stenella coeruleoalba*', 'eye', 'vision', 'ecology', 'environment'. Specifically, both 'eye' and 'vision' were alternated in the search process to identify with relevant information/literatures. Google scholar platform was chosen given its wider capture/coverage capacity to identify with scientific research materials/publications. A number of publications captured (initially) were based on the combination of above key words. Within these captured publications those deemed not relevant/sufficient to the context of objective of current study were then excluded. Authors applied their discretion to keep those publications deemed relevant based on the context of objective of current study. Thus, contextualization of relevant literature was performed specific to how retina of the studied dolphin species related to its surrounding environment.

3.0 Results

Histological preparation of the dolphin eye tissue microscopically revealed slightly rounded cornea, seemingly well-developed iris as well as spherical-like lens, which clearly resembled pictorial description, as has been reported by Mass and Supin (2007) (Figure 2).

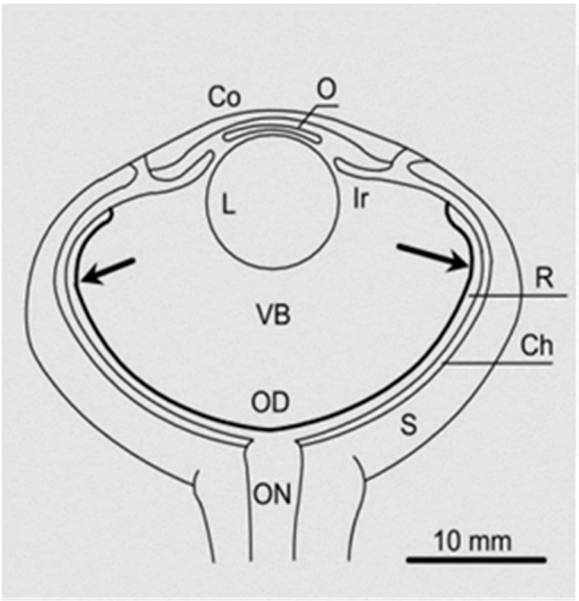


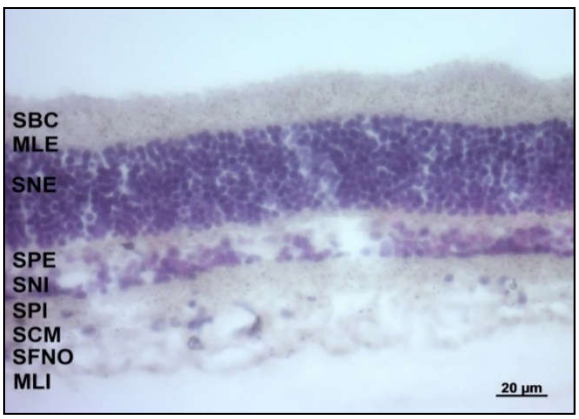
Figure 2. Horizontal section of eye of a typical dolphin species specimen indicating the different morphological aspects: Co, cornea; L, lens; Ir, iris; O, operculum; S, sclera; Ch, choroids; R, retina; ON, optic nerve; OD, optic disc; black arrows indicate the retina [Source: Mass & Supin (2007)]

Qualification criteria to further describe the retinal layer in terms of thickness ('very thick', 'thick' and 'not so thick') and visibility ('clearly visible', 'visible', 'not clearly visible' and 'imaginable') is showed in Table

1. We considered visual observation of retinal pigment epithelium, layer of rods and cones (SBC), external limiting membrane (MLE), outer nuclear layer (SNE), outer plexiform layer (SPE), inner nuclear layer (SNI), inner plexiform layer (SPI), ganglion cell layer (SCM), nerve fiber layer (SFNO), as well as inner limiting membrane (MLI), which is showed Figures 3 and 4 (All abbreviations in Italian as provided by microscopy facility).

Table 1. Qualification criterions to further describe the microscopically retinal layers in terms of thickness and visibility.

Layer type	Potential qualification	Potential ranking	Observable features/remarks
Retina layers	Thickness	Very thick	Densely/highly aggregated cells
		Thick	Less densely/highly aggregated cells
		Not so thick	Little-to-no cell aggregation
	Visibility	Clearly visible	Complete detectability of layer with border
		Visible	General detectability of layer
		Not clearly visible	Layer without border
		Imaginable	Layer seems impossible to clearly identify



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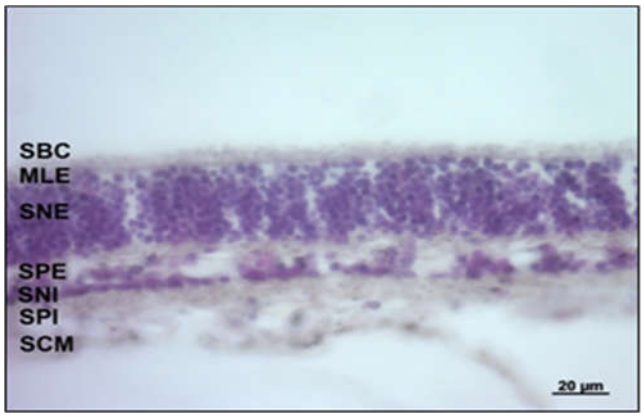

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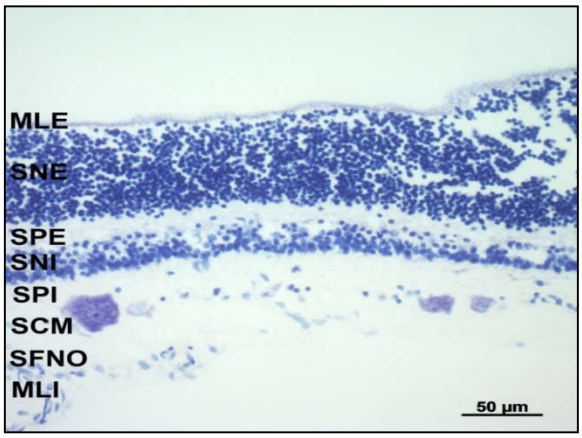

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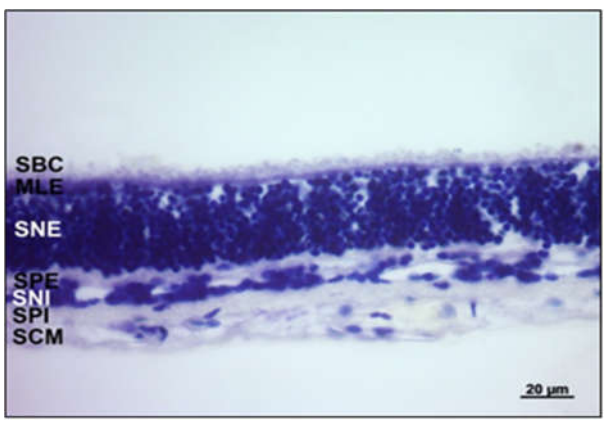


D

Figures 3 (A-D): Striped dolphin species retina specimen after staining by hematoxylin-eosin (Fig. 3 A and B) and thionine (Fig. 3 C and D). Abbreviations of retinal layers: SBC = layer of rods and cones; MLE = external limiting membrane; SNE = outer nuclear layer; SPE = outer plexiform layer; SNI = inner nuclear layer; SPI = inner plexiform layer; SCM = ganglion cell layer; SFNO = Nerve fiber layer and MLI = internal limiting membrane

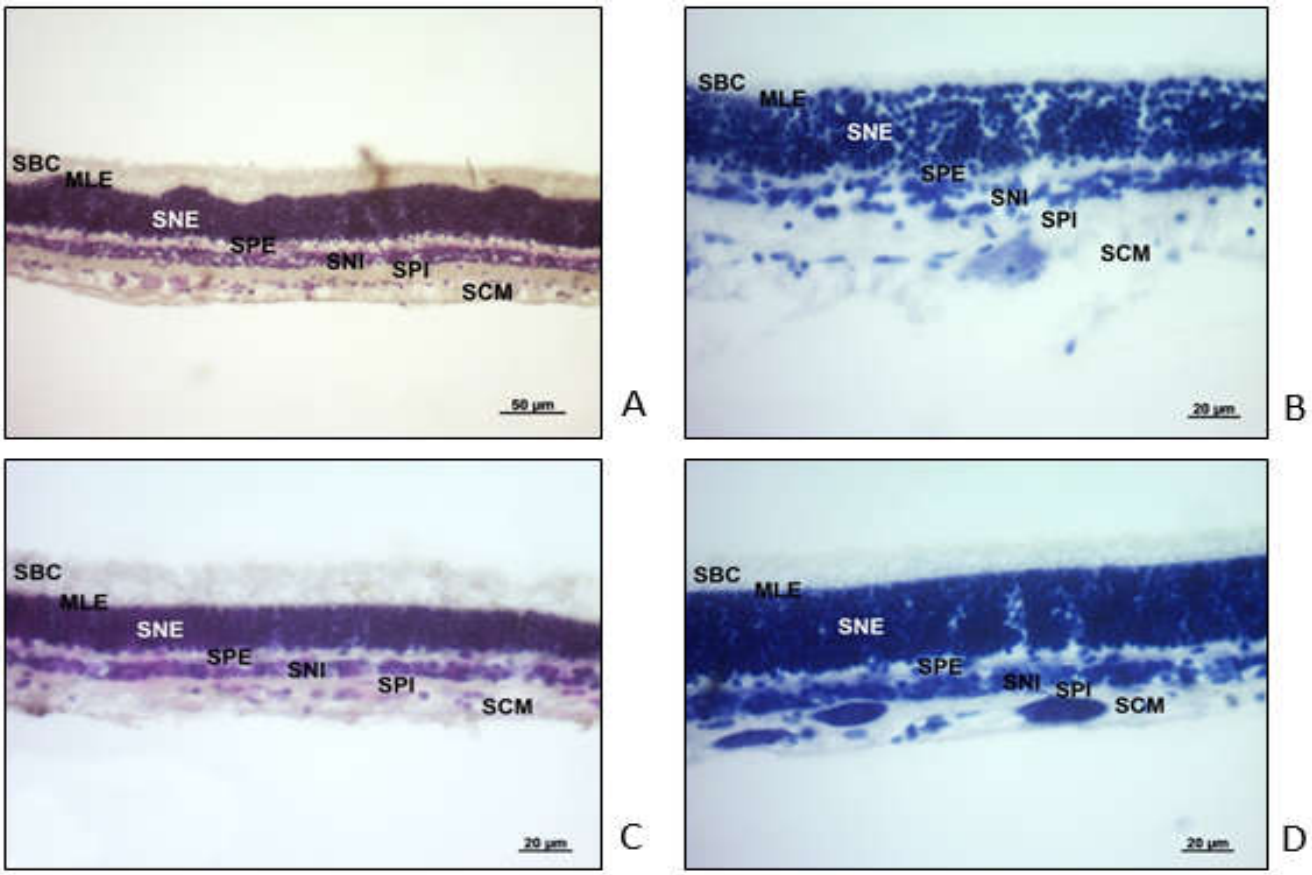


Figure 4 (A-D): Bottlenose dolphin species retina specimen after staining by hematoxylin-eosin (Fig. 4 A and C) and thionine (Fig. 4 B and D). Abbreviations of retinal layers: SBC = layer of rods and cones; MLE = external limiting membrane; SNE = outer nuclear layer; SPE = outer plexiform layer; SNI = inner nuclear layer; SPI = inner plexiform layer; and SCM = ganglion cell layer.

In terms of thickness, the 'SBC', 'SNE' and 'SCM' by hematoxylin–eosin and 'SNE' and 'SCM' by thionine staining respectively appeared 'very thick' in both dolphin species specimens. The 'SNI' and 'SPI' by hematoxylin–eosin and only 'SNI' by thionine staining appeared 'thick' for both species, except the 'SBC' specific to thionine staining for bottlenose species specimens, respectively. The 'SPE' by hematoxylin–eosin staining and thionine at both species respectively appeared 'not so thick' except the 'SBC' and 'MLE' specific to thionine staining for those of striped dolphin species. But other layers seemed 'imaginable' at both dolphin species by hematoxylin–eosin staining, to include retinal pigment epithelium, 'MLE', 'SFNO' and 'MLI' and by thionine staining to include retinal pigment epithelium, 'SPI', 'SFNO' and 'MLI' except the 'MLE' specific to bottlenose species specimens (Figure 3-4).

In terms of visibility, 'SBC', 'SNE' and 'SCM' by hematoxylin–eosin as well as 'SNE', 'SNI' and 'SCM' by thionine staining of both species respectively appeared 'clearly visible' and consistent, except the 'SNI' and 'SPI' specific to bottlenose dolphin species specimens. Further, 'SPE', 'SNI' and 'SPI' by hematoxylin–eosin staining appeared 'visible' for striped dolphin whereas only layer of rods and cones by thionine staining for bottlenose species specimens. However, by thionine staining the 'SBC', 'MLE' and 'SPE' appeared 'not clearly visible' for striped dolphin whereas by hematoxylin–eosin staining it was so only at 'SPE' for bottlenose dolphin species specimens. Other layers that would seemed 'imaginable' at both species by hematoxylin–eosin staining include 'SBC', 'MLE', 'SFNO' and 'MLI' and by thionine staining include retinal pigment epithelium, 'SPI', 'SFNO', 'MLI' except the 'MLE' specific to bottlenose species specimens (Figure 3-4).

Regardless of the staining method applied, the retinal layers after microscopy that appeared "very thick" up to "not so thick" such as 'SBC','SNE', 'SPE', 'SNI', 'SPI' and 'SCM' were further quantified by mean thickness. Table 2 shows the mean retinal layer thickness measurements with corresponding minimum and maximum ranges of the studied dolphin species specimens. Clearly, the thickness found peak ranges at 'SNE' (37.8 – 38.5 µm) and lower ranges at 'SPE' (7.8 – 8.7 µm). Comparing the two species specimens, the overall thickness of individual retinal layers across the two dolphin species specimens show statistical differences in some cases as indicated by ANOVA, which were as follows: 'SPE' (P=0.015, F-change =6.06, Rsq (adj)=4.29%), 'SPI' (P=0.04, F-change =4.13, Rsq (adj)=2.20%), and 'SCM' (P=0.0001, F-change =15.26, Rsq (adj)=9.24%), added with pooled SD that ranged between 1.943 and 9.746. Specifically, T-test confirmed statistical differences between the two dolphin species specimens at 'SPE', 'SPI' and 'SCM' ($p<0.05$). Overall, the mean thickness of retina of bottlenose dolphin was 101.23 µm ± 17.24 (maximum = 132.48 µm; minimum = 67.41 µm), whereas that of striped dolphin was 108.35 µm ± 18.29 (maximum = 166.06 µm; minimum = 72.84 µm).

Table 2: Retinal cell layer thickness measurements (mean, minimum, and maximum) of the studied (two) dolphin species specimens

Layers	Dolphin species specimens	Thickness (µm)		
		Mean± SD	Minimum	Maximum
Layer of rods and cones (SCB)	Bottlenose dolphin	16.93 ± 3.01[a]	11.06	22.37
	Striped dolphin	17.21 ± 3.46[a]	11.81	26.21
Outer nuclear layer (SNE)	Bottlenose dolphin	38.53 ± 7.09[a]	19.52	55.03
	Striped dolphin	37.82 ± 8.86[a]	10.38	58.89
Outer plexiform layer (SPE)	Bottlenose dolphin	8.74 ± 2.20[a]	4.58	17.63
	Striped dolphin	7.79 ± 1.79[b]	3.19	13.76
Inner nuclear layer (SNI)	Bottlenose dolphin	12.03 ± 2.84[a]	7.45	22.10
	Striped dolphin	12.98 ± 3.57[a]	6.46	20.43
Inner plexiform layer (SPI)	Bottlenose dolphin	12.85 ± 2.34[a]	6.81	16.18
	Striped dolphin	14.81 ± 5.82[b]	6.95	32.65
Ganglion cell layer (SCM)	Bottlenose dolphin	18.45 ± 4.57[a]	8.60	27.87
	Striped dolphin	25.62 ± 11.08[b]	11.20	59.45

Key: Different letters ([a,b]) between samples per layer indicate significant differences ($p<0.05$) of replicated measurements; SD = Standard deviation

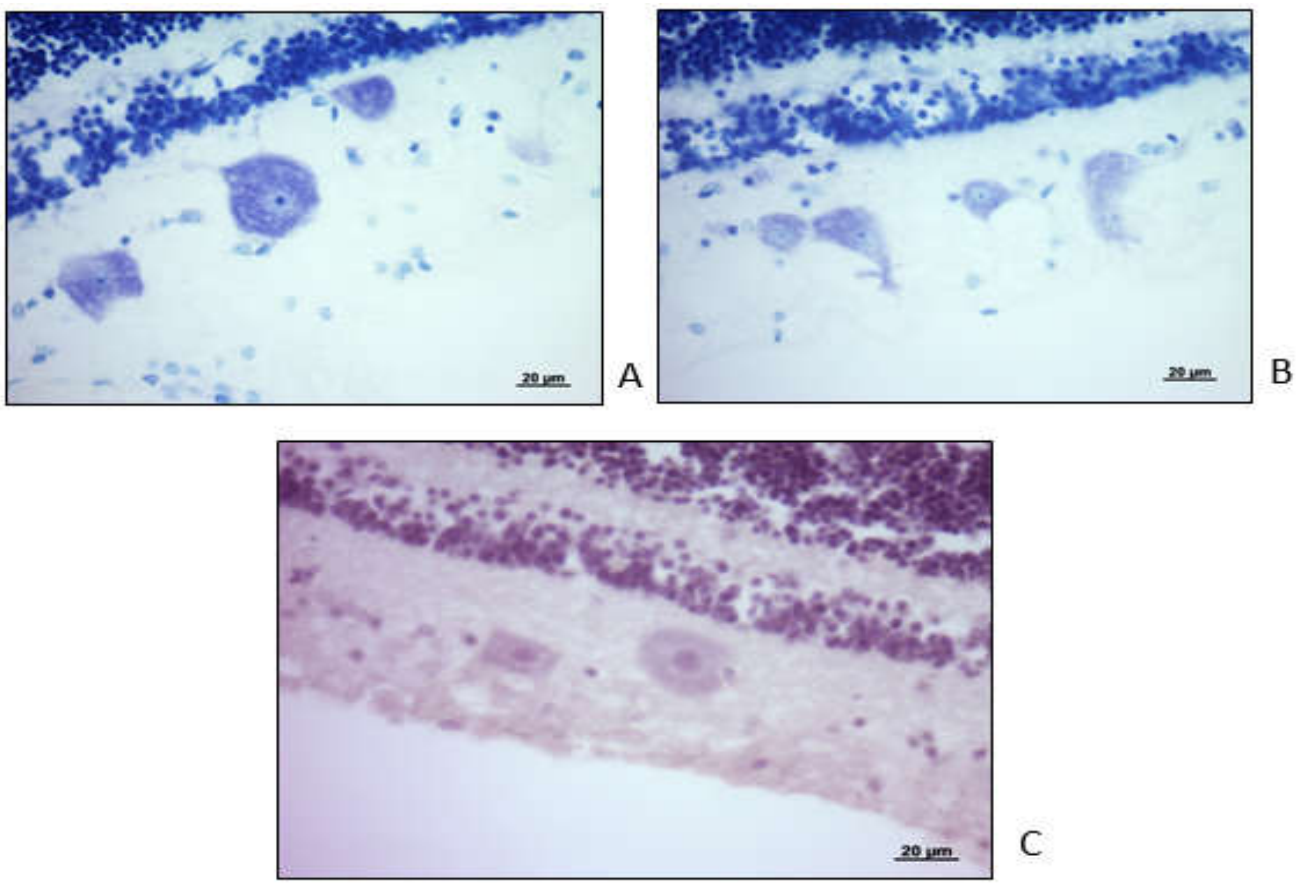


Figure 5 (A-C). Striped dolphin species retina ganglion cell layer (SCM) by staining methods. **Fig. 5A** shows the giant multipolar situated around the middle, and **Fig. 5B** shows the small or medium size neuron by thionine, whereas **Fig. 5C** shows the small up to medium size neurons after staining by hematoxylin – eosin.

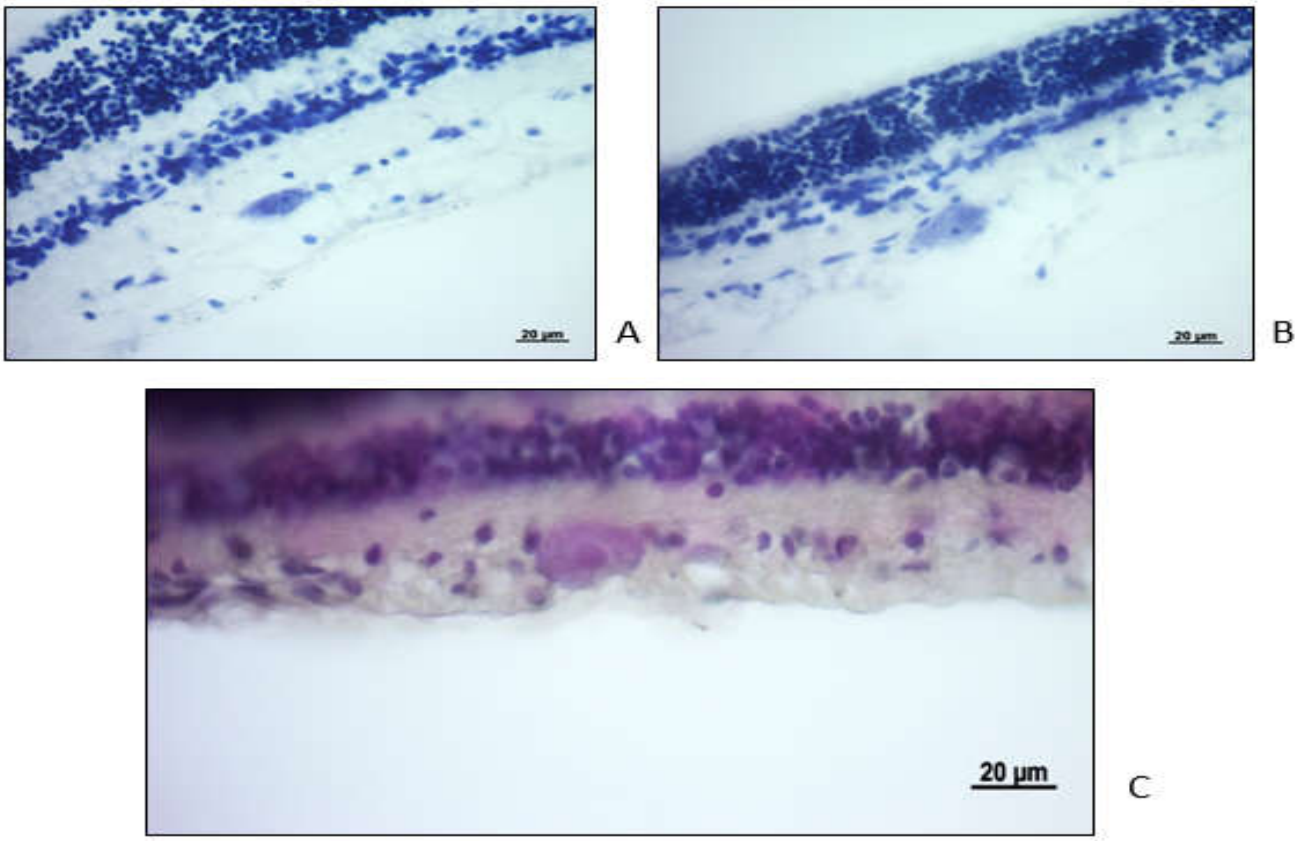
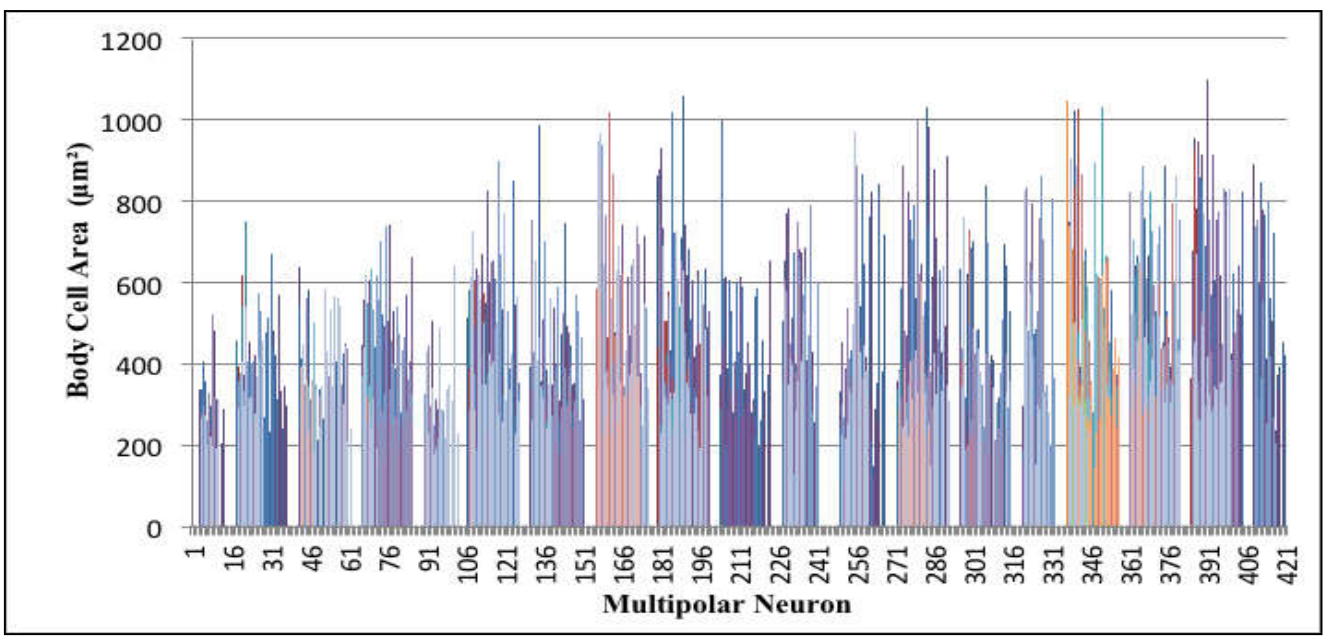


Figure 6 (A-C). Bottlenose dolphin species retina ganglion cell layer (SCM) by staining methods. **Fig. 6A** shows the small sized and **Fig. 6B** shows the large and medium sized multipolar neurons by thionine, whereas **Fig. 6C** shows the medium size neurons by hematoxylin – eosin.



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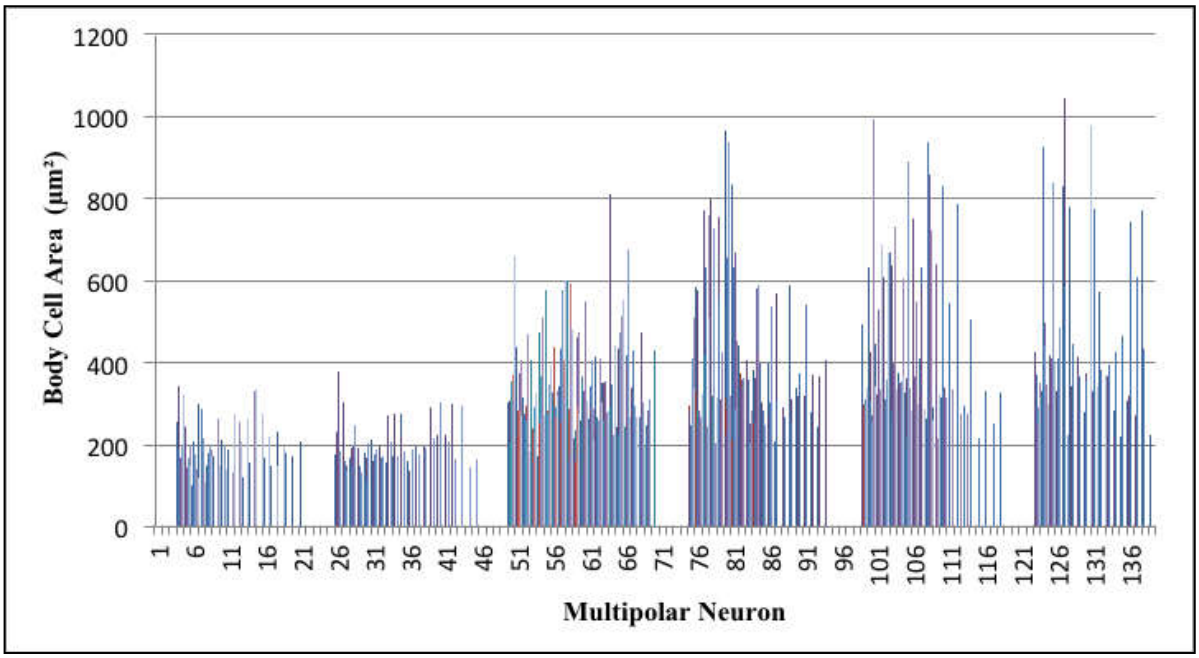

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Figure 7A-B. Size distribution of retinal multipolar neuron in striped (**Fig. 7A**) and bottlenose (**Fig. 7B**) obtained from different microscopic section measurements of dolphin retina species specimen

Figure 5 (A-C) shows the observed small/medium up to giant multipolar neurons in ganglion cell layer of retina of striped dolphin, whereas Figure 6 (A-C) shows the observed medium up to large multipolar neurons of bottlenose species specimens.

Figure 7 (A and B) shows the detected area of body cell (μm^2) ganglion neuron cell at striped and bottlenose dolphin species specimens, respectively. Body cell mean ± SD area had 330 μm^2 ± 165.32 (maximum = 1046.56 μm^2; minimum = 102.63 μm^2) and 390.66 μm^2 ± 167.95 (maximum = 1101.21 μm^2; minimum = 106.29 μm^2) for bottlenose dolphin and striped dolphin species, respectively. With the help of retina sagittal sections the number of multipolar neurons has also been quantified. The mean ± SD length of retina sagittal section had 3841.84 µm ± 561.89 and 6315.73 µm ± 875.62 whereas the mean number ± SD of multipolar neurons had 21.12 ± 8.96 and 32.25 ± 6.49 for bottlenose and striped dolphin species specimens, respectively.

To relate the retina of studied dolphin species with its surrounding environment, we found/selected up to 20 publications, which comprised of both analytical/experimental and review articles that captured topics ranging between behavior and ecology of related/studied cetacean species. Out of these, some had in one way or another reported about the visual capacity of either one or two of the currently studied dolphin species. Thus, these specific publications were succinctly synthesized, in the view to extract some associated/relevant information specifically within the context of how the retina of studied dolphin species related to its surrounding environment.

4.0 Discussion

Essentially, there is paucity of relevant literature(s) about comparisons of retina cell morphology and its corresponding ganglion neurons between such dolphin species as *T. truncatus* and *S. coeruleoalba*. The microscopic observation of sectioned eye presented typical features that appeared consistent with those descriptions at published relevant literature(s) (Refer to Figure 2) (Mass *et al.,* 1986; Mass and Supin, 1999, 2007). By retinal stratigraphy, the layers were arranged in the sequence of retinal pigment epithelium/ layer of rods and cones (SBC)/ external limiting membrane (MLE)/ outer nuclear layer (SNE)/ outer plexiform layer (SPE)/ inner nuclear layer (SNI)/ inner plexiform layer (SPI)/ ganglion cell layer (SCM)/ nerve fiber layer (SFNO)/ inner limiting membrane (MLI). The layer arrangement appeared consistent with reported retinal laminar structure of related/similar cetacean species such as common dolphin (*Delphinus delphis*) (Dral, 1983; Mass and Supin, 2007), minke whale (*Balaenoptera acutorostrata*) (Murayama *et al.,* 1992, 1995) and pilot whale (*Globicephala melaena*) (Peichl *et al.,* 2001).

To authors' best knowledge, there appears no relevant information that further qualifies the arrangement of retinal layers in eye of such cetaceans as dolphins (as well as other related aquatic mammals), specifically in terms of either cell thickness and or visibility. In the current study, via microscopic visualization, we attempt to compare the retinal layers in terms of cell thickness and visibility. This (retinal) cell qualification criteria showed that thickness could rank between 'very thick' – 'not so thick', whereas visibility could rank between 'clearly visible' – 'imaginable'. Whilst qualifying retinal cell layers by thickness and visibility criteria may likely be considered subjective because it is based on authors' visual observation of (microscopic) images, it should still be deemed relevant because it provides additional understanding about how consistent and related the retinal cell layers features/trends along 'thickness' and 'visibility'. Essentially, 'SNE' and 'SCM' layers showing 'very thick' and same time 'clearly visible' makes this descriptive ranking approach of retinal layers somewhat credible. Further, some generated/resultant data of thickness were equally quantified, which sought for differences in

thickness of retinal layer of the studied dolphin species, as showed in Table 2. A previous study has reported that thickness of cetacean retina could reach up to 425 µm (Mass and Supin, 2007). Besides, other researchers (Dral, 1983; Murayama *et al.,* 1992, 1995; Mass and Supin, 1997, 2007) had only provided some descriptions about the retinal laminar structures of eye of related/relevant aquatic mammal species, which were largely based on both composition and corresponding function(s).

The ganglion cell layer of retina of two dolphin species of this current study given by multiple neurons body cells ranged between small/medium and large/giant size. In addition, the body cell mean area ranged between 330 and 390 μm^2, and this result seemed consistent with cetacean species reported elsewhere (Mass and Supin, 2007). The estimated mean retinal sagittal section and number of multipolar neurons ranged between 3841 – 6315 µm and 21 – 32, respectively. In a number of cases, cetacean ganglion cells would feature as a single profile of large neurons (Bjerager *et al.,* 2003; Mass and Supin, 2007) of low-density yet with ample intercellular space (Mass and Supin, 2007).

Multipolar neuron cells of some aquatic mammal species could be termed 'great' if its diameter reached 30 µm and 'giant' if it surpassed 80 µm. An example of aquatic mammals with such 'giant' multipolar neuron cells is finnwal (*Balaenoptera physalus*) (Pilleri and Wandeler, 1964; Mass and Supin, 2007). Multipolar neurons can equally assume a polygonal shape (Bombardi *et al.,* 2013) somewhat resembling those shown in Figures 7 A-B. Previously, the body cell area of multipolar neurons of cetacean species has been reported to surpass the 800 μm^2 mark (Mass and Supin, 2007). Meanwhile, such cetacean species as baleen whales are believed to possess ganglion cell diameter of up to 160 µm (Mass and Supin, 2007), which suggests the 'diameter' concept an increasingly key/relevant concept in determining multipolar neuron size within ganglion cell layer(s) (Pilleri and Wandeler, 1964; Mass and Supin, 2007; Mass *et al.,* 2012). Notably, diameter values can mathematically relate with area measurements, which may well allow for effective comparisons. Specifically, these (abovementioned) diameter values of 30 and 80 µm would result in respective (estimated) area values of 700 and 5000 μm^2. If body cell area of multipolar neurons of our data surpassed the 800 μm^2 mark, it is therefore in this context that we would consider it to be of 'giant' type. Consistent with already published data (Mass and Supin, 2007), the body cell area of multipolar neurons of the currently studied dolphin retina specimens would surpass 800 μm^2.

Notwithstanding the relevant knowledge increasingly available about the characteristic features of cetacean eyes and among species (Bjerager *et al.,* 2003; Mass *et al.,* 2012), it is to find out how the eye/vision of dolphin species potentially relate to its surrounding (ecological) environment that further justifies this current study. Through terse contextualization of some relevant literatures (Shane, 1977; Würsig ans Würsig, 1979; Shane *et al.,* 1986; Würsig *et al.,* 1986; Kastelein *et al.,* 1990; Mobley and Helweg, 1990; Frantzis and Herzing, 2002; Constantine *et al.,* 2004; Bearzi *et al.,* 2005), how the retina of studied dolphin species can relate to its surrounding environment can be feasible to delineate. There appears some increase in evidence and knowledge about retinal morphology of cetaceans and its constituent/surrounding features (Dral, 1983; Li et al., 1983; Mass et al., 1986; Murayama and Somiya, 1998; Buono et al., 2012). In general, ganglion cells play an important role of junctions for the visual information before it leaves the retina (Waller, 1982). Usually confined to low cell density areas within the retina, giant ganglion cells, which support a large dendritic tree with (large) axon that form one fiber of optic nerve tract, facilitate rapid communication between the retina and brain (Kastelein *et al.,* 1990) and integrate the signals of photoreceptors over a larger area compared to smaller ganglion cells (Kastelein *et al.,* 1990; Mobley and Helweg, 1990). Moreover, the large axons that provide high-speed neural pathways for communication of motion cues in the peripheral field, allow for rapid detection of moving targets (Waller, 1982; Stone and Halasz, 1989; Mobley and Helweg, 1990; Mass and Supin, 2007). The importance of motion cues is accounted for by elliptical shape of delphinid eye, having short axis from pupil that provides the "barrel distortion effect", enhancing the movement detection capabilities (Mobley and Helveg, 1990; Mass and Supin, 2007). In particular, objects moving toward peripheral visual field of horizontally ellipsoid eye sweep across a broader area of retina, resulting in an optical enhancement of the size and speed of object's projection (Mobley and Helveg, 1990). These (retina) features suggest an enhanced motion detection system with an arguably improved detection of elusive prey, avoidance of predation, coordination of rapid social group movements and visual communication through coloration, body postures and facial signals (Mobley and Helveg, 1990; Wursing et al., 1990).

Certainly, the retina plays an important role in the dolphin's eye, providing it with robust capacity to effectively/efficiently function within its habitat and fully capture/view its surrounding environment. Most likely, the manner in which dolphin's retina is shaped may well account for the power of its vision. This can corroborate with why bottlenose dolphins farther from shore can significantly travel much longer distances well before turning compared to when they were seen within 0.5 km of shore (Würsig and Würsig, 1979). In this current study, the fact that the retino-features of studied cetacean species somewhat resemble, may possibly account for why there exists (around Eastern Mediterranean) mixed species' association between striped and short-beaked common dolphins (Frantzis and Herzing, 2002). Nonetheless, there still exist rare associations between common and bottlenose dolphins (Bearzi *et al.,* 2005). Reports about (permitted) dolphin-watching (tour) boats when they came by, whilst monitoring the bottlenose dolphin species, the latter became far much less at rest, which points to their visual capacity as robust in identifying with those specific boats (Costantine *et al.,* 2004). Meanwhile, from the macro-viewpoint, the powerful nature of dolphin's vision within its surrounding environment has been reported in some published works (Shane, 1977; Wursig *et al.,* 1986), for example, bottlenose dolphin, able to thrive well both day and night with very flexible behavioral movement. To relate both micro- and macro-viewpoints is therefore conceivable/feasible given that the retino-morphological analysis of studied dolphin can be associated with the power of dolphin's vision within its immediate environment/surroundings. Indeed, all the above-mentioned do point to the fact that, the physical specializations of eyes remain a very crucial aspect within the functionality of vision in cetacean life-time (Mobley and Helweg, 1990).

5.0 Conclusion

Together with how it can relate with surrounding environment, retinal cell morphology of dolphin species of *T. truncatus* and *S. coeruleoalba* has been compared. The qualification criterion of retinal layers comparisons has allowed for some ranking of its thickness and visibility with good clarity. Indeed, this current study does supplements existing literature concerning dolphin's retina cell layer(s), because it has provided some additional knowledhge and understanding about (both) consistent and related the (observed) thickness and visibility of area/density of ganglion neurons and its corresponding stratigraphy can be across two dolphin species. Through synthesis of relevant existent literature, the retino-morphological data/pictorial analysis of this current studied dolphin

species can plausibly serve as a useful micro-view, which considering that retina plays an important role in the eye, such that the latter's functionality within its habitat can therefore be translated into a macro-view, given its capacity to fully capture/view its surrounding environment. To wrap up, it would be worthwhile if future studies can test this proposed retinal layer qualification criteria, applying it to similar (microscopic) details of other cetacean and non-cetacean species, in the view to validate/verify its consistency. Importantly, such future studies would help to generate new/relevant data as well as literature that supplements existing information.

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Conflicts of Interest

Authors' declare there is no conflict of interest is associated with this study.

Authors Contribution

Conception: GS, CB and EF
Design: GS, CB
Laboratory execution: GS
Data interpretation: GS, CORO, SV, CB and EF
Writing the paper: GS, CORO, SV, CB and EF
Proofreading the paper: CB, CORO, EF

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