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A preliminary investigation into the morphology of oral papillae and denticles of blue sharks (*Prionace glauca*) with inferences about its functional significance across life stages

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Abstract

Sensory organs in elasmobranchs (sharks, skates, rays) detect and respond to a different set of biotic and/or abiotic stimuli, through sight, smell, taste, hearing, mechanoreception and electroreception. Although gustation is crucial for survival and essential for growth, mobility, and maintenance of neural activity and the proper functioning of the immune system, comparatively little is known about this sensory system in elasmobranchs. Here we present a preliminary investigation into the structural and dimensional characteristics of the oral papillae and denticles found in the oropharyngeal cavity of the blue shark (*Prionace glauca*) during embryonic development through adulthood. Samples were obtained from the dorsal and ventral surface of the oropharyngeal cavity collected from embryos at different development stages as well as from adults. Our results suggest that development of papillae occurs early in ontogeny, before the formation of the oral denticles. The diameter of oral papillae gradually increases during development, starting from 25 μm in stage I embryos, to 110 μm in stage IV embryos and 272–300 μm in adults. Embryos exhibit papillae at early developmental stages, suggesting that these structures may be important during early in life. The highest density of papillae was observed in the maxillary and mandibular valve regions, possibly related to the ability to identify, capture and process prey. The oral denticles were observed only in the final embryonic stage as well as in adults. Accordingly, we suggest that oral denticles likely aid in ram ventilation (through reducing the hydrodynamic drag), to protect papillae from injury during prey consumption and assist in the retention and consumption of prey (through adhesion), since these processes are only necessary after birth.

Key words: embryonic development; feeding behavior; gustation; oropharyngeal cavity; ram ventilation.

Introduction

Elasmobranchs (sharks, skates and rays) are well known for their impressive range of specialized sensory systems that aid in chemoreception, mechanoreception, electroreception and magnetoreception (Collin, 2012). The gustation capacity has been found in all classes of vertebrates (Northcutt, 2004; Atkinson & Collin, 2010). However, there have been few detailed studies on the gustatory system in elasmobranchs despite its documented importance for survival, growth, mobility, and maintenance of neural activity and immunity in numerous taxa (Whitear & Moate, 1994a,b; Hueter et al. 2004; Atkinson & Collin, 2010; Ferrando et al. 2012; Gardiner et al. 2012).

In vertebrates, the gustatory system consists of peripheral sense organs (oral papillae), located in the epithelium of specific body parts involved in food handling and intake. After direct contact, gustation, along with other sensory

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systems, leads to the decision to eat or reject prey. In elasmobranchs, the oral papillae are composed of taste buds, comprising multicellular peripheral chemoreceptors with axons at its base, as well as solitary chemoreceptor cells sparsely distributed in the oropharyngeal cavity and the external surface of the shark skin (Fahrenholz, 1915; Whitear & Moate, 1994a; Northcutt, 2004; Atkinson & Collin, 2010; Collin, 2012).

In addition to the papillae, oral denticles are also found in the oropharyngeal cavity of all sharks and guitarfishes studied so far (Nelson, 1970; Atkinson & Collin, 2012; Rangel et al. 2016). In some species, they fill the entire surface, protecting against abrasion during food consumption, increasing adhesion during prey intake and assisting in the reduction of hydrodynamic drag (Atkinson & Collin, 2010, 2012). Oral denticles, as well as dermal denticles, exhibit interspecific variation, ranging from simple to highly differentiated shapes (Imms, 1905; Fahrenholz, 1915; Daniel, 1928; Nelson, 1970; Atkinson & Collin, 2012).

The blue shark *Prionacea glauca* (Linnaeus, 1758) is an oceanic predator of global distribution, occurring in temperate and tropical waters, being considered the species with the widest distribution (Compagno, 1977; Nakano & Stevens, 2008; Ebert & Stehmann, 2013). It exhibits one of the highest fecundities among sharks, with an average of 30 embryos per breeding cycle. The gestation period lasts for 9–12 months and size at birth ranges from 30 to 35 cm (total length, TL; Pratt, 1979; Nakano & Stevens, 2008). Development and sexual maturation are relatively fast as compared with most sharks; male blue sharks reach sexual maturity at 4–6 years and females at 5–7 years (Nakano & Stevens, 2008). Blue sharks are the most heavily exploited shark species as both bycatch and target (Mejuto, 1985; Gallagher et al. 2014; Vandeperre et al. 2014; Queiroz et al. 2016). *Prionace glauca* is a pelagic species at all stages of life, with a full set of morphological adaptations to a lifestyle in the open ocean, including relatively long pectoral fins, a long dorsal lobe of caudal fin and a lower abundance of electrosensitive pores compared with other species of Carcharhiniformes (Kajiura et al. 2010), since pelagic species appear to rely more on smell and sight than other senses (Yopak et al. 2007; Lisney & Collin, 2008).

Despite the prevalence and biological/ecological importance of gustation in elasmobranchs, the gustatory system remains poorly understood, especially with regard to how the structure and function of oral papillae and denticles change throughout ontogeny. To address this knowledge gap, we conducted a preliminary investigation of structural and dimensional characteristics of the oral papillae and denticles found in the oropharyngeal cavity of blue sharks (*Prionace glauca*) during embryonic development through adulthood. This was accomplished through analysis of oral papillae and denticles via light microscopy and scanning electron microscopy.

Materials and methods

Sample collections

The specimens were obtained from a commercial fishing vessel (*Marbella I*, Kowalsky Ind. and Com. de Pescados Ltda company) in South and Southeastern Brazil. Animals from embryonic stages I–III were obtained in one location (32°46′S, 50°05′W), and individuals from embryonic stage IV as well as adults in another (19°34′S, 26°00′W – 23°28′S, 30°03′W). The animals were donated to the Surgery Department of *Faculdade de Medicina Veterinária da Universidade de São Paulo* (FMVZ-USP). Sample use was approved by the Brazilian Ministry of the Environment and IBAMA through SISBIO license number 48348-7 and Animal Ethics Committee (CEUA) no. 9623050214, from FMVZ-USP.

Samples were obtained from the ventral and dorsal surfaces of the oropharyngeal cavity of four adult blue sharks (2000 mm TL, $n = 2$; 2200 mm TL, $n = 1$; 2700 mm TL, $n = 1$) and 16 embryos divided into different groups based on body size, based on published age at total length (Caltabellotta, 2009): stage I – 100 mm TL ($n = 2$) and 120 mm TL ($n = 2$); stage II – 180 mm TL ($n = 2$) and 210 mm TL ($n = 2$); stage III – 220 mm TL ($n = 2$) and 250 mm TL ($n = 2$); stage IV – 460 mm TL ($n = 4$). For embryos, the entire ventral and dorsal region of the oropharyngeal cavity was analyzed, whereas for adults, only samples from the anterior region were analyzed (Fig. 1A,B). Individuals > 2000 mm TL were considered adults (Nakano & Stevens, 2008).

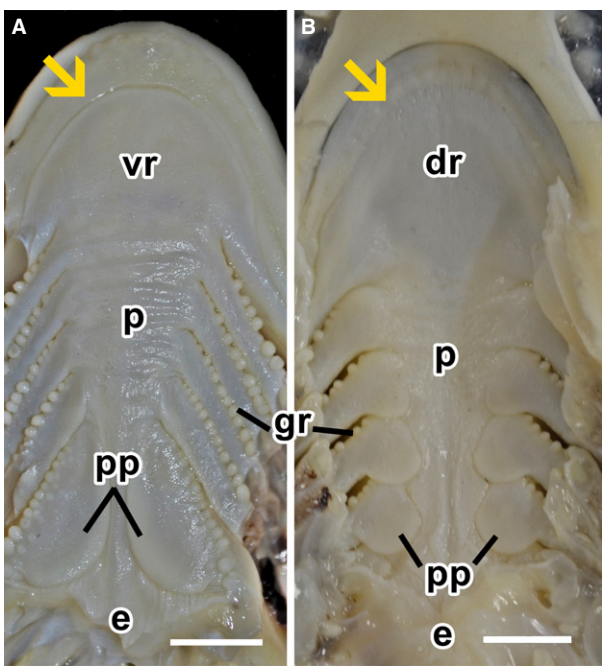

Fig. 1 Oropharyngeal cavity in *Prionace glauca* (model; embryo 460 mm). (A) Mandibular valve (yellow arrow); the ventral region (vr), the basihyal, later the pharynx (p) where the gills are located; presence of gill rakers (gr) and finally the pharyngeal pad (pp); esophagus (e). (B) Maxillary valve (yellow arrow); the dorsal region (dr); pharynx (p) and gill rakers (gr) of the second to the fifth pair of gills; esophagus (e). Scale bars: (A–B) 10 mm.

The terminology used for oropharyngeal cavity, oral papillae and denticles was based on Whitear & Moate (1994a,b), Atkinson & Collin (2012) and Thies & Leidner (2012).

Light microscopy (LM)

Light microscopy (Nikon Eclipse E-800) was used to examine the two-dimensional microscopic profile of the oral denticles and papillae through development. Samples were gathered from two adults and two embryos of each age group (I, II, III, IV) to facilitate the morphological description. Tissue from two individuals of each stage of the oropharyngeal cavity were fragmented and fixed in 10% formaldehyde solution. After 20 days, the samples were rinsed for 15 min and stored in 70% alcohol and then dehydrated in ascending ethanol series (from 70 to 100%), and thereafter cleared in xylene for subsequent embedding in paraplast. Paraplast blocks were sectioned (5 μm) using a microtome (Leica, German) and stained with hematoxylin and eosin (HE).

Scanning electron microscopy (SEM)

Scanning electron microscopy (LEO 435VP) was used to examine the three-dimensional microstructure and distribution of taste buds and oral denticles through development. For a better understanding of the morphological features, samples were gathered from two adults and two embryos of each age group (I, II, III, IV). The tissue from the oropharyngeal cavity was fragmented and fixed in 10% formaldehyde solution and then dehydrated in series of increasing ethanol density (70–100%). After dehydration, the samples were dried in a Balzers CPD 020 critical-point device mounted onto metal stubs with carbon adhesive and sputtered with gold in an Emitech K550 sputter apparatus.

Count of papillae and denticles

Given low sample sizes in this study, we were unable to perform statistical analyses on the data obtained. However, we provide qualitative and quantitative morphological measurements, including descriptive size and density for papillae and denticle. To estimate the density per cm^2 in the oral cavity of *P. glauca*, the photographs obtained by SEM were edited (contrast and sharpness) for analysis using the cell counter function from ImageJ software (version 1.48): 120 mm TL, $n = 1$; 210 mm TL, $n = 2$; 220 mm TL, $n = 1$; 250 mm TL, $n = 2$; 460 mm TL, $n = 2$; 2000 mm TL, $n = 1$; 2700 mm TL, $n = 1$). To establish a count parameter, random areas were selected and separated by mm^2. After determining the number of papillae in the areas standardized, an estimation in cm^2 was determined (x = number of papillae per $mm^2 \times 10$). All photographs were analyzed in triplicate, and the mean was used to estimate the density of papillae and denticles per cm^2, by selecting random areas (four per photography; Figs 1–4).

Results

Macroscopic aspects

The macroscopic aspects of the oropharyngeal cavity in *P. glauca* show the presence of both a maxillary valve and a mandibular valve. The basihyal revealed insertion in the

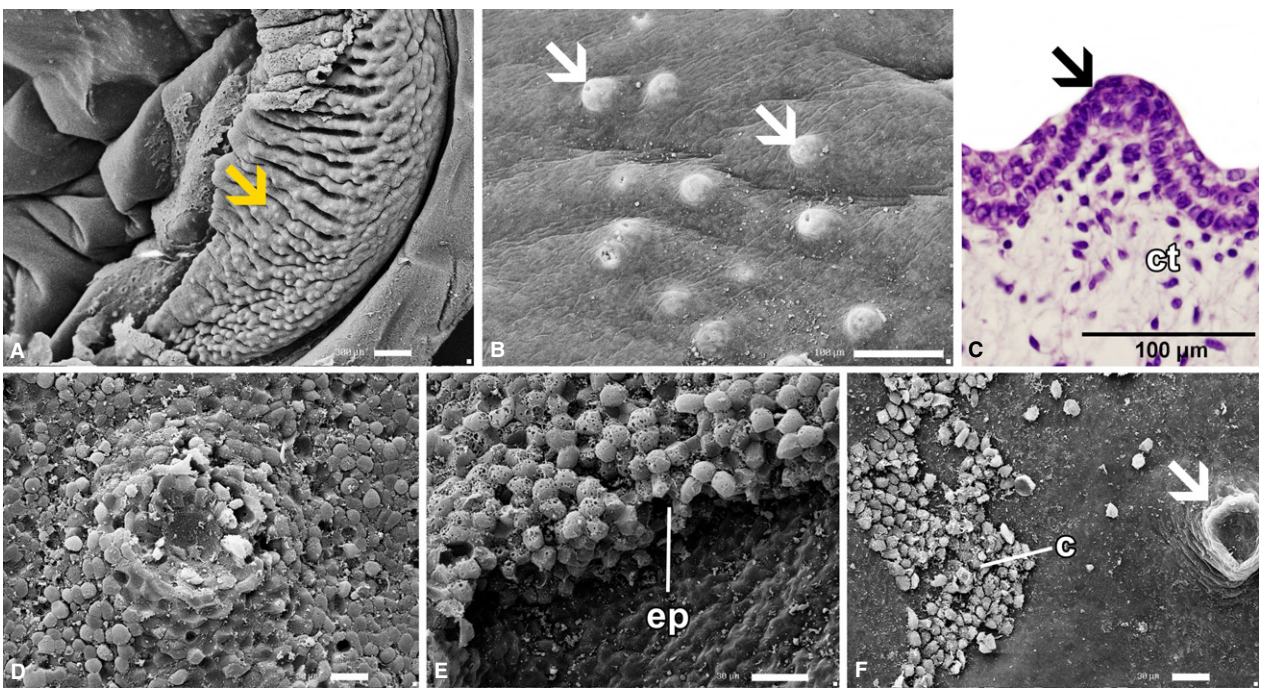

Fig. 2 Oropharyngeal cavity in *Prionace glauca* (stage I). (A,B) SEM of the dorsal oral region in the 120-mm embryo, with emphasis on the papillae (white arrows) revealing higher concentration in the maxillary valve surface (yellow arrow). (C) LM of the epithelial projection (arrow) below connective tissue (ct). Oropharyngeal cavity in *P. glauca* (stage II) (D–F) cell (c) layers detaching from the epithelium (ep); papillae projections (white arrow). Scale bars: (A) 300 μm; (B,C) 100 μm; (D–F) 30 μm.

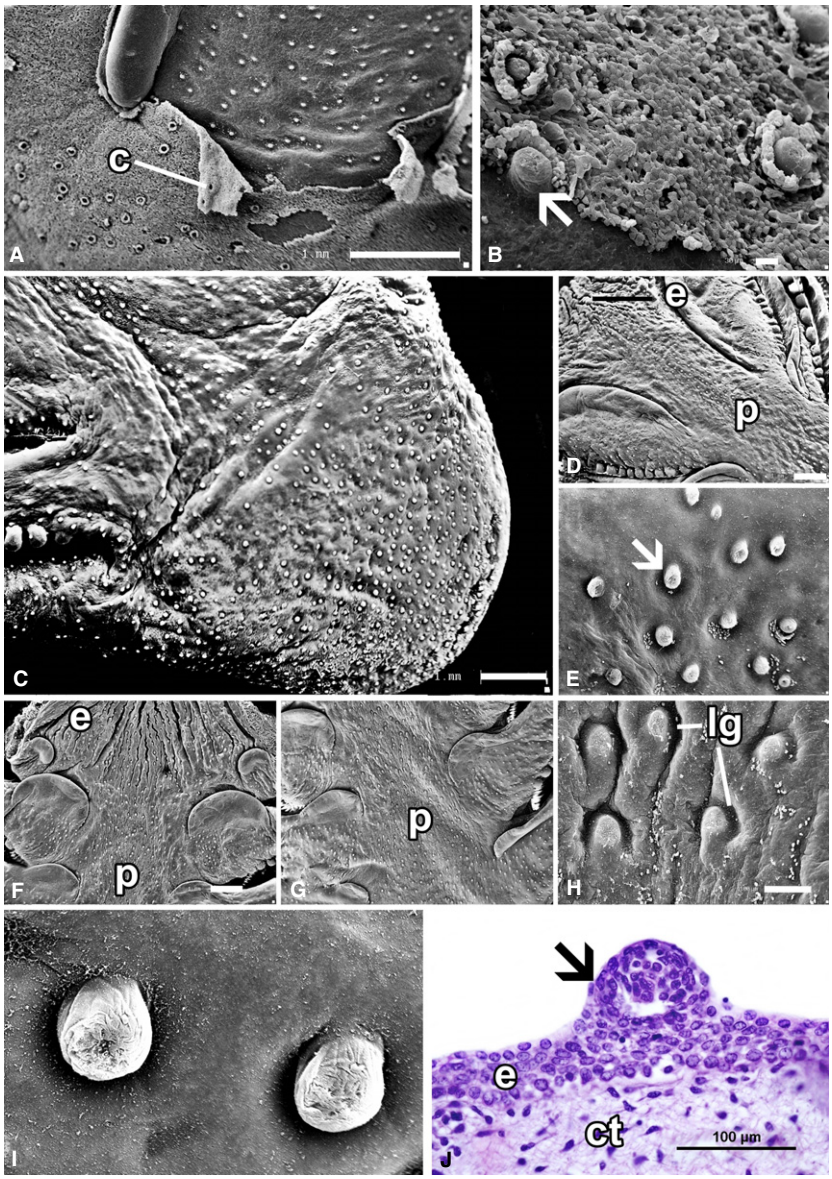

Fig. 3 Oropharyngeal cavity in *Prionace glauca* (stage III). (A,B) Cell (c) layer above the epithelium and papillae projections (arrow). (C) Anterior ventral region (basihyal) showing a high density of papillae. (D) Ventral region of the pharynx (p) and esophagus (e). (E) Projections of the oral papillae (arrow). (F,G) Dorsal region and pharynx. (H) Papillae projections in the region next to esophagus, lateral grooves (lg). (I) Cylindrical shape of oral papillae. (J) LM of the oral epithelium (e) with papillae projections (arrow) and connective tissue (ct). Scale bars: (A,C,D,F) 1 mm; (H,J) 100 µm; (B) 30 µm.

anterior region of the oropharyngeal cavity, attached to the mandible, as well as the pharyngeal, which was embedded in the last gill pair located in the ventral region (Fig. 1A). Rakers were observed within the gills.

In the dorsal region, it was possible to observe the pharyngeal from the second and fifth gill pairs, characterized as rigid structures, composed of cartilage and present in the base of each gill (Fig. 1B). They were distinguished by a differentiation in size, the one exhibited by the second gill being smaller than the one exhibited by the fifth gill. The increase in size was gradual from the anterior to the posterior region (pharynx to esophagus).

Embryos

The general morphological aspect from the oropharynx was relatively consistent across the embryonic development stages; however the papillae and oral denticles exhibited more significant changes, as described below. The measured density and diameter of the papillae are provided in Table 1.

Oral papillae

SEM of the oropharyngeal cavity dorsal region in the 120 mm embryo (n = 1; stage I) revealed a sparse arrangement of papillae that were projected relatively (Fig. 2A,B). Light microscopy of the oropharyngeal cavity ventral region in the other stage I embryo (n = 1; 100 mm) revealed epithelial projections in development (Fig. 2C). In the stage II embryos, layers of detached cells were observed across the epithelium (Fig. 2D–F). The morphology of the oral papillae is shown in Fig. 2F (cylindrical projection with concave opening in the central region). In stage III embryos, layers of detached cells were observed, forming aggregations around the oral papillae (Fig. 3A,B). The ventral

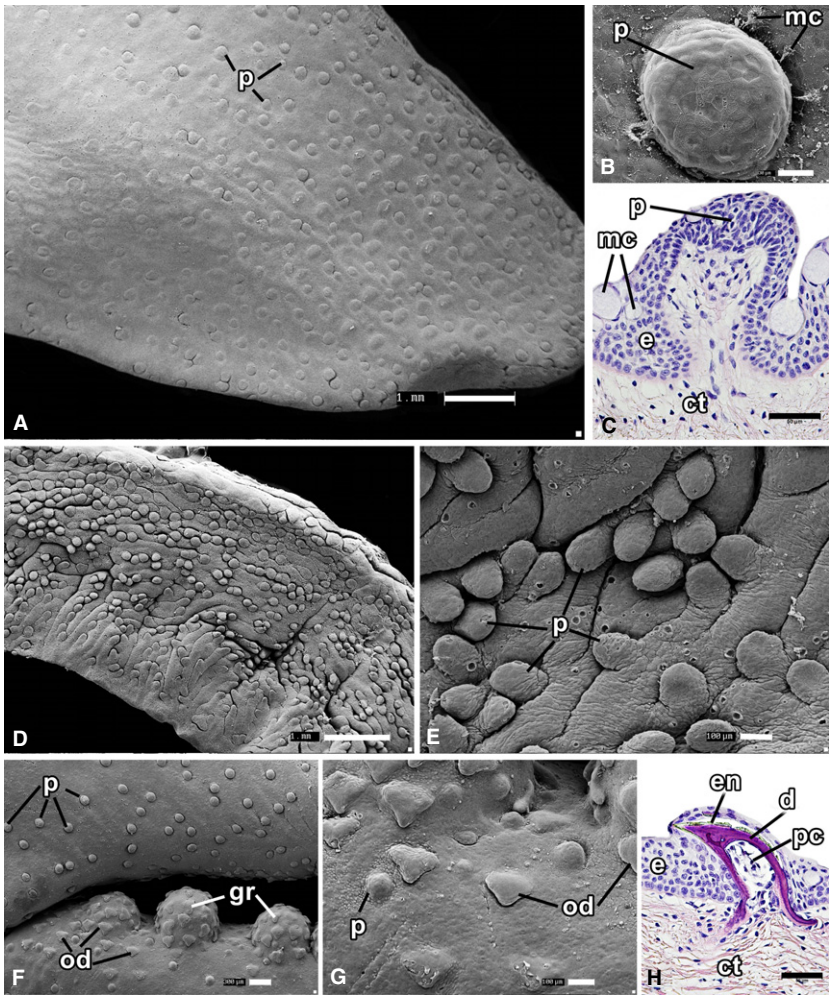

Fig. 4 Oropharyngeal cavity in *Prionace glauca* embryos (46 cm). (A) SEM of the ventral region, arrows (black and white) identify the oral papillae and denticles (d). (B) Papillae rounded by glandular mucous cells (C) ML of the connective tissue (ct) and projection (p) of papillae in the epithelium (e), with the presence of glandular mucous cells (mc). (D,E) SEM of the mandibular valve showing a high concentration of papillae (arrows). (F,G) Gill arch region showing denticles and papillae in gill rakers (gr). (H) LM of connective tissue (ct) and the oral denticle composed of enamel (en), dentin (d) and pulp cavity (pc) covered by one layer of epithelial tissue. Scale bars: (A,D) 1 mm; (F) 300 μm; (E,G) 100 μm; (C,H) 50 μm; (B) 30 μm.

Table 1 Diameter and density of papillae in the oropharyngeal cavity measures of blue sharks, separated by total length and stage.

TL	Stage	Papillae diameter	Papillae density
120 mm	I	25 ± 3.9 μm	280/cm^2
180 mm	II	38 ± 4.7 μm	–
210 mm		42 ± 4.3 μm	40/cm^2
220 mm	III	43 ± 11.7 μm	322/cm^2 (b) 150/cm^2 (p)
250 mm		77 ± 6.4 μm	420/cm^2 (b) 200/cm^2 (p)
460 mm	IV	110 ± 13.89 μm (b) 174 μm (mv)	90/cm^2 (b) 180/cm^2 (mv)
2000–2700 mm	Adult	295 ± 81.2 μm	10/cm^2

Some separated by region of the basihyal (b), palate (p), and mandibular valve (mv).

region of the oropharyngeal cavity (basihyal) exhibited a round conformity (Fig. 3C). The papillae were found throughout the epithelium (Fig. 3E), except in the posterior region of the fifth gill, where the ventral pharyngeal pad is located (Fig. 3D).

The dorsal region of the oropharyngeal cavity revealed epithelial projections on the surface, distributed widely (from anterior to the beginning of the esophagus) and evenly, except on the surface of the pharyngeal pads, where the papillae were absent (Fig. 3F,G). The pharyngeal pads exhibited different sizes: those observed in the first gill pair (Fig. 3G) were relatively smaller than the ones observed in the last gill pair, with a gradual increase in size anterior to posterior (Fig. 3F). At this stage (III), the papillae were rounded in shape and more elongated than those observed in other embryonic developmental stages (Fig. 3I,J). The papillae observed nearest to the esophagus exhibited lateral grooves (Fig. 3H).

In stage IV (460 mm TL), SEM of the ventral region revealed a sparce distribution of the denticles (Fig. 4A), composed of round papillae (slightly lifted; Fig. 4B,C). In some regions of the epithelium, secretory pores were observed (Fig. 4B,C). In LM, epithelial projections of oral papillae were observed as well as adjacent regions of the epithelium with the presence of mucus cells (Fig. 4C). The papillae density in the maxillary and mandibular valve was twice as high as that measured in the oropharyngeal cavity

(Fig. 4D,E). In the gills and gill rakers, papillae and partially exposed denticles were identified (apex facing the inside of oropharyngeal cavity; Fig. 4F).

Oral denticles

In samples from stages I–III, individuals did not exhibit oral denticles. However, oral denticles were observed at stage IV (Fig. 4G), but still above the epithelium (Fig. 4H), with a width of 174.74 ± 18.42 μm.

Adults

Oral papillae

Papillae were present in adults, sparsely distributed between the denticles (Fig. 5A–D). The papillae were round, with a large amount of adjacent glandular cells, some identified as pores open to the epithelial surface (Fig. 5B). The approximate diameter of the papillae measured ranged from 272 to 350 μm (Fig. 5A,B; Table 1).

Oral denticles

In adults (2000–2700 mm TL) oral denticles were distributed evenly within the epithelium, overlapping each other, with the apex of all denticles always pointing in a posterior direction – similar to that observed in dermal denticles. The denticles were mostly tricuspid (Fig. 5A), with the central cuspid being more pronounced, pointed and triangular in shape. The density was 190/cm^2 and width 337.15 ± 38.74 μm (Fig. 5A–C). Monocuspid denticles were also observed in the oral cavity (Fig. 5B). Three to five folds were identified in the crown of denticles, with hexagonal trimmings at the base of the central region (Fig. 5B,C,E).

In the mucosal epithelium, we observed a relatively large number of glandular cells and oral denticles anchored in the epithelium, with the presence of two rods on the base (Fig. 5F). Rods in the anterior region, the base of the denticles, appeared relatively more elongated than rods observed in the caudal region. In the central area of the denticles, we identified the pulp region (Fig. 5F).

Discussion

Here we provide a preliminary investigation into the morphology of the oral papillae and denticles found within the oropharyngeal cavity of embryos and adults of blue sharks followed by ecological and behavioral inferences about its

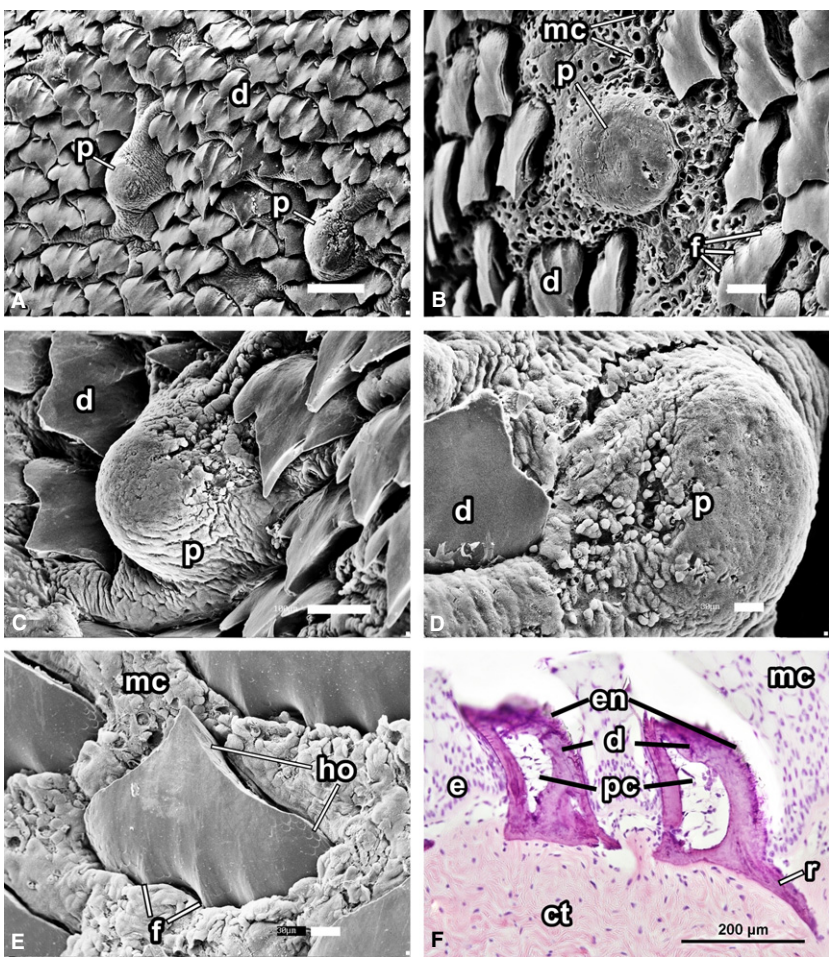

Fig. 5 Oropharyngeal cavity in *Prionace glauca* embryos (Adults). (A–D) SEM of oral papillae (p), which are projected sparsely distributed in the epithelium between the denticles (d) and mucous cells (mc). (E) Hexagonal ornamentations (ho), mucosal epithelium (mc), and folds (f). (F) LM of oral denticles (d), rods (r) and the epithelium showing a high amount of mucous cells (mc),. Scale bars: (A) 300 μm; (F) 200 μm; (B, C) 100 μm; (D–E) 30 μm.

functional importance among life stages. Although form and function of sensory structures in elasmobranchs have been well studied, there are few investigations of the morphology of the oral papillae and denticles within the oropharyngeal cavity of elasmobranchs. These structures, however, may play important role in gustation (Collin, 2012), phylogenetic differentiation (Mello et al. 2011), ventilation and prey grasp (Atkinson & Collin, 2012). In this study, we examined structural and dimensional characteristics of the oral papillae and denticles found in the oropharyngeal cavity of blue sharks from embryonic development to adulthood.

Oral papillae and implications for gustation

We found that development of papillae occurred prior to oral denticles, first appearing in the earliest embryonic stage (stage I). The papillae of *P. glauca* are round, distributed along the ventral and dorsal epithelium of the oropharyngeal cavity, gill arches, gill rakers as well as maxillary and mandibular valve, similar to the description of Cook & Neal (1921) for *Squalus acanthias*, a small deep-sea shark, suggesting that the regions of the oropharyngeal cavity that exhibit papillae, may represent a pattern among elasmobranchs, differing only in shape, size, and density.

We found that papillae diameter increased with ontogenic development (from Ø 29 μm to Ø 272–350 μm); however, overall papillae density in the oral cavity showed the opposite pattern (from 280/cm^2 in stage I embryo to 10/cm^2 in the adult). This suggests a possible trade-off in importance of papillae size and density in the ontogeny of blue sharks. Accordingly, we propose that during early ontogeny, as compared with late stages, gustation may play a significant role in learning and distinguishing prey preferences (facilitated via higher papillae density) by sharks (Kasumyan & Døving, 2003). Moreover, the earlier development of oral papillae in embryos may be indicative of an ability to taste in the uterus, since nutritive substances are secreted by the uterus during embryonic development in placental sharks (Hamlett et al. 2005).

Blue sharks are a dietary generalist, consuming a wide variety of prey (Vaske Junior et al. 2009), coupled with euthophagous habits (Henderson et al. 2001; Campana et al. 2011). The species appears to hunt in deeper water in during daylight hours and in shallower surface waters during nighttime (Tricas, 1979; Henderson et al. 2001; Campana et al. 2011; Doyle et al. 2015). Under these low-light feeding conditions, the electrosensory system and olfaction are likely important for prey detection (Kajiura et al. 2010). However, these sensory modalities may not provide sufficient information in the decision to swallow the prey, which may be more related to gustation and other tactile information during prey capture.

Distribution of the papillae varied by area of the oral cavity, with higher density around the maxillary and mandibular valves (oral valves), which are among the first areas of tactical contact with items taken in to the oral cavity. In benthic species, the oral valves are located in the anterior region of the mouth, assisting in water flow for respiratory purposes (Motta & Wilga, 1995; Whitear & Moate, 1998b; Motta et al. 2002; Tomita et al. 2012). Specifically, this structure facilitates the direction of water flow, preventing its escape by the oral cavity during increases in pressure within the mouth (Gudger, 1946). In Carcharhinid sharks (e.g. *Negaprion brevirostris*) the valves are reduced, composed of extensions of the connective tissue (Motta & Wilga, 1995), where the regulation of water flow it is not needed, leading us to believe that the structure it is part of the gustatory system. This is consistent with the need for sharks to taste and select potential prey at the time of capture (Atkinson & Collin, 2010), permitting selection and subsequent consumption of preferred prey and avoiding the ingestion of harmful and/or nutritionally poor items.

The epithelium attached to maxilla and mandible possesses ridges of different depths adjacent to the papillae. The large number of ridges and papillae may be related to the act of hunting (mandibular protrusion), and also increases the surface area of contact with the prey, consequently exposing a greater number of sensory structures, increasing efficiency during predation. In adults, the papillae of the maxillary and mandibular valve region are more projected than the papillae found in the oropharyngeal cavity. The same has been observed in *Orectolobus maculatus*, *Orectolobus ornatus*, *Chiloscyllium punctatum*, *Hemiscyllium ocellatum*, *Carcharhinus melanopterus* and *Negaprion acutidens* (Carla Atkinson, personal communication). Accordingly, the oral valves may have two main functions in sharks: ventilation and gustation. However, a more detailed investigation is needed to determine whether the increased surface area of the oral valves also leads to a larger number of papillae, which would suggest a gustatory role of the structure in the group.

Oral denticles and use in ventilation and feeding

As with dermal denticles, oral denticles display intra- and inter-specific variation (Atkinson & Collin, 2012). Many species of shark exhibit denticles throughout the oropharyngeal cavity as a primitive condition in the group (Nelson, 1970). In the blue shark, denticles are located adjacent to each other, influencing the distribution of oral papillae and mucosal cells. The amount of keels and cusps of denticles varied among individuals, with the presence of mono- and tricuspid denticles in the same area. According to Mello et al. (2011), the number of keels is homoplastic, with different selective pressures triggering the emergence or re-emergence of such traits in organisms that do not share a recent common ancestor. So, it is possible that two types of denticles in the same individual could be advantageous for several reasons. First, much like dermal denticles, which

help reduce hydrodynamic drag for efficient locomotion (Reif, 1978; Oeffner & Lauder, 2012), oral denticles may decrease drag of water flow through the oral cavity and over the gills, thus improving ram ventilation. Secondly, the oral denticles may also help protect the oral papillae against injury from ingested items during feeding. Finally, oral denticles may facilitate prey retention due to abrasion from the cusps, thus improving feeding efficiency especially for small planktonic prey items that are consumed via ram feeding and collected via gill rakers in blue sharks.

In terms of design, hexagonal ornaments restricted to the ventral and dorsal anterior portion of the crown (simpler) were observed in the oral denticles. The disposition of these structures in the crown of denticles varies between species and may have different patterns (Ciena et al. 2015; Rangel et al. 2016). The absence or reduction of denticle ornaments is considered a plesiomorphic feature for the group (Mello et al. 2011), being absent in basal species such as *Rhinobatos horkelii* (Rhinobatiformes) and *Zapteryx brevirostris* (Rangel et al. 2016). The microstructures positioned in the anteroposterior direction (cusps) probably evolved to cover the entire dorsal surface of the scale, covering hydrodynamic gaps in mouth and thus increasing efficiency in ram ventilation, especially in coastal species (Mello et al. 2011). Such organization (complex in order to reduce hydrodynamic drag) is important in species that live nearer the coast and typically swim relatively more slowly (due to space limitation and presence of obstacles), since reduction in swim speed can reduce ram ventilation efficiency. On the other hand, oceanic species tend to have ornamentations that do not cover the entire surface in the crown of denticles (Mello et al. 2011), which are simpler as a consequence of the higher swim speed observed in open water that, as result, improves ram ventilation efficiency.

Oral denticles were also observed only in the last stage of development (CT = 46 cm) and in adulthood (absent from early embryos), likely because this species does not need to ram ventilate or feed until birth. During embryonic development, *P. glauca* perform placental viviparity (Calzoni, 1935), characterized by the use of a yolk reserve, which later is modified into a placenta, allowing direct connection to the female and maternal–fetal exchanges related to nutrition and ventilation (Otake & Mizue, 1986). Right after birth, pups start to forage and ram ventilate. Manta rays (*Manta alfredi*) also seem to change ventilation strategy during development, with buccal-pumping during embryonic stages that changes to ram ventilation after birth (Tomita et al. 2012).

Additional studies of this kind across species, life stages and gender, coupled with immunohistochemical analysis, are needed to better understand the functional, behavioral, ecological and evolutionary significance of morphological structures within the oropharyngeal cavity of elasmobranchs. Such an understanding may have implications for the vulnerability of elasmobranchs to fisheries capture, ingestion of harmful foreign objects, and even negative interactions with people.

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Authors' contributions

B.R. was responsible for the examination of the samples, analysis and manuscript writing. N.W. and N.H. contributed the design and development of this study as well as manuscript writing and final edits. A.C. assisted with data analysis and manuscript editing. R.E.G.S. was responsible for examining scanning microscopy equipment and final manuscript edits, and J.R.F.J. contributed to final manuscript edits.

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