3-D Aspects of the dentition in rays of genus: *Atlantoraja, Rhinobatos and Zapteryx* from Southeastern and South of Brazil


1 FMZV-USP, School of Veterinary Medicine and Animal Science, Surgery Department, University of Sao Paulo, São Paulo, Brazil.
2 UNINOVE, University Nove de Julho, São Paulo, Brazil.
3 APTA Center of Marine Fish, Institute of fishing, APTA, SAA, SP, Santos, Brazil.

The development of the dentition in elasmobranchs is driven by selective pressures to maximize food and reproductive efficiency. Were described and compared to the structures of the dentition in male and female of three species of rays in the family Rajidae and three species of the family Rhinobatidae employing scanning electron microscopy techniques. The samples were fixed in buffered 10% formal solution, processed and documented photo. In the rays of the genus *Atlantoraja* and *Zapteryx* heterodontia occurs between males and females. In the teeth of the males grabber type, and females teeth crusher type. In the rays of the genus *Rhinobatos* all teeth of the jaws are similar, not showing heterodontia between males and females. The variation in dental morphology observed in these rays shows great importance for future comparative studies and adaptive mechanisms used in feeding and reproduction.

**Keywords:** guitarfish; reproduction; sexual dimorphism; feeding; scanning electron microscopy

1. Introduction

The suspension-feeding mechanisms and suction are the most common in modern aquatic organisms [1]. The teeth contribute to such systems, having important implications for the analysis of evolutionary patterns of these mechanisms [2].

Teeth in elasmobranchs (sharks and rays) are of type polyphyodont, organized on the square palate and Meckel's cartilage, which develop in lines and are replaced at regular intervals throughout their lives, as in most elasmobranchs, or in the form of large paved plates, found in the Myliobatidae family rays [3-6].

There are six main classifications of dentitions of chondrichthyans [7.8], based on form and function when capturing prey or rendering: These dentitions include: (1) clutching, (2) tearing and (3) cutting types (present in species that feed primarily on soft-bodied prey such as fishes, mammals and soft-bodied invertebrates) and (4) crushing, (5) grinding and (6) clutching-grinding types typically present in durophagous (hard prey feeding) species that consume crustaceans and hard shelled mollusks [4,7].

The development of the dentition in elasmobranchs is driven by selective pressures to maximize food and reproductive efficiency [9-12]. The teeth in the most of the rays has the presence of a ventral sucker mouthparts, which combined with crusher jaws strengthened and a more robust musculature produces crushing, which is the typical power supply mechanism in many rays, where the majority has the feeding habit benthic [4.13]. Despite the adaptive functions molariform teeth in rays for power, this form is ineffective to hold the pectoral fins of females by males during mating [12].

The stingrays of the genus *Atlantoraja*, family Arhynchobatidae, consist of three species are endemic to the Western Atlantic Ocean coast of South America [14]. The spotback skate *Atlantoraja castelnau* (Ribeiro, 1907), is found in the state of Rio de Janeiro in Brazil to Argentina [15], having a distribution in greater depth in the South of Brazil, where the species occurs in most of the continental shelf from 20 to 220 m [16]. Is a species subject to fishing pressure due to its large size and have high commercial value. As a result, their biomass decreased by 75% between 1994 and 1999, being classified by the International Union for Conservation of Nature (IUCN) as "endangered" [16]. The La Plata skate *A. platana* (Günther, 1880) varies from coast of the state of São Paulo, in Brazil, Argentina, and can be found between 40-100 m depth [15], which is listed on the IUCN as vulnerable [17]. The eyespot skate *A. cyclophora* (Regan, 1903) occurs from Cabo Frio in Rio de Janeiro, Brazil, and is found from the coast to depths of 300 m [15] due to the exploration is also classified as vulnerable on the red list of the IUCN threatened species [18].

The rays the Rhinobatidae family, known popularly as guitarfish, in Brazil are known two genus, *Rhinobatos* and *Zapteryx* comprising four species of small to medium-sized (between 60 cm and 1.5 m long), inhabitants of the continental shelf, about sandy and mud funds [3, 15,19]. The species to be studied, *Rhinobatos horkelii* (Müller and Henle, 1841) is endemic to Southwest Atlantic, recorded from Brazil to Argentina [15.20]. Due to large declines documented due to fishing pressure, the species is evaluated as "critically endangered" by the IUCN, and may become extinct in about 10 years if urgent measures are not taken conservation [20]. The *Rhinobatos percellens* (Walbaum, 1792) has a wide distribution in the Western Atlantic, the Caribbean to Brazil, currently classified as "near threatened" by the IUCN [15.21]. *Zapteryx brevirostris* (Müller and Henle, 1841), registered since the Espirito Santo, in Brazil,
Argentina [3,15], classified as “vulnerable” by the IUCN, although no commercial value, the species is in decline due to fishing pressure and low fecundity [22].

We analyzed the characteristics of dental arches by scanning electron microscopy, comparing the structures between species and with the feeding habits of each. The species chosen for this study are due to the large ecological concern and measures for each conversation, and the abundance of specimens captured incidentally by trawling in the Southeast and South of Brazil.

2. Material and Methods

Dental arches were obtained from males and females of rays of the families Arhynchobatidae: *Atlantoraja castelnaui* (n = 6), *A. platana* (n = 4) and *A. cyclophora* (n = 4); and Rhinobatidae: *Rhinobatos horkelii* (n = 4), *R. percellens* (n = 3) and *Zapteryx brevirostris* (n = 4). The specimens were captured incidentally by trawling for shrimp-pink in the southern and southeastern Brazil (23°-26 S and 42-47° W), with the permission of research paragraph 35614-3 (IBAMA – SISBIO). The copies were donated to the Institute of fishing, from Santos, São Paulo, identified and sampled. Later were transferred to the Department of Anatomy, Faculty of veterinary medicine and Zootechny of the University of São Paulo (FMVZ-USP). This study was approved by the Ethics Committee on the use of Animals (CEUA) nº 5785050214, FMVZ-USP.

For scanning electron microscopy samples of teeth were immersed in fixing solution of formaldehyde 10%. Then were washed in distilled water, subject to dehydration in growing series of alcohols and dried at room temperature. In the sequel the samples were positioned and glued with glue in carbon aluminum metal bases and submitted to metallic coating "spitting" with gold ions in the unit being analyzed EMITECH-K550 and photographed in scanning electron microscope LEO 435VP (FMVZ-USP).

The description of the teeth of the rays was based on nomenclature Moss [4] and Cappetta [7]. Sexual maturity in males was determined by the stiffness of the clasper and presence of spines in the fins [23].

3. Results

3.1 *Atlantoraja castelnaui*

In females of *A. castelnaui* (Fig. 1A), the teeth are monocuspidate type crusher (Fig. 1B, C and D). Present the occlusal and buccal flattened and smooth and a little pronounced cusp and rounded (Fig. 1D). Teeth with a cusp in the central region are larger than the teeth of distal region, where these are practically molariform (Fig. 1C). The upper jaw has fewer teeth than the upper jaw, where at the symphysis there is an elevation of three rows of teeth (Fig. 1C).

In mature males the teeth are of the type with a grabber cusp, pointed and well pronounced (Fig. 1E, F and G). As well as in females the crown face is smooth. The teeth of the central region of the upper and lower jaws are most prominent and pointed cusps, with apices facing lingual region (Fig. 1F), possessing two side protrusions to the central cusp, seen sideways in figure 1G. The cusps become gradually softer side teeth, until the distal region, where the teeth are molariform (fig. 1E).

3.2 *Atlantoraja cyclophora*

The dentition of *A. cyclophora* (Fig. 1I, J and K) is similar to that found in females of *A. castelnaui*, shredder and type monocuspidate, but instead of having the smooth crown, as noted on another streak, this has a few knobs (Fig. 1K).

In mature males the teeth are of type grabber (Fig. 1L, M and N), having three different settings when the form, size and position in the jaws. The teeth from the ranks of the central region feature a cusp pronounced and pointed at the tip (Fig. 1N), positioned vertically. The lateral teeth are also monocuspidate, but with inclination where the apices are geared towards the distal region (Fig. L). A third type is found in the distal region, bigger teeth with cusps and flat Crown less pronounced rounded (Fig. 1M).

3.3 *Atlantoraja platana*

In female (Fig. 1O) your teeth are of type crusher (Fig. 1P, Q and R). In the central region of the jaws teeth feature a flat with rounded Apex cusp and little pronounced (Fig. 1P and Q). The crown features a rectangular shape in the teeth of this region. The teeth of the distal region are molariform, oval-shaped Crown (Fig. 1R).

In mature males of *A. platana* streak is four different teeth configurations regarding the shape, size and position, gradually altering the symphysis distal region (Fig. 1S, T and U). In the central region of the jaws are present monocuspidate teeth, with long, sharp cusps in vertical position (Fig. 1S). In the lateral region are similar, but with the tip of the cusps wider and tilting toward the distal region (Fig. 1Q). Teeth present in the distal region possess the crown flattened, having the occlusal smooth face. Some closest to the teeth with a pointed cusp, have small rounded cusps.
towards the distal region/lingual (Fig. 1Q and U), which gradually change until teeth with rounded oval-shaped crown (Fig. 1U).

Fig. 1  Diagrams and photomicrographs of stingrays of the genus Atlantoraja - (A) dorsal view of A. castelnaui female. Dentition of the female of A. castelnaui: (B) the central region of the lower jaw and (C) above; (D) the central region in greater increase, monocuspidate teeth, with cusp flattened and rounded apex. The dentition of male A. castelnaui: (E) monocuspidate teeth and pointed lower jaw and (F) above; (G) central tooth side view. (H) dorsal view of A. cyclophora male. Dentition of the female of A. cyclophora: (I) teeth distal region monocuspidate and (J) the central region; (K) central teeth in greater increase. The dentition of male A. cyclophora: central (L) and side of the lower jaw and (M); (N) monocuspidate pointy teeth lower jaw plants in side view; (O) dorsal view of A. platana female. Dentition of the female of A. platana: (P-Q) central monocuspidate lower jaw teeth; (R) molariform teeth of the distal lower jaw. The dentition of male A. platana: (S) teeth sharp monocuspidate of the central region of the upper jaw; (T) lateral teeth of the upper jaw, teeth and left teeth with crown flattened; (U) distal region of the upper jaw, teeth monocuspidate little pronounced on the right and molariform left. Bar scale – (B, C, E, F, I, J, L, M, P, Q, R, S, T and U): 1 mm; (D and K): 300µm; (N): 100µm.
3.4 Rhinobatos horkelii e Rhinobatos percellens

Unlike the stingrays of the genus Atlantoraja, R. horkelii and R. percellens shows no sexual heterodonty, being the teeth of males and females are similar.

The teeth of R. horkelii female (Fig. 2B, C, D and G) as male (Fig. 2E and F) and R. percellens female (Fig. 2I, J and K) and male (Fig. 2 L, M and N) are monocuspidate to crown smooth with rectangular shape with rounded tips. The cusp is wide and rounded and the occlusal face is toward the lingual side. The teeth of the distal region are smaller than the other.

3.5 Zapteryx brevirostris

The ray Z. brevirostris (Fig. 2O) presents sexual heterodontia. In females (Fig. 2P and Q) teething is of type shredder. The teeth are molariform in the labial, and the lingual region teeth feature a small cusp rounds. The crown is smooth, molariform teeth possess the occlusal face oval shaped (Fig. 2Q).

In young males (Fig. 2R and S) dentition is similar to that observed in females, where the majority of teeth is molariform, but with the presence of teeth with a cusp little pronounced.

In mature males (Fig. 2T and U) are of type grabber. In the central region of the jaws teeth are monocuspidate, showing conical cusp, with the apex rounded, geared toward the lingual region. The teeth of the distal region are molariform, with the occlusal face oval-shaped crown, as well as in females (Fig. 2U).

4. Discussion

The morphological pattern of the dentition of the studied species corresponds to the general pattern found in Batoidea [3,4,13], where the ventral mouth position and individual teeth are in accordance with the benthic habit of the species. Sexual dimorphism is evident in dental four of the six species studied, with the females being relatively flat and sharp males. Dental morphology between species in the same family is similar in shape and layout.

The dentition between the rays of the genus Atlantoraja is similar, but in each species there are particularities. In the three rays of this genre to heterodonty course, where it is observed in males plus a variation as the morphology also on rotation of teeth. Even in homodonty condition, where all teeth share the same form, a single morphology can serve for various functions, facilitating the capture and handling, if the orientation angle in relation to the arrested or captured item is varied in different parts of the jaw [8,24].

In relation to the food item in these rays, each species shows a tendency to a preference for different prey. The streak A. castelnaui feeds mostly on benthic teleost fish, followed by cephalopods, elasmobranchs and decapods [25]. The feeding of A. cyclophora consists mainly on crabs, but also with great importance of Teleost [26]. The ray A. platana the main stomach contents found was decapods [27].

The rays the Rhinobatidae family noticed a great similarity in the two rays of the genus Rhinobatos, where present few differences among themselves and between male and female, but which differ from the observed dentition in the Z. brevirostris, with evident heterodonty. The anatomical structures of R. percellens (opening of relatively larger mouth and body characteristics) allows swimlanes violas crawl and swim with greater speed and to capture highly mobile organisms [28,29]. For this reason this species preys on fish, large prawns and crabs [28,30], including R. horkelii [19].

Z. brevirostris has a smaller mouth, allowing the consumption of small prey, such as small shrimps and Polychaeta [29].

Differences in diet are often correlated with morphological specializations in species [4]. As noted by BORNATOWSKI [29] the rays Z. brevirostris, R. percellens, Rhinoptera bonasus and Rioraja agassizi, rays of this study present a high degree of interspecific variation in tooth morphology, showing different performances in power and allowing a widely varied diet [30,31].

The functional significance of dentition in elasmobranchs differs from other fish because it is used not only for food, but also in breeding [10,11,12,32].

The elasmobranchs exhibit complex reproductive behaviors a serious involving the use of the mouth by males to grab the female in order to provide a proper alignment for insertion of the clasper (copulatory organ) [33]. During copulation the male will bite aggressively the fins and body of the female [10,33,34,35]. This behavior was suggested by Springer [36] to act as a mechanism that facilitates cooperation precopulatory release the female to mate.

Based on observations in other species of rays as Urolophus concentricus sample [32], Urolophus halleri [10] and Dasyatis Sabina [12,35], it is suggested that the rays of the genus Atlantoraja and Z. brevirostris sexual dimorphism is advantageous especially in reproductive behavior, where the male holds the female with his teeth for copulation.

In these rays there are secondary sexual characteristics in addition to sexual heterodony, as the presence of spines at the tips of the dorsal fins. As observed and suggested for teeth, appear in male in the juvenile phase for an adult, being used to hold the female during copulation [23,37,38]. The rays R. horkelii and R. percellens sexual dimorphism was not observed in mature males, dental and other secondary sexual characters such as the presence of thorns was not observed in specimens studied nor in the bibliographical revision of works with these species.
Fig. 1  Diagrams and micrographs of the rays in the Rhinobatidae family - (A) dorsal view of ray *R. horkelii*. (B, C, D and G) female and dentition (E-F) male *R. horkelii*. (H) dorsal view of ray *R. percellens*. (I, J and K) female and dentition (L, M and N) of the male of *R. percellens*. (O) dorsal view ray *Z. brevirostris*. (P-Q) female and molariform dentition (R-S) young male. (T) monocuspidate teeth of mature male of *Z. brevirostris*; (U) differentiation of central and lateral teeth (monocuspidate) and the distal region (molariform) in mature males. Scale bars – (B, C, E, J, L, M, P, R and T): 1 mm; (D, F, G, I, K, N, S and U): 300µm; (Q): 100µm.
5. Conclusion

Was made possible by the scanning electron microscopy analysis of morphological variation in the dentition in species of rays studied, mainly the difference between species in the same family and between males and females. With observation of sexual heterodony in species A. castelnaui, A. cyclophora, A. platana and Z. brevirostris suggests that this is advantageous primarily as reproductive function, whereas for power of those rays, blunt teeth and with a larger surface area is more suitable [39,12].

For rays R. horkelii e R. percellens, teething doesn't seem to collaborate for use in reproduction. Behavioural observations are essential to analysis of reproductive repertoires in these species, and the use of teething for mating.

Seasonal analysis of dentition in these species is required in order to verify that the dental sexual dimorphism is static or seasonal change occurs so as noted Kajiura and Tricas [12].

Only the anatomical study is not sufficient to determine functions of the dentition of the rays in the feeding and reproduction. But it is an important tool to assist future comparative studies, descriptive and taxonomic.

References


