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Methods of investigations of shark heterodonty and dental formulae's variability with the blue shark, *Prionace glauca* taken as an example.

Litvinov F.F. and V.V. Laptikhovsky

Abstract

The blue shark Prionace glauca is the most abundant and widespread elasmobranch and one of the most prominent large predatory nektonic species of the World ocean. This is one of the keystone elements of the oceanic ecosystem, influencing living resources in the open oceanic and neritic waters, populations of submarine mounts, etc. The same time its morphology and life story are poor investigated and understood. The present paper is aimed to quantitative assessment of heterodonty in the blue shark and its modifications through the life story. There were two methods applied, the area indices and linear and discriminant analysis, which allowed to clearly define the two shapes of teeth: the awl and the knife ones. There are demonstrated the allometrical changes of teeth through ontogenesis, beginning of sexual heterodonty in subadult sharks which result in use of the awl-shaped teeth by males in copulation. The sexual heterodonty emerged as adaptation for copulation in pelagial, leads to different food preferences and spatial segregation of sexes. The methods used allow tracing and quantitatively assessing the group and individual variability in the teeth shapes. These methods were successfully tested and may be recommended for zoological practice in analyses of variability of curvilinear projections: scales and bones, body patches etc. The dental formula is described in details indicating high variability of this parameter.

Keywords: blue shark, heterodonty, sexual dimorphism, allometric changes.

Contact author:

Feodor Litvinov: Atlantic Scientific Research Institute of Marine Fisheries and Oceanography (AtlantNIRO), 5, Dmitry Donskoy Str., Kaliningrad, 236000, Russia [tel: +7 0112 225887, fax: +7 0112 219997, e-mail: flit@atlant.baltnet.ru].

Introduction

Elasmobranch fishes are the important top predators in all marine ecosystems. Despite their biological and commercial importance, many aspects of their morphology and life histories are poorly understood and insufficiently investigated. Teeth shape is an important taxonomic feature in modern sharks, and nearly the only diagnostic feature in taxonomy of extinct elasmobranchs. However, intraspecific and ontogenetic changes in teeth morphology are poorly known. Even papers describing a comparative morphology of shark teeth (Herman et al., 1990; 1991) do not provide the whole picture of teeth modifications, the most common and abundant species included. Shark specialists usually do not consider the finer variations in tooth morphology to be important in shark taxonomy. A few studies revealed that that elasmobranch teeth shape is a possible subject of ontogenetic changes, inter-population variability and sexual dimorphism (Bass et al., 1973; Gruber, Compagno, 1981; Litvinov, 1982; 2003).

One of these rare examples is the blue shark *Prionace glauca.* In this species the two main types of teeth on the lower jaw revealed: awl-shaped toothed or knife-shaped toothed. They exist everywhere throughout the species range, and almost every specimen could be assigned either as one or another (Litvinov, 1982; 2004). In these teeth a single cusp may be more or less elongated, more or less widened, more or less serrated, and subjected to a cline variability making a classification sometimes quite subjective. Initial investigation of a small sample from Northeast Atlantic allowed supposing that the existence of two types is not related to sex and age (Litvinov, 1982).

The blue shark is the most abundant and widespread living elasmobranch with a circumglobal distribution between 55°N to 55°S (Compagno, 1984a).

Despite this species is known since Pliocene (Case, 1982), in sediments of every oceanic region its teeth were relatively common but still rare, whereas those of *Isurus spp.* strictly predominated even in Holocene (Belyaev and Glikman, 1970). It allows supposing that the blue shark became the most abundant oceanic elasmobranch very recently, probably in historical time expelling *Isurus spp* from its dominance in oceanic pelagia, being so an example of the actively evolving species (Litvinov, 1989).

The species range in *Prionace glauca* is complicated with a spatial segregation between sexes, adult males aggregating over submarine mounts, forming so-called “bachelor clubs”, and waiting subadult females for the first copulation (Litvinov, 2004**).** The species abundance in such aggregations is about tens times higher that in the adjacent open oceanic waters, providing an opportunity for decimation of stocks, which is very real, bearing in mind a great demand for shark fins on one hand, and location of such aggregations beyond EEZs on another hand.

This paper is based on numerous samples collected after the previous short communication was published in 1982, and is aimed to provide unambiguous quantitative features to confirm or to reject an existence of both teeth types. Another task is find out probably reasons of existence of such a dimorphism, its distribution between sexes and populations and its role in biology and population structure.

Materials and methods

To describe the teeth morphology we used 129 teeth rows, collected by the first author in Atlantic (127) and East Pacific (2) between 1978 and 1984 onboard Russian (AtlantNIRO) RVs exploring resources of tuna, billfish and sharks (Fig.1).

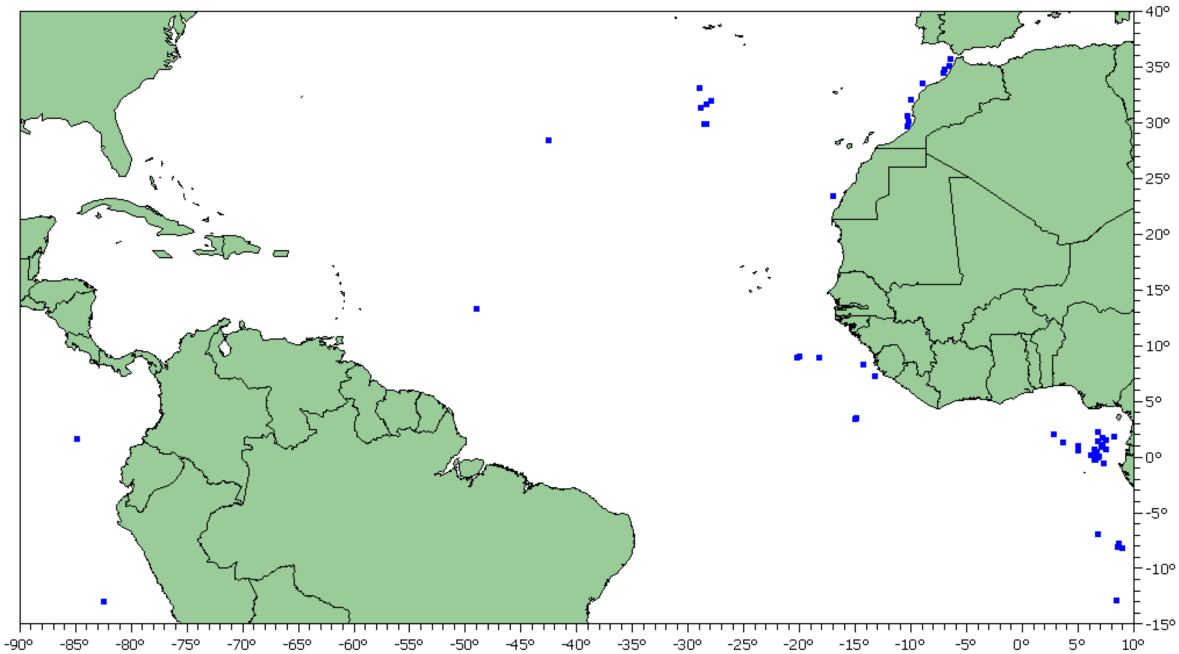

Fig. 1. Position of sampling sites.

In every specimen jaws were extracted, cleaned from soft tissues and dried. Then, upon returning on shore, jaws were put in the boiling water for a few minutes. After cartilage became soft, the teeth of the first, second and third rows were extracted, washed, dried and glued to the black matte paper. The teeth of further rows were insufficiently calcified and too soft for the further treatment. All further measurements were done using enlarged teeth images (perpendicular projections), which may be easily obtained by scanning. The teeth of the first (working) row were measured, with some teeth from the successive rows used to substitute broken and missing. First, all the teeth sets were visually assigned as either awl-shaped (AS) (Fig. 2) or knife-shaped (KS) (Fig. 3). Then a range of meristic features were measured in every tooth (Fig.4).

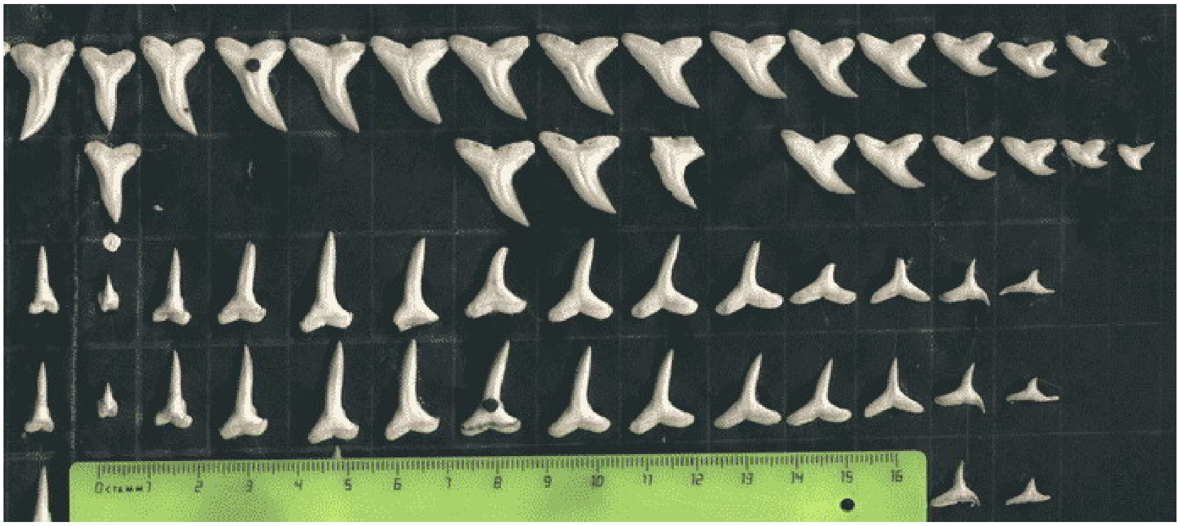

Fig. 2. Teeth preparation of the Blue shark, according to the natural position, face to observer, so the right side is from the left for observer. The functional teeth are in the inner position, the second and third rows are beyond them, in outside position. The awl-shaped teeth, an extreme case. The lower teeth are long, thin, not serrated and nearly round in the middle of cusp; cutting edge is pronounced in the apical part only. The upper teeth are elongated as well, the distal part is relatively narrow, serration is very fine and nearly absent from the apical point. Some teeth in the first row are absent due to substitution process or broken when capturing. Some teeth of the third row were not well calcified and hardened. The symphysis series is marked with white circle; teeth of the second series in upper row and fifth series of the lower raw are marked with black points. Male, TL=253 cm, 08°55.3N, 18°13.1W, 11.05.1982.

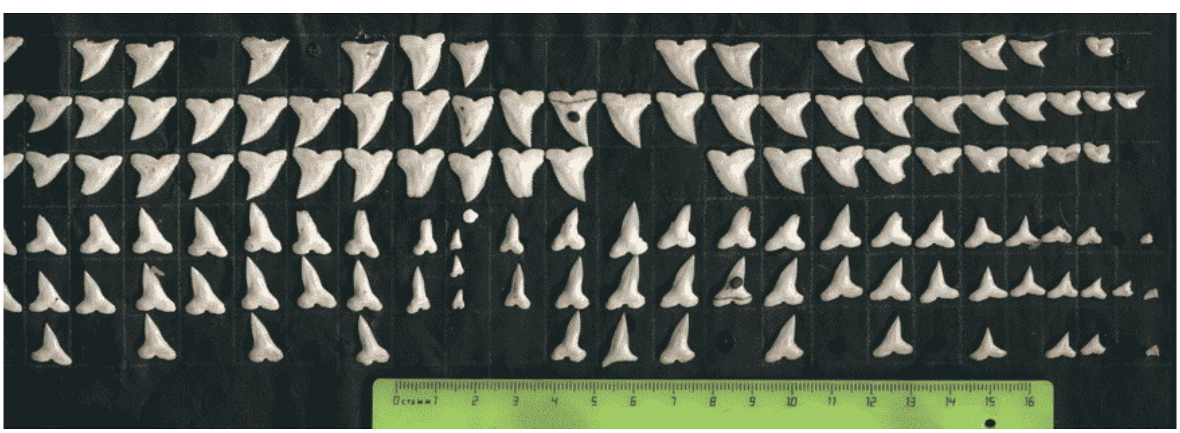

Fig. 3. The knife-shaped teeth, an extreme case. The upper teeth are very wide and roughly serrated. The lower teeth are flat, knife-shaped, widely triangular and well-serrated. The cutting edge is well-pronounced along all the cusp. It may be traced, that the teeth of the second and third rows are narrower comparing the first one, both in upper and lower jaws. Female, TL=247 cm, 00°29.6N, 06°42.1E, 03.06.1982.

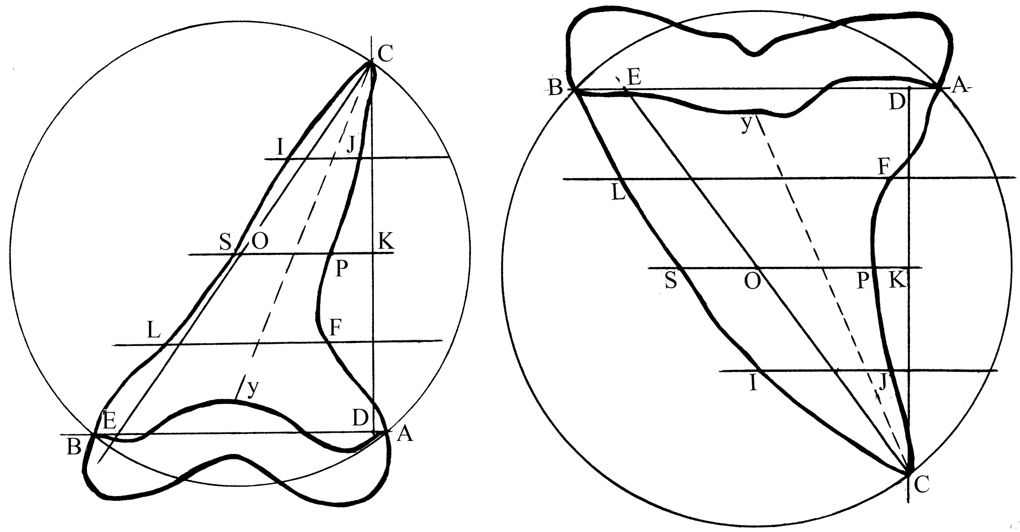

Fig. 4. The images of the lower (left) and upper teeth, grid and letters denote the measurements carried out to make the linear discriminant analysis.

To reveal and to quantify difference in teeth shape, two methods were applied:

1. Area indices method (Glickman, 1980; Averianov, Martens, 1998)
2. Linear discriminant analysis method using Statistica Software (Litvinov et al., 2004).
3. Routine statistical procedures using Graph Pad Prism

The two lengths were measured for the area indices method: 1) enamel cusp length which is equal to the distance CY; 2) width of the enamel cusp, distance AB. Then the three indices were calculated:

$$K_1 = \frac{BxC}{S};\ K_2 = \frac{\sqrt{S}}{B};\ K_3 = \frac{\sqrt{S}}{C}.$$

The eight measurements were taken from teeth images for the linear discriminant analysis method. Due to the curvilinear nature of the teeth images, three points only may be determined unambiguously: the top (C) and side ends of the base of enamel cusp (A and B). To get more points and measurements the following drawing to be applied:

- perpendicular from C to AB determines the D position;
- perpendiculars from A, B and C determines the position of the circumscribed circle, O;
- CO line determines E position on AB;
- perpendicular from O to CD determines K position;
- from the middle of KC and KD the lines parallel to AB determine positions J, L, S, P, L, F.
- The distances OC, AB, CD, AD, DE, FL, PS, JI were measured and related to the radius of the circumscribed circle in order to obtain the comparable values.

At the first stage of the linear discriminant analysis there were prepared the two tuitional and one test samples. One tuitional sample included 15 sets of AS, another one – 13 sets of KS. The teaching samples were used to create discriminant functions in the attribute space and decision rule to identify the certain teeth as belonging to AS or KS. Then the testing sample, which included all 129 sets, was analyzed.

RESULTS

1. Dental formula

For the dental formula there were used data of 144 sets, 98 of them from the same collection used for morphology and 18 additional ones, from Atlantic and Eastern Pacific. There were 39 females (34%) and 75 males (66%); 73 of the form (64%) and 41 of the form 2 (36%).

Symphysis (central) upper teeth: 113 sets of 114 had 1 central upper tooth; one male from Guinea Gulf had 1 upper tooth. The 37 upper central teeth of 113 were curved to the right, 13 (35%) females and 24 (65%) males, 29 (78%) of the form 1 and 8 (21%) of the form 2. The 56 of 113 were curved to the left, 20 (36%) females and 36 (64%) males; 29 of the form 1 (51%) and 27 of the form 2 (49%). The 21 of 113 central upper teeth were not curved, but rather straight, 7 (33%) females and 14 (67%) males, 15 of the form 1 (71%) and 6 of the form 2 (29%). Thus, upper central teeth did not reveal any sexual preference. The teeth curved to the left (49%) prevail in both sexes comparing curved to the right (32%) and straight teeth (18%).

Symphysis (central) lower teeth: 5 sharks, 4 males and 1 females, had no central lower teeth al all. Three of them were of the form 1 and 2 of the form 2. 83 sharks, 23 (28%) females and 60 (72%) males had one central tooth, 58 (70%) of them of the form 1 and 25 (30%) of the form 2. 24 sharks had 2 lower central teeth,13 (54%) females and 11 (46%) males, equally 50% of the form 1 and 2. The18 sharks had the lower central teeth in parallel, (males – 33%, females – 67%), and 6 were placed in steps mode (males – 67%, females - 33%). The two teeth were more frequent in females, but the step mode was more frequent in males; such position is very similar to the one central tooth, one may say these sharks had 1.5 lower central teeth. Two females had 3 central teeth. Generally speaking, females have more lower central teeth comparing males.

Lateral upper teeth. The teeth of the 1st, 2nd and 3rd series are the largest in the both forms. Sometimes the 2^{nd} upper tooth is slightly longer comparing 1^{st} and 3^{rd}. The 4^{th} and subsequent teeth become narrower and shorter; the length decrease more rapidly comparing width. The distal teeth are relatively wider and more curved to the mouth corners comparing the proximal ones (Fig. 1, 2).

Lateral lower teeth. The 1^{st} tooth is significantly shorter comparing the 2^{nd} one. The 3^{rd}, 4^{th} and 5^{th} are the largest lower lateral teeth. The 6^{th} and subsequent teeth become narrower and shorter; the length decrease more rapidly comparing width, like upper teeth. The distal teeth are relatively wider comparing the proximal ones, but their cusps but the two extreme series are still rather straight, and even may be inclined to the center.

The 64 sets of the 114 were completely symmetric, i.e. the number of teeth to the left of symphysis was equal to the number of teeth to the right of symphysis, in upper and lower jaws. The 6 sets had 13 upper lateral teeth and 58 14 ones (Table 1).

Table 1. The number of central and lateral teeth in the blue shark. The lateral teeth are calculated in 114 sets; the lateral teeth are calculated in 64 sets (symmetrical only).

	Number of lateral teeth			Number of central teeth			
	13	14	15	0	1	2	3
Upper jaw, number of sharks having the	6	58	0	0	113	1	0

corresponded number of lateral teeth							
Upper jaw, % of sharks of 64 (lateral) or 114 (central)	9.4	90.6	0	0	99.1	0.9	0
Lower jaw	16	47	1	5	83	24	2
Lower jaw, %	25.0	73.4	1.6	4.4	72.8	21.1	1.7

The 4 sets of 64 had 13 upper and lower lateral teeth (2 males and 2 females), 12 sets had 14 upper and 13 lower lateral teeth (6 males and 6 females), 45 sets had 14 upper and lower teeth (15 females and 30 males) two males had 13 upper and 14 lower lateral teeth, one male had 14 upper and 15 lower lateral teeth.

Asymmetry. The 50 sets of 114 (43.9 %) revealed the pronounced asymmetry, i.e. the number of lateral teeth to the right of symphysis was not to the number of teeth to the left of symphysis I upper jaw (14 sets) or in lower jaw (24 sets), or in both jaws (12 sets).

The data on the asymmetrical sets are summarized in Table 2.

Table 2. The number lateral teeth in the blue shark in asymmetrical sets.

	The number of lateral teeth to the left of symphysis						The number of lateral teeth to the right of symphysis				
	10	11	12	13	14	15	8	12	13	14	15
Upper jaw, the number of sharks having the corresponded number of lateral teeth	1	0	3	7	37	2	0	2	15	29	14

Upper jaw, %	2.0	0	6.0	14.0	74.0	4.0	0	4.0	30.0	58.0	28.0
Lower jaw	0	1	3	20	24	2	1	14	25	16	4
Lower jaw, %	0	2.0	6.0	40.0	48.0	4.0	2.0	28.0	50.0	32.0	8.0

Morphology. Indices K_1 and K_2 were not found useful to demonstrate numerically the heterodonty. In contrast to this, K_3 values, calculated for the lower teeth only, demonstrated evident bimodal distribution and ranged from 1.59 to 2.55 (Fig. 5). Assigning teeth with index value less or equal 1.95 as awl-shaped, those with index value of 2.05 or more as knife-shaped, and of 2.0 as awl-knife shaped it become evident that both forms could be met both in males and females. However, awl-shaped teeth happen in females occasionally, whereas in males its share increases with growth, upon attaining 2 meters most of males have teeth of this shape (Fig. 6). Both Pacific shark were large males and had awl-shaped teeth that was an agreement with the bulk of material.

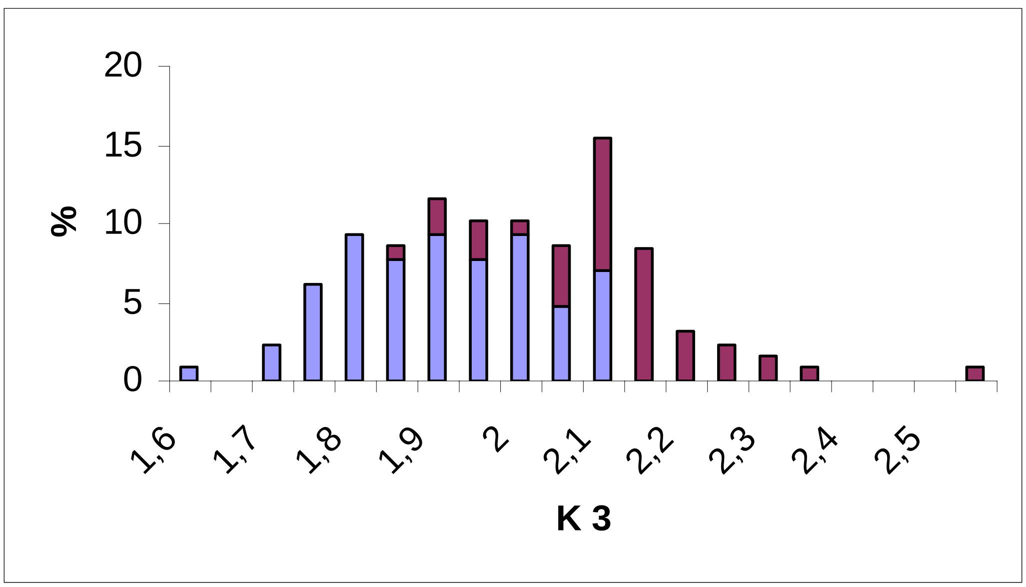

Fig. 5. Distribution of the area index K_3 vales calculated for the lower tooth of the fifth series. Form 1 is given in light color, form 2 is dark. Both sexes combined, N=129.

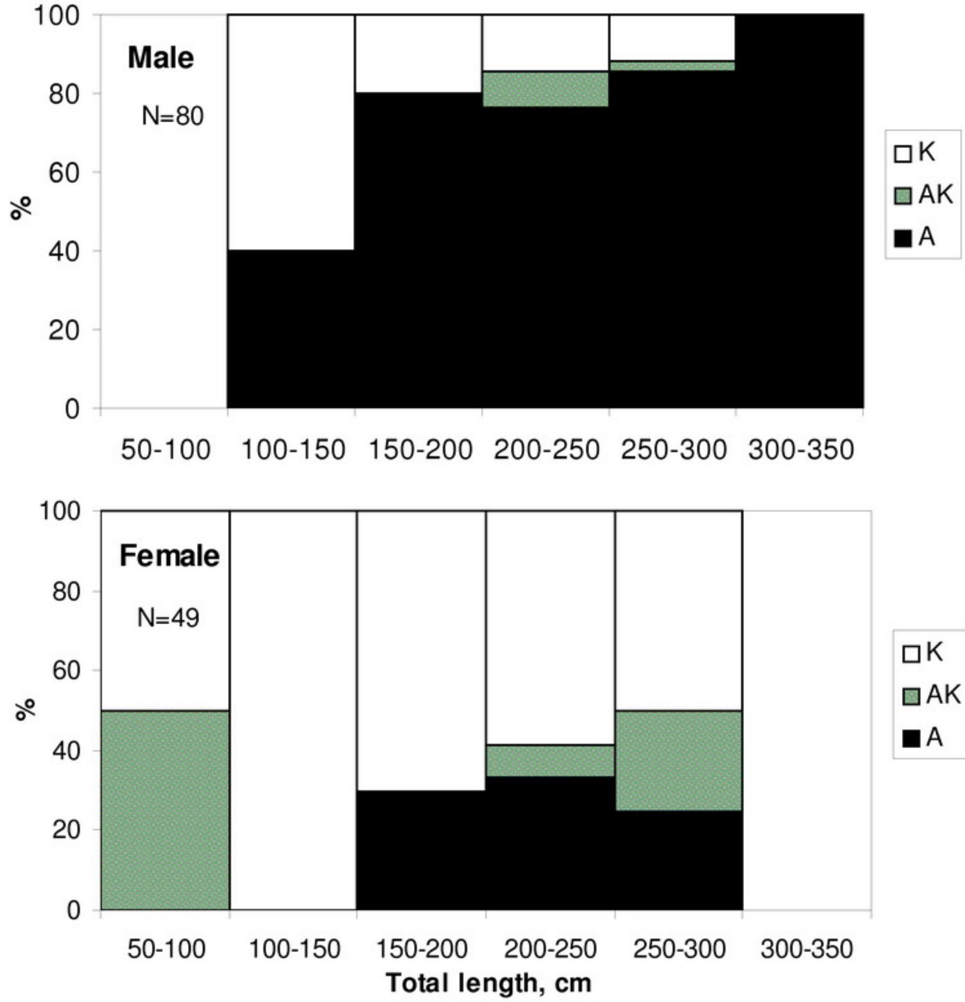

Fig. 6. Occurrence of the different types of the teeth shapes at the different body length. K – knife type. A – awl type, AK – transit type.

A total of 82% of teeth were assigned by LDA method as either awl-shaped or knife-shaped with probability higher 0.9 and 2.4% only with probability lower 60% (Fig. **7**) confirming the reality of these forms. All the assignments but one were in agreement with visual evaluation.

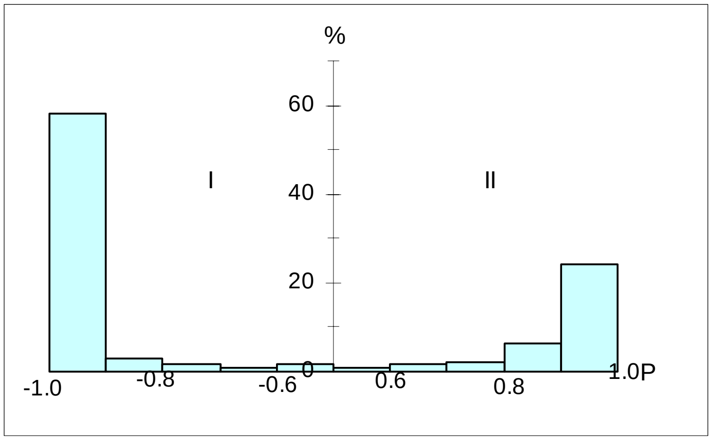

Fig. 7. The distribution of the probabilities (P) corresponding to the highest discriminant function, for the lower teeth of form 1 and 2.

Sexual differences in the teeth growth patterns could be seen also with cusp base width (CBW) taken as an example. In males CBW of upper teeth increases monotonously up to 200 cm TL and faster afterwards (Fig. 8**),** and sharks longer 200 cm have relatively wider teeth. The lower teeth in males demonstrate rather uniform increase of CBW related to bogy length, and even slight decrease of its rate in larger males with awl-shaped teeth. Vice versa, females demonstrate monotonous increase of CBW up to 250 cm TL and slighter or even zero increase afterwards, in both jaws and in both forms.

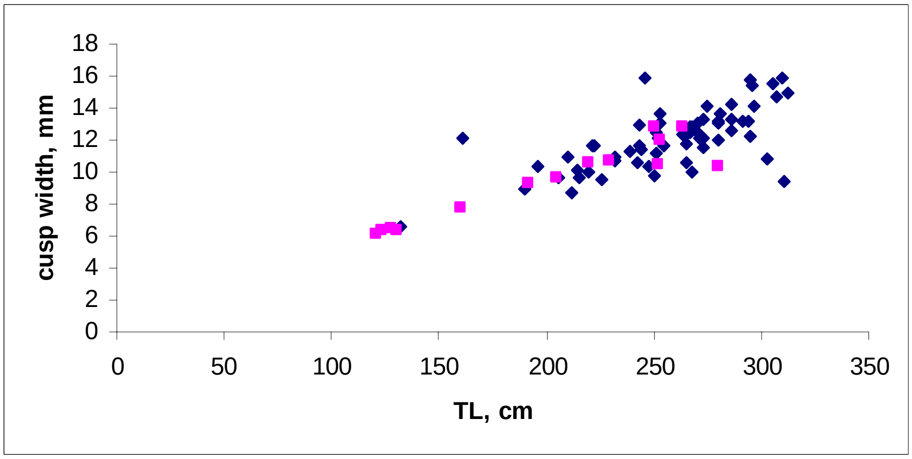

Fig. 8. The relationship between cusp base width and shark body length, males, upper jaw. Diamonds=form 1, squares=form 2.

DISCUSSION AND CONCLUSION

The most part of the sharks' descriptions present the dental formula in general, giving the possible quantity of the symphysis and lateral teeth. There is no any literature on the quantitative assessment of the dental formula and predominate number of the teeth. The same time the quantitative assessment of the predominate values is the integral part of the phenotype, it is important for the description of the modern species and paleontologic studies. Bass et al. (1975) observed that the usual tooth count in 13 jaws of the blue shark was 14-1-14/13 or 14-2-13 or 14. Central teeth varied between none and one in the upper jaws and between one and two in the lower jaws. According to Compagno (1984) there were 24 to 31/25 to 34 rows of teeth.

Our data correspond to the previous observations in general, but demonstrate the pronounced variability of the dental formula. It may be concluded, that conventional dental formulae do not describe sufficiently the real picture and more detailed descriptions are required, for carcharhinids family at least.

Our data confirmed a previous assumption about an existence of two distinctive teeth shapes in blue sharks (Litvinov, 1982). We suppose that appearance of awl-shaped teeth in males at about 170-250 cm is related to maturation and to use of jaws in mating behavior. In the North Atlantic male blue shark mature at 193-210 cm, with a length of 50% maturity of 201 cm (Campana et al, in press), around New Zealand it happens at 190-195 cm (Francis, Duffy, 2004) and at 166 cm in North Pacific (Nakano, 1994). These lengths generally coincide with changes in male teeth shape.

Courtship behaviour and copulation has not been observed in the blue shark, but these apparently involve biting of females by males. It is very likely that in copulation male holds female by teeth and in order to avoid a serious damage to female skin. Among adult and subadult sharks, this behaviour is sufficiently consistent with sex that sharks in the field can be sexed accurately merely by the presence or absence of bite wounds or scars. The blue shark has an unusual morphological adaptation for this behaviour; subadult and mature females develop skin about three times as thick as males (Compagno, 1984; Pratt, Castro, 2005**).** To minimize a harm to female body, the knife-shaped teeth of the young age which are able to cut away anything, are transformed into kind of fork, which holds but not cuts.

Also, in *Alopias superciliosus* females have broader teeth (Gruber, Compagno, 1981). In *Carcharhinus brachiurus* the upper teeth of large males are distinctly hooked near the tips as compared to those of females; in *C.sealei* the cusps of lower teeth are very finely serrated and this phenomenon is more pronounced in young specimens of both sexes and in adult females than in males where these cusps are virtually smooth (Bass et al., 1973). All these examples illustrate that adult male teeth look relatively harmless in respect to those of females.

Such transformation in the small spotted catshark *Scyliorhinus canicula*, when heterodonty designed first as adaptation to copulation, resulted also in spatial segregation of sexes and in different feeding spectrum (Litvinov, 2003, Patokina, Litvinov, 2004). It highly probable, that heterodonty serves also to separate feeding niches of males and females allowing the species to use wider food resources and decreasing a competence for food between sexes.

Our data allows supposing that the heterodonty could be distributed among sharks more widely than it is assumed to date. It could be a rule rather than exclusion. To investigate its occurrence, meristic features and statistics should be applied, and the index K_3 probably would be a very useful tool. Also, it looks promising to investigate a degree of maturity-related sexual heterodonty with spatial segregation of sexes and feeding spectra.

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