Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v46i1.71273

Morphological and morphometric sperm analysis of dwarf sperm whale (*Kogia sima*)

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ABSTRACT. Nowadays, an increase in the number of marine species at risk of extinction has been observed. Therefore, studies on the reproductive characteristics of these animals are essential. This animal is considered relatively rare, and there is scarce information regarding its reproductive biology and physiology. Thus, this study aims to describe the morphology and morphometry of sperm obtained from dwarf sperm whale. The material used in this work was collected during the necropsy of a dwarf sperm whale specimen. Thus, seminal samples were fixed and stained with panoptic stain. In morphometric analysis, the results obtained were: acrosome length of 0.83±0.01 µm, head length of 1.5±0.02 µm, intermediate part of 0.4±0.00 µm and total length of 27.3±0.51 µm. In terms of morphology, the defects observed were double head, heavily curled tail, abnormal small head, simply bent tail, piriform head, heavily bent tail and specimens within the normal range. In this context, the morphometric and morphological sperm analysis of *Kogia sima* described in this study can assist future studies regarding the reproductive physiology of these animals.

Keywords: seminal analysis; reproduction; dolphin; aquatic mammal.

Received on February 13, 2024
Accepted on May 20, 2024

Introduction

Human activities have impacted and depredated marine environments, increasing the risk of extinction of many species. All these factors are potentially responsible for threatening the conservation of cetacean populations and diversity (Farina & Braga, 2019). Cetaceans are represented by aquatic mammals such as whales, porpoises and dolphins (Jacobina, 2000). These animals are taxonomically divided into Mysticeti and Odontoceti. Among Odontocetes, the genus *Kogia* is represented by two species*, Kogia breviceps* or pygmy whale (Blainville, 1838) and *Kogia sima* or dwarf sperm whale (Owen, 1866).

According to the International Union for Conservation of Nature (IUCN), there is few information regarding the population structure and number and physiology of *Kogia sima* (dwarf) species. Among justifications, the shy behavior stands out, which makes them extremely challenging to be studied and observed (Caldwell & Caldwell, 1989). Thus, *Kogia sima* was classified in 2008 as lacking of data on the IUCN Red List and still remains in this condition (Kiszka & Braulik, 2020).

In this context, the analysis of sperm morphology and structure has been a fundamental tool to understand the reproductive biology and phylogeny of wild species, which technique has helped to collect data for phylogenetic studies, evaluate reproductive strategies and for the development of reproductive biotechniques (Amaral, Silva, Domingos, & Martin, 2017). Although artificial insemination proved to be a good tool for generating information on the reproduction of cetaceans in captivity, as observed in *Tursiops aduncus* (Kinoshita et al., 2004), there is still lack of information on the reproductive physiology of free-living cetaceans specially those of the genus *Kogia* spp. in particular males of that species.

Mammalian sperm is composed of head and tail, the head is composed of the acrosomal and post-acrosomal region, while the tail is composed of neck, intermediate part, main part and terminal part (Farias Junior, 2018). Among mammals, sperm head morphology is variable (Miller, 2007). In addition, sperm size and shape are different between individuals of different species, and even between animals of the same species (Plön & Bernard, 2006**)**. Thus, it is believed that these variations are related to particular aspects of the phylogenetics and reproductive strategies of animals (Amaral et al., 2017).

According to Plön and Bernard (2006), *Kogia breviceps* and *Kogia sima* spermatozoa are similar in shape and size, with spatulated and very thin head when viewed from the side. The difference is that the head of *K. breviceps* sperm is more rounded and in *K. sima* specimens, it is bullet-shaped (Mothé, 2015). Furthermore, in *K. breviceps* and *K. sima*, the intermediate part is short and constituted by spherical mitochondria distributed in layers and columns (Plön & Bernard, 2006). In animals of the genus *Kogia*, five to six spherical mitochondria layers were observed, with total estimate of 25 to 30 mitochondria per sperm (Plön & Bernard, 2006). In most cetaceans, mitochondria are spherical, and have random or layered pattern. This morphological pattern also appears to be characteristic of odontocetes among cetaceans (Amaral et al., 2017).

However, despite such information, the in-depth morphological characterization of the *Kogia* sperm is necessary. In this way, the present project aimed to analyze the morphology and morphometry of the spermatozoa of *Kogia sima* (dwarf sperm whale) to elucidate the reproductive aspects of this species.

Materials and methods

Sperm samples from one (n = 1) adult male dwarf sperm whale that stranded on a beach located on the northeastern coast of Brazil (state of Sergipe) (Latitude: 10°.83'94.3" S; Longitude: 36°.94'34.66" W) was used. The animal died of natural causes, and the presence of fecaloma and pulmonary edema were observed at necropsy.

During necropsy, seminal material was collected, fixed and stained with Panoptic stain (Romanowsky) (García-Herreros, Aparício, Barón, García-Marín, & Gil, 2006). Subsequently, for the description of the sperm morphology and morphometry, the Image J software was used.

Four hundred and fifteen (n = 415) sperm cells were counted during computerized evaluation. Cells were evaluated for length (measured from the apical portion of the acrosome to the tip of the flagellum), sperm head diameter, intermediate part diameter and acrosome diameter (Yavetz et al., 1995). Furthermore, morphological characteristics were described, as well as the classification of major and minor defects (Barth & Oko, 1989).

All data were evaluated using the SAS System for Windows (SAS Institute Inc., Cary, NC, USA). Response variables were submitted to Mean and Standard Error analysis. Thus, the results were described as mean and standard error.

Results

After the morphometric analysis of each segment of the *Kogia sima* sperm, it was possible to observe acrosome length equal to 0.83±0.01 µm (Table 1). Head length was 1.5±0.02 µm, total sperm length was 27.3±0.51 µm and intermediate part length was 0.4±0.00 µm (Table 1).

Regarding sperm morphology, normal morphology (Figure 1) and defects (Figure 2) such as slightly bent tail (n = 1), severe bent tail (n = 10), curled tail (n = 2), short tail (n = 15) and double-headed sperm (n = 1) were observed.

Discussion

Regarding the morphometric analysis of the *Kogia sima* sperm, the total sperm length was previously described as 32.6 µm (Plön & Bernard, 2006). In this study, mean length of 27.3±0.51 µm was observed, which is similar to previously reported results.

Head size was observed with average length of 1.5±0.02 µm, while another study has shown head length of 4.0±0.58 µm, which was carried out with sample size of 2 *K. breviceps* males and 4 *K. sima* males (Plön & Bernard, 2006). This difference may be due to the fact that there is great variation between individuals of the same species than between individuals of different species (Ward, 1998; Schulte-Hostedde & Millar, 2004); in addition to the sample size and the different methods used. In this study, the panoptic technique was used while in the comparative study, other techniques were applied (Plön & Bernard, 2006). Furthermore, the size of the samples can also influence, as the tissue condition is an interfering factor. Plön and Bernard (2006) reported that the tissue shrank, influencing the difference in the result when compared to that of the present work.

Plön and Bernard (2006) observed that the intermediate part had 3.5±0.51 µm in length, which was not demonstrated in this study, which the mean size of the intermediate part reached 0.4µm. This difference may also originate from different measurement units used at the time of counting or the type of method used and the state of the tissue evaluated, which demonstrates that the size and shape of spermatozoa can vary between individuals of the same species (Ward, 1998; Schulte-Hostedde & Millar, 2004).

Table 1. Morphometric analysis of *Kogia sima* sperm (n = 415).

	Mean	Standard Error
Acrosome (µm)	0.833	0.014
Head (µm)	1.505	0.025
Intermediate part (µm)	0.474	0.007
Length (µm)	27.365	0.516

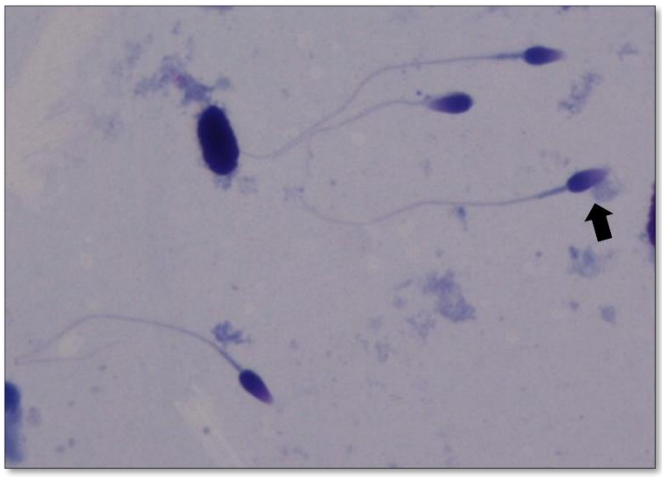

Figure 1. *Kogia sima* sperm in light microscopy. Head in fusiform shape (Arrow). 400x.

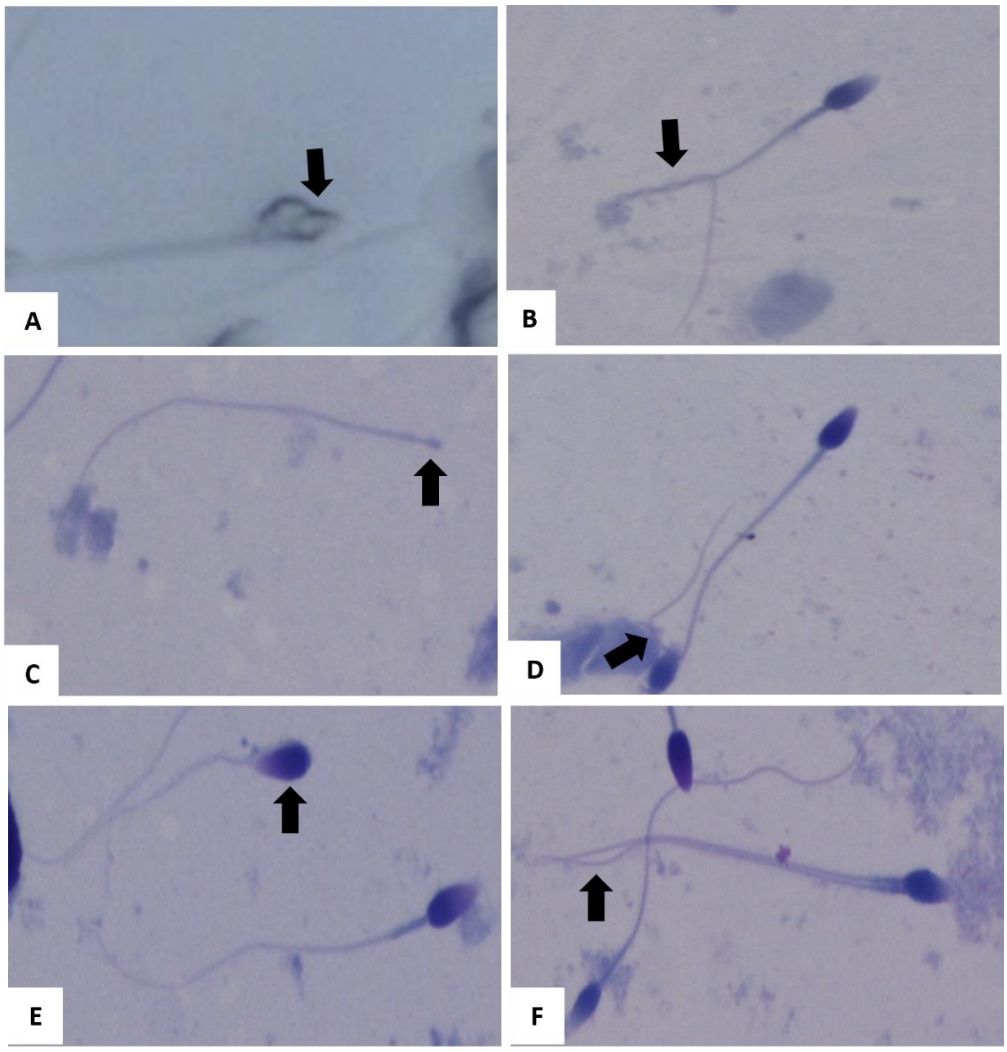

Figure 2. A. Double head; B. Tightly curled tail; C. Abnormal small head; D. Bent tail; E. Piriform head; F. Double tail. 400x.

When compared with other species (Figura 3), there is also variation in the shape of the sperm head, with some species having rounder head and others elongated one. In addition, the measurements of the sperm head dimensions found in the study carried out by Meisner, Klaus, and O'Leary (2005) differ from those found here, with length of only 4.6 µm. Furthermore, the intermediate part of the cetacean sperm is considered shorter than that of other mammals (Plön & Bernard, 2006), which can be compared to the intermediate part of the dwarf sperm whale, with length of 0.4µm (Woodall & Johnstone, 1988). The size of the intermediate part is linked to the number of mitochondria per column, that is, when shorter, there is fewer number of mitochondria (Cummins & Woodall, 1985; Plön & Bernard, 2006), as is the case with the *Mirounga leonina* southern elephant seal (Cummins & Woodall, 1985), which shows a relatively short intermediate part. Therefore, mitochondria are apparently not extremely necessary for *Kogia sima* sperm, unlike what is observed in dogs, for example (Angrimani et al., 2017).

This morphometric difference is also observed between cetaceans species, the total length of the dolphin sperm cell (*Delphinus delphis*) is 70.59±0.11 µm (Kita, Yoshioka, Kashiwagi, Ogawa, & Tobayama, 2001), the pink dolphin (*Inia geoffrensis*) has total length of 62.32±5.61 µm (Amaral et al., 2017), while *Kogia sima* presents 32.6µm (Plön & Bernard, 2006) and in the present study, the value is 27.3±0.51 µm. Moreover, the *Kogia sima* sperm has spatulated and thin head when viewed from the side, so that it is exclusively in the species under study in the fusiform shape (Cummins & Woodall, 1985; Plön & Bernard, 2006). The same results were found in the present study, and most specimens were bullet-shaped, and some oval-shaped, dorsoventrally flattened, as observed by Ballowitz (1907).

Cetaceans exhibit spherical mitochondria arrangement in layers and columns along the intermediate part, which is exclusive of mammals (Ballowitz, 1907), and the only other vertebrate in which spherical mitochondria are reported is tuatara *Sphenodon punctatus* (Ballowitz, 1907; Healy & Jamieson, 1992). Although it was not possible to count the number of mitochondria and visualize their shape, Plön & Bernard (2006) confirmed such data in the study that indicated both species (*K. sima* and *K. breviceps*) to have five to six tiers of similar-size spherical mitochondria and a total of 25-30 mitochondria. In Kogia species, the number of mitochondria can vary from 20 to 30, which results are not in line with other eutherian mammals, since this number is much higher (Fawcett, 1965).

Sperm morphology is described as the anatomical study of the male gamete, that is, the sperm cell (Arruda et al., 2015) and therefore it is an indispensable factor for the fertile potential of animals. Early 20th century studies on morphological changes have demonstrated a relationship with low fertility (Williams, 1920; Lagerlöf, 1936). Thus, each sperm cell compartment must not only be intact, but also respond correctly to intra and extracellular signals, which determine its fertility and viability (Arruda et al., 2015).

According to (Garcia, 2004), spermatogenesis is an extremely delicate process, so that any factor can influence the production of abnormal sperm. Barth and Oko (1989) found that the type of morphological defect is almost always indicative of its origin, and according to Arruda et al. (2015), they are: irregular nuclear division during meiosis, abnormal transformation of the nucleus, golgi gallbladder, centriole, spermatid mitochondria during spermiogenesis, cytoplasmic droplet migration failure during epididymal maturation, inability to maintain flagellar coherence during migration in the epididymis, deterioration of membrane stability during storage in the epididymis and disintegration of senescent sperm in the epididymis or ampulla.

Thawing can also influence the formation of folds in the tail, while sudden manipulations can cause heads to detach or tails to fracture (Lagerlöf, 1934). For Nöthling and Irons (2008), freezing and thawing processes can cause tail bending and acrosomal changes. The defects found in the present study were not considered in large numbers, as follows: double head, tightly curled tail, abnormal small head, bent tail, piriform head and double tail.

Regarding defects, they can be classified according to their importance as major defects, minor defects and the sum of both, total defects (Blom, 1973). The most serious anomalies, which interfere with fertility, are within the major defects, including severe head defects such as piriformis, abnormal small and abnormal isolated head, in addition to abnormal, underdeveloped contour, defective acrosome, diadem, defects of the intermediate part, proximal cytoplasmic droplet and tightly bent coiled tail (Blom, 1973). While minor defects are characterized as thin head, abnormal small head, giant head, short or wide head, loss of acrosomal membrane, abaxial implantation, distal cytoplasmic droplet, normal decapitated head (isolated), tail simply bent or curled. Among the defects mentioned, the following were found in the analyzed spermatozoa: piriform head, abnormal small head, tightly curled tail, being the largest and, as the smallest, simply bent or curled tail.

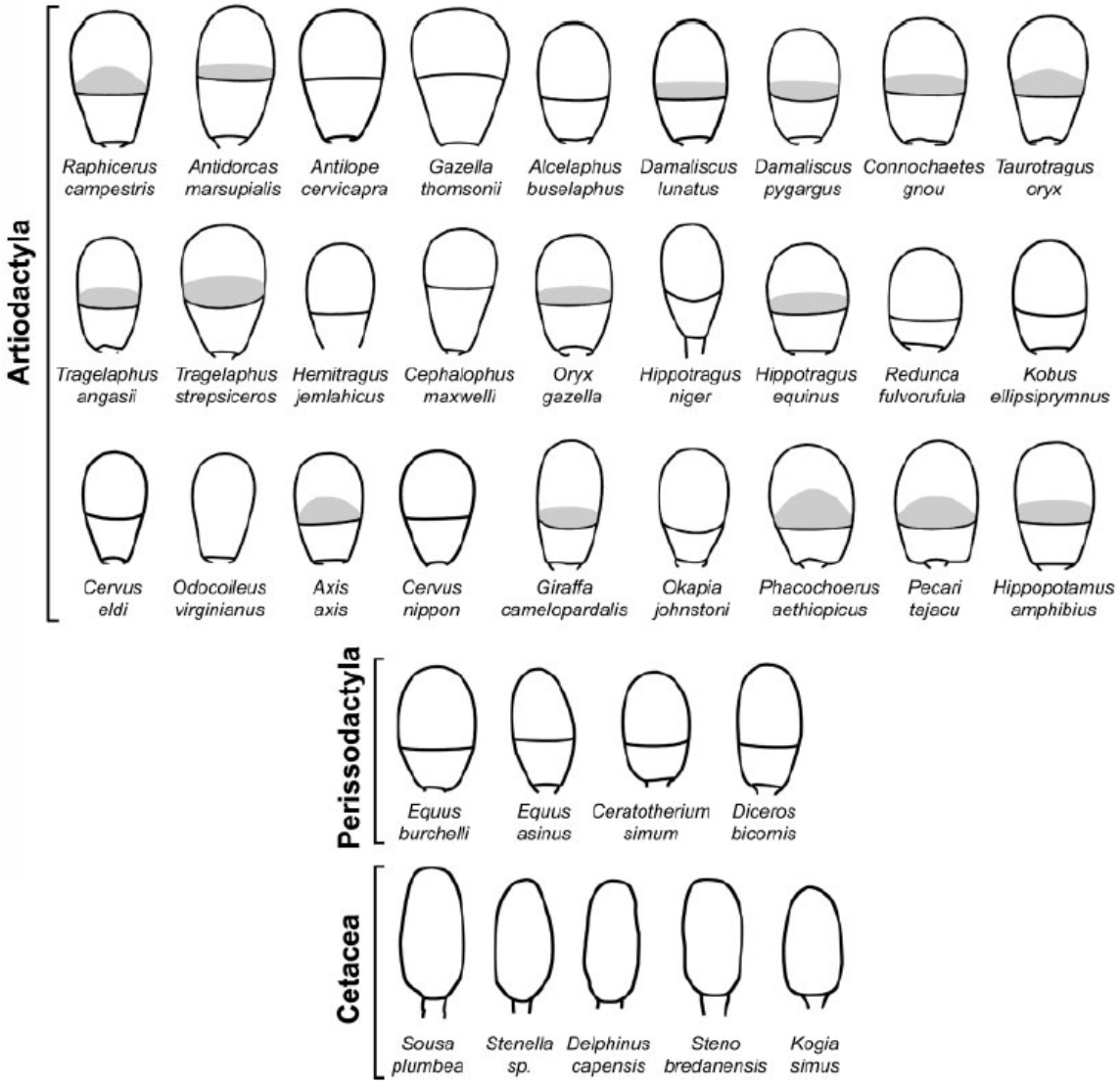

Figure 3. Generalized drawings comparing the shape of sperm heads in artiodactyl, perissodactyl and cetacean species (Meisner et al., 2005).

Conclusion

In conclusion, the morphometric and morphological sperm analysis of *Kogia sima* was described, which can assist future studies regarding the reproductive physiology of these animals. However, further studies using greater number of animals should be carried out in order to make an in-depth description of the sperm characteristics of the species.

Acknowledgments

The authors would like to thank the 'long Live the Manatee' project of the Aquatic Mammal Foundation and sponsored by Petrobras through the Petrobras Socio-environmental Program; Petrobras; The implementation of the Regional Program for Monitoring Strandings and Abnormalities in the area of influence of the Sergipe-Alagoas Basin is a measure of environmental impact assessment required by the federal environmental licensing conducted by Ibama; and the Laboratory of Animal Morphophysiology (Labma).

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