

## SPERM MORPHOLOGY AND MORPHOMETRY OF BURMEISTER'S PORPOISE (*PHOCOENA SPINIPINNIS*)

Knowledge of sperm morphology and morphometry in mammals may contribute to the understanding of their phylogeny and evolution of reproductive systems. The evolution of features such as sperm shape and sperm number is probably the result of two major selective forces: female reproductive physiology and sperm competition (Roldan *et al.* 1992). Characterization of the sperm shape and size has been utilized as a taxonomic tool (Rouse and Robson 1986, Harding *et al.* 1987), and in some analyses sperm abnormalities have been related to inbreeding (Wildt *et al.* 1987). Morphometric data on the sperm of some cetacean species have been previously reported (Matano *et al.* 1976, Fleming *et al.* 1981, Cummins and Woodall 1985).

Burmeister's porpoise inhabits temperate waters of South America, from Tierra del Fuego to Río de la Plata (Uruguay) on the Atlantic coast, and from Tierra del Fuego to Paita (Peru) on the Pacific coast (Honacki *et al.* 1992). The aim of this work was to characterize the shape, dimensions, and the percentage of abnormalities of Burmeister's porpoise spermatozoa and to compare the results with those for other cetaceans.

Spermatozoa of two adult male Burmeister's porpoises were obtained from individuals accidentally caught in gillnets in Necocha, Argentina (38°37'S, 58°50'W). The individuals had more than three growth layer groups (GLGs) (Corcuera *et al.* 1995). Testes and epididymis were fixed in 10% phosphate-buffered formalin solution within 24 h of the death of the animal. Sperm were

Table 1. Percentages of different sperm abnormalities in two individuals of *Phocoena spinipinnis*.

Morphology type	Male 1 <i>n</i> = 400 (%)	Male 2 <i>n</i> = 400 (%)
Large oval head	0.25	0.75
Small oval head	0.25	0.00
Tapering head	0.75	0.75
Pyriform head	0.25	0.50
Pin head	1.50	3.00
Round head	0.25	0.00
Amorphous head	0.75	0.25
Midpiece defect	0.25	0.25
Total abnormalities	4.25	5.50

obtained by squeezing the *cauda epididymis* into a known volume of 4% phosphate-buffered formalin solution.

To study sperm shape, a small amount of the sperm suspension was placed between a slide and coverslip and examined with a 40 $\times$  objective using a phase-contrast microscope. Other small amounts were used for counting in a hemocytometer in order to determine testis activity. Sperm smears were stained with silver nitrate (Howell and Black 1980) or with Giemsa. Sperm dimensions were measured from at least 50 spermatozoa of each animal in two ways: from photographs, and directly from the stained spreads using a micrometric eyepiece and a 40 $\times$  objective. Two hundred spermatozoa from each individual were counted and characterized twice from stained smears in order to obtain the percentages of sperm abnormalities. The spermatozoa were classified using the categories and the percentages for human sperm abnormalities of the World Health Organization (World Health Organization 1992).

The epididymal sperm concentration were  $2.7 \times 10^6$  and  $2.16 \times 10^6$  spermatozoa per mm<sup>3</sup> in males 1 and 2, respectively. The percentages of total sperm abnormalities observed were 4.25% and 5.50%; these percentages were considered normal with respect to human World Health Organization criteria (Table 1). The shapes of the sperm heads were ellipsoidal in frontal view and ensiform in lateral view. The acrosome covered more than the anterior half of the sperm nucleus. The sperm shape did not present great variation from that reported for other cetaceans. The tail insertions were central, and tail shapes were similar to those of most eutherian mammals (Fig. 1). The means and standard deviations obtained for the linear dimensions of spermatozoa are shown in Table 2.

The percentages of total sperm abnormalities were very low. Low percentages were also reported for *Tursiops truncatus* (Fleming *et al.* 1981). We believe the percentage of sperm abnormalities observed cannot be attributed to damage of the samples before fixation because we did not observe typical signs of incipient sperm degeneration, such as acrosome or midpiece swelling. Cassinello *et al.* (1998) demonstrated that shape and size were not affected by the

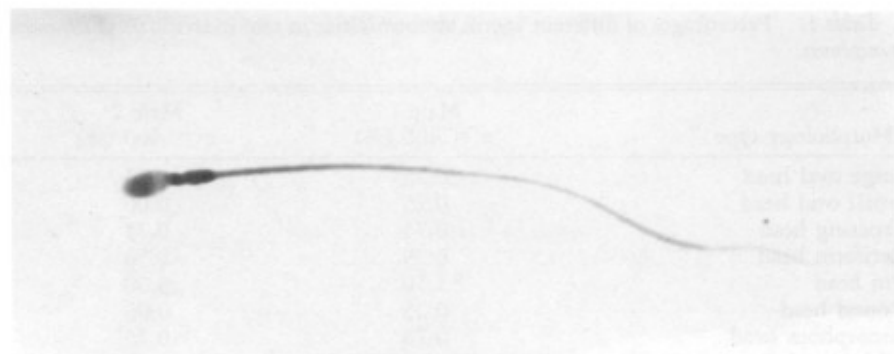


Figure 1. Spermatozoon of *Phocoena spinipinnis* stained with Giemsa (1,450 $\times$ ).

different fixation or staining techniques used in this work. The morphology of the sperm head and head sizes in this species are similar to those described for other cetaceans and especially to those for the Delphinidae (Fleming *et al.* 1981, Cummins and Woodall 1985). The total sperm length in *P. spinipinnis* is very similar to that in *T. truncatus*, and both are approximately 20  $\mu\text{m}$  longer than that in *Physeter macrocephalus* (Cummins and Woodall 1985). The middle piece is short and the neck is long compared to those of the sperm of other mammals; both characteristics have also been found in *P. macrocephalus* (Matano *et al.* 1976). The sperm morphology and morphometry of *P. spinipinnis* correspond, as in other cetaceans reported, to the ancestral type described for mammalian spermatozoa (Cummins and Woodall 1985, Gomendio and Roldan 1994). Although there are few species of cetaceans for which spermatozoa have been studied, there seems to be little diversification in this order, as compared to that observed in rodents (Roldan *et al.* 1992) and armadillos (Cetica *et al.* 1998). The main differences are only in some morphometric parameters, as in other eutherian orders (Cummins and Woodall 1985). Further sperm studies will be necessary in order to decide whether sperm evolution is congruent with data from comparative anatomy and evolutionary biology in this order.

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Table 2. Means and standard deviations of sperm linear sizes in *Phocoena spinipinnis* ( $n > 100$  for each variable; units in  $\mu\text{m}$ ).

Head		Total tail length	Principal length	Midpiece length	Total sperm length
Length	Width				
5.29 $\pm$ 0.3	2.55 $\pm$ 0.2	59.67 $\pm$ 2.2	56.25 $\pm$ 1.9	3.31 $\pm$ 0.3	66.04 $\pm$ 2.7

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ADELA BEILIS, P. CETICA and M. S. MERANI, Centro de Investigaciones en Reproducción, Facultad de Medicina, Universidad de Buenos Aires. Paraguay 2155, Piso 10°, 1121 Buenos Aires, Argentina; e-mail: cir@fmed.uba.ar. Received 14 October 1998. Accepted 15 October 1999.