EVALUATION OF AGING PARAMETERS
IN *TORPEDO MARMORATA*

by

Eugenia ALOJ TOTARO, Francesco Aurelio PISANTI (1),
Paola RUSSO and Patrizia BRUNETTI
Insegnamento di Biologia e Zool. Gen.,
I Fac. di Medicina e Chir., Università di Napoli,
Viale M. Cristina, 18/d, I-80122 Napoli (Italy)

SUMMARY

In an investigation on the aging in *Torredo marmorata*, the authors have studied and compared the number of calcification rings present in the vertebral body, the length of the alfactory pedicles, the total length of the animal, the maximum diameter of the body disc, weight, and the quantity of the neuronal lipofuscin granules. The investigation was performed on randomly fished animals, and also on specimens born in captivity and kept in the laboratory until the age of 90 days. There is a good correlation between the length of the alfactory pedicles and the other evaluated aging parameters.

Key words : *Torredo*, aging, lipofuscin, alfactory pedicles.

INTRODUCTION

To date, lipofuscin has been used as a marker of cellular age by various authors, using in the majority of cases vertebrates, where the age of the subjects could be accurately recorded (SEKHON and MAXWELL, 1974). In order to use an animal of a lower evolutive level, such as *Torpedo*, which has interesting biochemical characteristics (particularly some neuronal metabolic patterns, see PISANTI, FRASCATORE, ALOI TOTÀRO and UUTTARIELLO, 1983) it is necessary to design a method to evaluate lipofuscin in order to overcome the uncertainty of the age of subjects casually collected. In the case of *Torpedo* collected in the Bay of Guascogna (MELLINGER, 1971) and those collected in the Bay of Naples (ALOI TOTÀRO, 1979), both authors experienced difficulties in maintaining animals in aquaria. It has been possible, however, to collect newborn specimens, bred in captivity, and rear them until 90 days of age (ALOI TOTÀRO, 1979). On the other hand, the central and peripheral nervous system in *Torpedo*, which above all is interesting for the peculiar metabolism of the electric lobe which controls the activity of the electric organ (GRASSÉ, 1958), may help us to understand the histogenesis of neuronal lipofuscin (PISANTI, FRASCATORE, ALOI TOTÀRO, CRISCI, 1983). Thus we have studied various parameters of *Torpedo* and compared them to published data in order to dertermine the age of casually collected specimens.

MATERIALS AND METHODS

Thanks to facilities of the Zoological Station of Naples, we have obtained specimens of *Torpedo marmorata* from the Bay of Naples of both sexes and various dimensions : of these, several gave birth in captivity. The animals were kept in aquaria with circulating sea water and were fed artificially as described by ALOJ TOTÀRO, 1979). After opening the cranial roof, the rectilinear distance of the olfactory pedicles (Fig. 1 b) was measured from their rise on the forebrain to the foramen olfactorium by using compasses. The evaluation of the number of calcified rings present in the vertebral bodies was made according to the methods of LA MARCA (1966) and MELLINGER (1971). The morphometric evaluation of lipofuscin granules was carried out on sections prepared for the TEM according to the technique des-
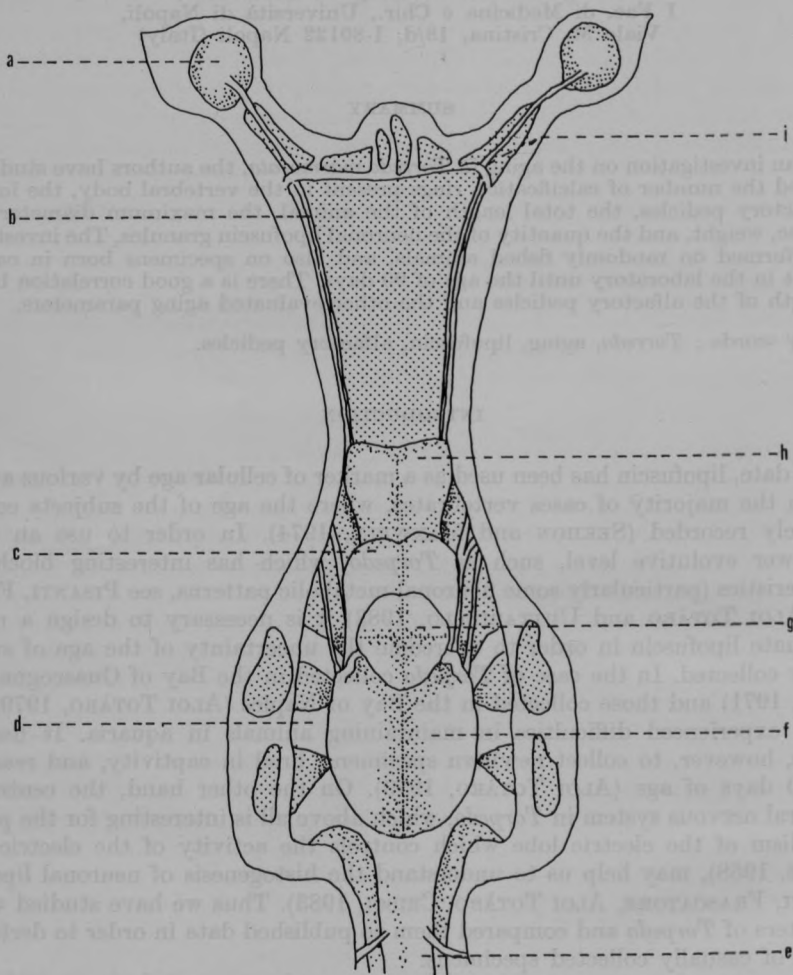

Fig. 1. — Dorsal view of the central nervous system in *Torpedo marmorato;* a : olfactory bulb, b : olfactory pedicles, c : optic lobe, d : electric nerves, e : medulla oblongata, f : electric lobes, g : cerebellum, h : forebrain, i : foramen olfactorium.

cribed by ALOJ TOTÀRO and PISANTI (1979, 1981). Teeth from newborn and adult *Torpedo* and pieces of dorsal and ventral skin from the same animals were fixed in glutaraldehyde 3,5 % buffered according to ALOF TOTÀRO and PISANTI (1979). The specimens were processed for SEM by critical point drying and treatment with gold-palladium. They were observed with a Philips SEM 505 at 15 kW.

RESULTS

The data examined refer to measurements taken from 100 adult (65 females and 35 males). 31 animals were born in captivity from three pregnant females. However, it was only possible to follow the growth of 15 of these upto 90 days of age. We present in Fig. 2 the parameters studied in males, females, newborn and 90 days old individuals.
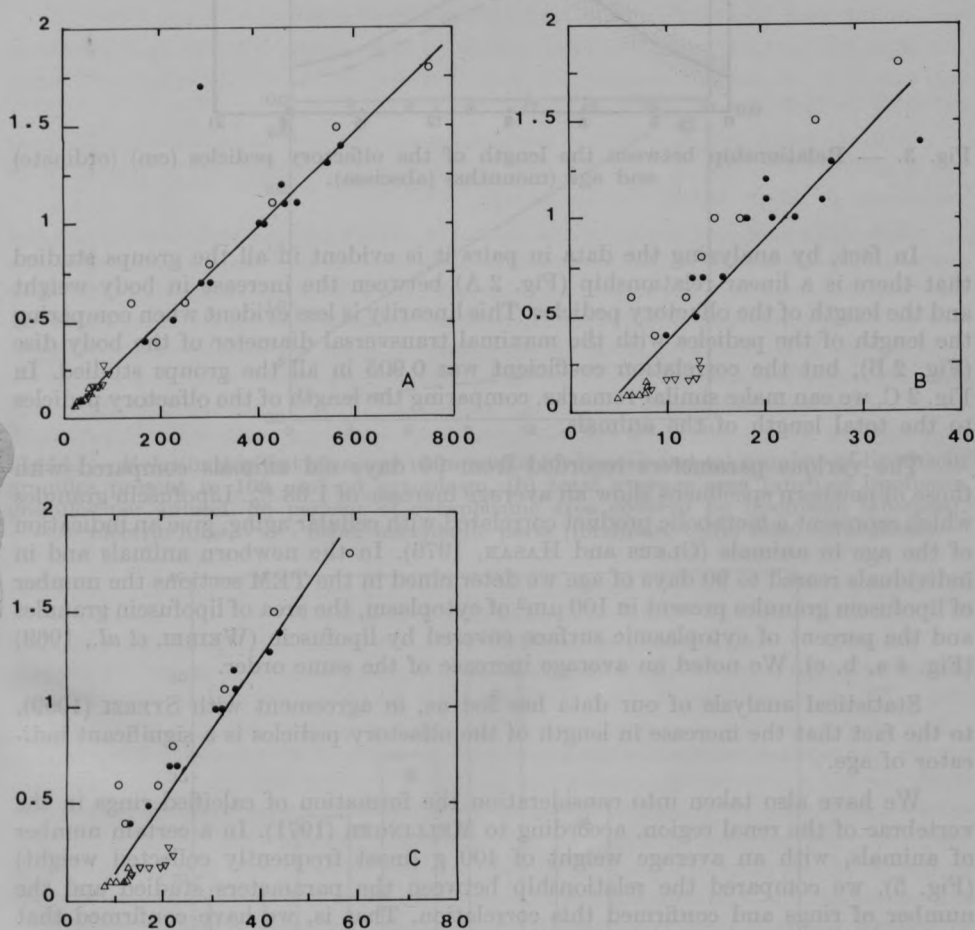

Fig. 2. — Regression lines between the length of the olfactory pedicles (cm) (ordinate) and (A) weight (g), (B) transverse diameter of body disc (cm), (C) maximum length of the animal (cm) (abscissa). ● = males, ○ = females, Δ = newborn, ▽ = 90 days old animals.

Comparing the data related to the various parameters, we observe a good correlation (r = 0.987) between the increase in body weight and the increase in length of the olfactory pedicles, defined by Sterzi (1909) as a way of estimating the age of this species (Fig. 3).
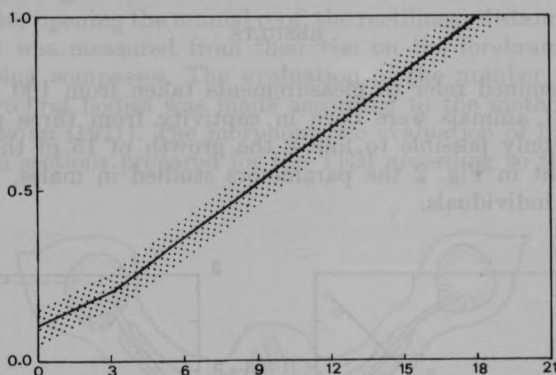

Fig. 3. — Relationship between the length of the olfactory pedicles (cm) (ordinate) and age (mounths) (abscissa).

In fact, by analyzing the data in pairs it is evident in all the groups studied that there is a linear relationship (Fig. 2 A) between the increase in body weight and the length of the olfactory pedicles. This linearity is less evident when comparing the length of the pedicles with the maximal transversal diameter of the body disc (Fig. 2 B), but the correlation coefficient was 0.905 in all the groups studied. In Fig. 2 C, we can make similar remarks, comparing the length of the olfactory pedicles to the total length of the animals.

The various parameters recorded from 90 days old animals compared with those of newborn specimens show an average increase of 1.68 %. Lipofuscin granules which represent a metabolic product correlated with cellular aging, give an indication of the age in animals (GLEES and HASAN, 1976). In the newborn animals and in individuals reared to 90 days of age we determined in the TEM sections the number of lipofuscin granules present in 100 μm^2 of cytoplasm, the area of lipofuscin granules and the percent of cytoplasmic surface covered by lipofuscin (WEIBEL et al., 1969) (Fig. 4 a, b, c). We noted an average increase of the same order.

Statistical analysis of our data has led us, in agreement with STERZI (1909), to the fact that the increase in length of the olfactory pedicles is a significant indicator of age.

We have also taken into consideration the formation of calcified rings in the vertebrae of the renal region, according to MELLINGER (1971). In a certain number of animals, with an average weight of 400 g (most frequently collected weight) (Fig. 5), we compared the relationship between the parameters studied and the number of rings and confirmed this correlation. That is, we have confirmed that measuring the length of the olfactory pedicles is a suitable method for evaluating the age of this species.
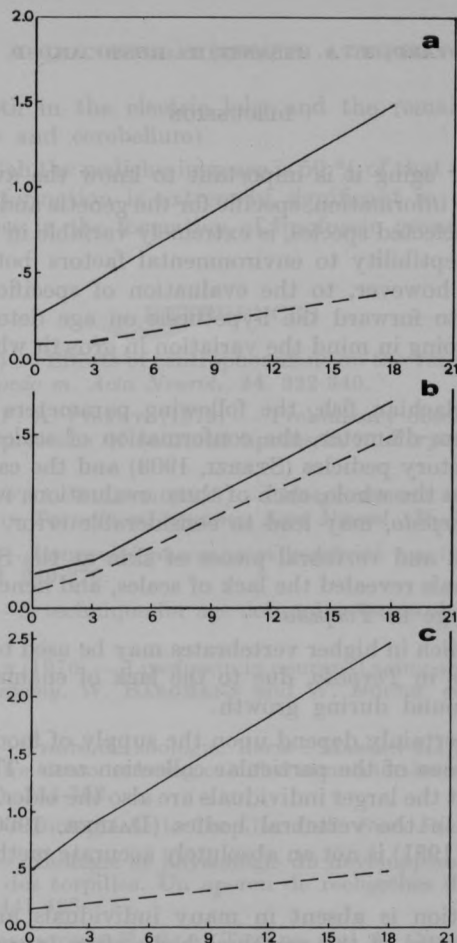
Fig. 4. — Relationship between age in mounths (ordinate) and (a) number of lipofuscin granules present in 100 μm² od cytoplasm, (b) total average area (μm²) of lipofuscin granules per animal, (c) percent of cytoplasmic area covered by lipofuscin (abscissa). ——— electric lobe; - - - - other encephalic parts (forebrain, optic lobe, cerebellum).
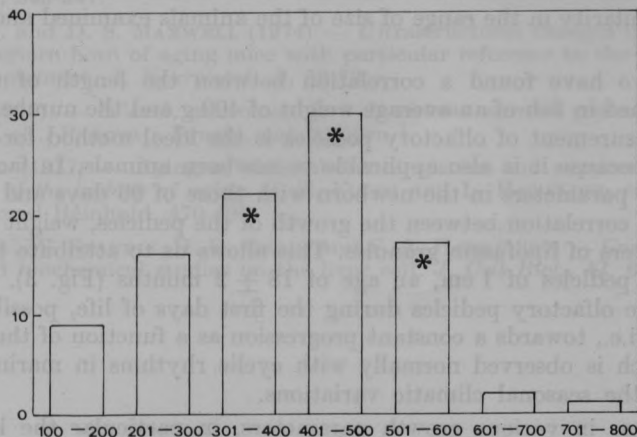

Fig. 5. — Percentage (ordinate) of weight classes in grams (abscissa) in the samples. * = one vertebral calcified ring present.

DISCUSSION

In order to study aging it is important to know the average lifespan of the species examined. This information, specific for the genetic and morpho-physiological characteristics of the selected species, is extremely variable in marine animals owing to their extreme susceptibility to environmental factors both autoecological and sinecological. Thanks however, to the evaluation of specific morphometric parameters it is possible to forward the hypothesis on age determination of animals casually collected, keeping in mind the variation in growth which may occur during the lifespan.

In teleost and selachian fish, the following parameters may be considered : body weight, maximum diameter, the conformation of scales and otoliths (BOUR-LIERE, 1946), the olfactory pedicles (STERZI, 1909) and the calcified vertebral rings (MELLINGER, 1971). On the whole, each of these evaluations when considered alone, also in the case of *Torpedo*, may lead to considerable error.

Analysis of dorsal and vertebral pieces of skin at the SEM in both newborn and one year old animals revealed the lack of scales, and hence these factors cannot be used to indicate age in *Torpedo*.

Also the teeth, which in higher vertebrates may be used to indicate age (TONNA, 1977), are of little use in *Torpedo*, due to the lack of enamel and of considerable variations in shape found during growth.

Size and weight certainly depend upon the supply of food and therefore on the environmental conditions of the particular collection zone. This therefore does not allow us to assume that the larger individuals are also the older. In addition, counting colcified rings found in the vertebral bodies (DAIBER, 1960 ; LA MARCA, 1966 ; MELLINGER, 1971 and 1981) is not an absolutely accurate method for age determination in *Torpedo*.

In fact, calcification is absent in many individuals and the stellate structure present in the centre of the vertebral body often impedes their observation. The individual rings often cannot be distinguished and are absent in young individuals. Probably for this reason, if not for the different environmental conditions which influence growth of animals taken at Arcachon (France) and Naples (Italy), we found differences between our results and those of MELLINGER (1971) ; although there was a similarity in the range of size of the animals examined and the number of rings found.

Instead, we have found a correlation between the length of the olfactory pedicles examined in fish of an average weight of 400 g and the number of vertebral rings. The measurement of olfactory pedicles is the ideal method for the determination of age because it is also applicable to newborn animals. In fact, comparing all the average parameters in the newborn with those of 90 days and 400 g individuals, we see a correlation between the growth of the pedicles, weight and morphometric parameters of lipofuscin granules. This allows us to attribute to individuals with olfactory pedicles of 1 cm, an age of 18 ± 2 months (Fig. 3). The increase in length of the olfactory pedicles during the first days of life, possibly goes to a normalization, i.e., towards a constant progression as a function of the deceleration of growth which is observed normally with cyclic rhythms in marine animals in opposition to the seasonal climatic variations.

The increase in various growth parameters, in particular the length of the olfactory pedicles, points to a correlation with some morphometric parameters of

lipofuscin studied both in the electric lobe and the remaining encephalic parts (forebrain, optic lobe and cerebellum).

The factor by which the pedicles increase is 50 % of that relative to the granules of lipofuscin. This information is extremely significant in order to consider also in *Torpedo* the increase in the formation of lipofuscin granules as an indicator of aging.

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