Some Approaches to Calibration of Age in Odontocetes Using Layered Hard Tissues

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ABSTRACT

Current methods in determining age of odontocetes rely upon untested assumptions. The relationship between layering and absolute age requires calibration. To achieve this goal, five approaches are suggested:

(1) the establishment of a reference collection for known-age, minimum known-age, and time-marked samples;

(2) the construction of developmental series of layered hard tissues representing individuals from various taxonomic and geographical stocks;

(3) the tagging and recovery of wild animals that have been time-marked;

(4) an extended program of multiple time-marking and extraction of teeth of live-captive animals; and

(5) the study of layered hard tissues in conjunction with clinical records of specimens treated with fluorescent antibiotics.

Pilot programs using these approaches are in various stages of implementation at the Southwest Fisheries Center, La Jolla.

INTRODUCTION

Recent studies of odontocete life histories have used counts of layers in teeth and in bones to estimate individual ages preparatory to constructing age-composition and growth models of dolphin populations. The models rely on the sweeping assumption that layers are deposited in hard tissues of odontocetes yearly in a manner analogous to the process of tree-ring formation.

Specifically, the working assumptions seem to be, first, that layering occurs at approximately constant rates; second, that yearly layers may be defined by convenient breaks that occur between dark and light zones in the tissue; and third, that the dark-light components of putative annual layers reflect physiological responses to seasonal change or to some other regular fluctuation in the external environment. Despite the 'soft' data used in interpreting layers in terms of age, it is also assumed that the results are sufficiently accurate for constructing meaningful life history models for various species of odontocetes.

Our present knowledge of the factors contributing to hard-tissue layering in odontocetes is insufficient to justify the use of the above assumptions as though they are proven hypotheses. The general physiology of cetaceans is poorly understood, and it is not known what causes layering in the hard parts of dolphins. Despite years of study, there exists little direct evidence that layering occurs at a constant or predictable rate, although it is true that a progressive increase in layers stands as some measure of increasing age. The few published studies that have used small samples of known-age teeth (Hui, 1978; Sergeant, Caldwell and Caldwell, 1973) or of single treatments to time-mark specimens (Best, 1976; Gurevich, Stewart and Cornell, this volume), have demonstrated only that the known elapsed time may be divided into a pattern of layers (i.e. that layers may be interpreted in such a manner as to obtain the age that is already known for the specimen). Results of a recent exercise reported by Kimura (this volume), involving experienced readers who counted dentinal layers of known-age specimens, underscore the problem that when the age is unknown, counts of number of layers are subjective and variable.

Although experience in defining growth layer groups (GLGs, terminology of workshop report, this volume) in teeth of specimens of known age is an important first step in the right direction toward calibration, such exercises are by no means a panacea for the problem of obtaining accurate age estimates. Nor should they seriously be regarded as more than a poor alternative to direct monitoring of layering rates in living animals. The exercises do not provide adequate support for inferring either that accumulation rates are constant, or, that 'annual' layers composed of dark and light subunits represent yearly records. Pulp-cavity occlusion, tooth wear, and poorly defined layering in dentinal, cemental, and periosteal tissues, as well as subjective interpretations of results using non-standardized techniques, may impair accurate reconstructions of life histories of even the most well known species.

This paper suggests various approaches that may be used to test the assumptions discussed above.

APPROACHES

Calibration of layered structure with absolute age (if indeed such is feasible) must be regarded as a goal of first priority and critical to accurate models of odontocete population dynamics. The appropriate tools are available for assessing the feasibility of real-time calibration, and opportunities now exist for applying these tools. The following approaches are suggested for assessment.

(1) The use of small numbers of specimens for which the layering period is known prevents confident conclusions about layering rates, but retrospective calibrations of large samples of such specimens could add measurably to this block of circumstantial evidence. A considerable amount of material exists from time-marked, e.g. tetracycline-injected, known-age (captive-born), and known minimum-age (wild-captured and held in captivity) animals representing a number of odontocete species, but at present it is held by a large number of individuals and institutions, some of whom do not fully realise its potential value in age/layer calibrations.

An international center (or centers) should be established as a permanent repository for all available time-marked, known-age, and known minimum-age specimens. The designated center would be charged with the responsibility of collecting and maintaining such specimens as a systematic reference library for use by all specialists engaged in age determination of odontocetes.

(2) A primary problem in calibration is the difficulty in defining countable units in layered tissue. Because hard-tissue layering patterns are complex and variable, it is important to understand to what extent differences in age, sex, and geographical stock influence such variability.

As available, teeth and samples of bone should be collected and prepared in thin section to construct ontogenetic series demonstrating developmental sequences of layer accumulation in individuals taken from discrete geographical and taxonomic stocks. The ontogenetic series would be ordered according to body length and sexual maturity and separated by sex. They should be curated centrally and be made available for study upon request.

(3) The possibility exists that layering rates and patterns among wild animals are different from those among animals maintained in captivity.

As economically and technologically feasible, experiments should be carried out to tag wild odontocetes *en masse* and time-mark their hard tissues by tetracycline injection. Eventual recovery of some animals through stranding salvage, incidental kill associated with commercial fisheries, or through other means would provide information on layering rates and patterns.

(4) Captive animals exist in numbers sufficient for comparative studies of layering rates and of factors that may influence layering in odontocetes.

Long-term studies should be carried out to time-mark and to periodically extract teeth of odontocetes maintained in captivity in order to monitor layering. Ideally, the experiments should be performed on reasonably large samples of dolphins representing both sexes and various age groups and should include controlled feeding exercises that would permit investigators to examine the possible effects changes in diet or in feeding frequency may have on layering. Experiments might be designed to alter water temperature, water salinity, or day length. Such experiments should require continuous observational records of any apparent changes in behavior, physiology, or proximate environment that may later be correlated with layering events. Eventually, results of experiments conducted near equatorial zones might be compared with results of experiments carried out on the same species in more temperate areas to examine the possibility that seasonality may alter layering patterns or rates.

(5) Many animals maintained in research and commercial oceanaria are treated routinely with medicines containing antibiotics, such as tetracycline, which combine with the hard tissues. When thin sections of layered tissue from these animals are examined microscopically under ultraviolet light, the markers fluoresce. By identifying the dates of the treatments, especially if the treatments were multiple, it is possible to calibrate the layered tissue with these fluorescent time envelopes, and thus, to calculate layering rates.

Veterinary records of marine mammals from commercial and scientific aquaria should be researched to identify preserved specimens that may have been incidentally time-marked, during medical treatment, with fluorescent or otherwise detectable drugs. If incidental marks are found to be multiple and incorporated into dentinal, cemental and periosteal layering systems, it may be possible to intercalibrate layers of all three systems with real time, allowing any system to be used alternatively. This would be valuable in cases of older specimens where dentinal deposition has ceased because of pulp cavity occlusion or where early periosteal bony layers have been resorbed.

PILOT PROGRAMS

Pilot programs with the above goals in mind are in various stages of implementation at the Southwest Fisheries Center in La Jolla.

(1) A literature search is underway to collect references to experiments or studies of known-age and time-marked specimens, or of captive animals for which time in captivity has been documented. Samples or specimen loans are being requested from individuals and institutions who are in charge of such specimens in order that we may prepare, examine and photograph them. Specimens that have been sequestered through donation will be organized into a permanent systematic collection. Photos and pertinent data for specimens in the collection and on loan to us will be maintained in a data file and eventually will be computerized. Requests for specimen donations or loans will be made also to acquaria and other institutions likely to have known-age or time-marked specimens in their charge.

(2) Sections of teeth and bone selected from early fetal through adult animals are being processed to construct comprehensive developmental series of males and females for *Tursiops truncatus, Delphinus delphis, Stenella attenuata* and *Stenella longirostris.* As adequate samples are accumulated, documented and catalogued, they will become available for study upon request.

(3) As part of school composition and cohesion experiments carried out on wild dolphins in the eastern tropical Pacific by NMFS scientists aboard the M/V *Queen Mary* chartered for dolphin/tuna research by the U.S. Tuna Foundation in September-October 1978, 331 offshore spotted dolphins, *S. attenuata,* were captured, measured, sexed, tagged, tetracycline-marked, and returned to the wild. Because this species is associated with the yellowfin tuna fishery, operations of which are being monitored by NMFS scientific technicians aboard many purse seiners, there is a good chance that at least some tagged specimens will be returned. Returns should enable us to compare hard tissue accumulation rates in wild dolphins with those in captive dolphins.

(4) A three-year project is being implemented, in cooperation with Hubbs/Sea World Research Institute, to monitor layering rates using tetracycline injections and tooth extraction. The study is using a large sample of healthy captive delphinids. In addition to developing data on layering rates, the study will attempt to identify such factors as age, sex, diet, handling and treatment, seasonal fluctuations in water temperature, and intergeneric vari-

ability, that may influence or alter layering patterns. The project will use a minimum of 12 specimens of *Tursiops truncatus*, representing approximately equal numbers of males and females and including animals of various relative ages. Animals of other species will also be used to develop data on delphinids in general.

Teeth will be prepared and examined under ultraviolet light to locate fluorescent marks, which will be used to calibrate growth layer groups in stained and unstained specimens.

(5) When microscopically examined under reflected ultraviolet light, thin-sectioned teeth from several captive specimens have revealed multiple fluorescent marks within their dentinal layers. Efforts are being made to obtain veterinary data for these specimens in an attempt to identify treatment dates, dosages, and the kinds of antibiotics used to treat these animals. In the absence of these data, I have identified growth layer groups to estimate the real time represented as bracketed by the fluorescent markers. This was done as a test of the assumption that growth layers are accumulated at a constant rate. Veterinary data, when obtained, will be used to test the estimates of time intervals represented between the markers.

CONCLUSION

The approaches and pilot programs outlined above offer means by which the long standing assumptions in age determination from layered tissues may be tested extensively. The progress made along these lines will depend upon the cooperation of members of the scientific community and of other persons in charge of animals, preserved skeletal materials, and medical records from commercial aquaria. Only through such cooperative efforts may we expect to address the many other difficult questions of dolphin life histories with some assurance.

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