

Whale Poaching Detection Based on Microscopic Characteristics of Bottlenose Dolphins' (*Tursiops truncatus*) Bone Fragments

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Abstract

Although many countries prohibit whaling, it remains a significant cause of whale population decline. Whale meal, likely from whales killed during illegal whaling or caught accidentally, can appear in a fish meal as a contaminant detectable by microscopic examination of bone fragments. To provide a rigorous basis for such a detection, microscopic characterization of bone fragments of 10 female and 10 male, less than a year old to 21-year-old bottlenose dolphins (*Tursiops truncatus*), a widespread cetacean species herein used as a whale model, was performed and compared to the reference bone fragments of fish, ruminants, poultry, and pigs. The processing of bones mimicked the process used in the production of meat and bone meals, while their description was based on qualitative characteristics (i.e., the shape of a bone fragment; the shape, density, and distribution of osteocyte lacunae; and the distribution and density of canaliculae). Bottlenose dolphin bone fragments are smoothly contoured. Their elliptical osteocyte lacunae are clearly visible, while the canaliculae radiate from the lacunae in all directions. The comparison of these qualitative bone characteristics with that of other vertebrata under study revealed that bottlenose dolphin bone fragments can definitely be differentiated from that of fish, may be differentiated from that of poultry and pig, but cannot be differentiated from that of ruminants. Measurements of the osteocyte lacunae showed the lacunar length to be strongly associated with an animal's age and lacunar shape, while their width was strongly associated with an animal's gender and lacunar shape. The results indicate the possibility of detecting a whale meal admixture in a fish meal using light microscopy, which should be followed by PCR to allow for the identification of the admixture source.

Key Words: whale poaching, bottlenose dolphin, *Tursiops truncatus*, osteocyte lacunae, fish meal, whale meal, canaliculae

Introduction

Whaling has significantly contributed to the decline of cetacean populations (Hoyt, 2005), especially when it comes to sei (*Balaenoptera borealis*) and fin (*Balaenoptera physalus*) whales (International Union for Conservation of Nature and Natural Resources [IUCN], 2018), which, together with minke (*Balaenoptera acutorostrata* and *Balaenoptera bonaerensis*), grey (*Eschrichtius robustus*), and blue (*Balaenoptera musculus*) whales represent the whale species currently most commonly exploited for commercial purposes (Thomas et al., 2016; International Whaling Commission [IWC], 2018). Whales are a protected animal species under many national and international laws (e.g., IWC, 1946; Marine Mammal Commission [MMC], 1972; Republic of Croatia, 2013), yet an illegal trade with, and the illegal use of, whale remains pose a global problem (Baker & Palumbi, 1994; Baker et al., 2010). With the intention of stopping commercial and scientific whaling as a source of black market products primarily dealt with in Japan and South Korea, Baker & Palumbi (1994) initiated the Molecular Monitoring Programme to identify whale species circulating on these two major black markets. The authors developed the molecular whale identification protocol that made use of Polymerase Chain Reaction (PCR) (Baker et al., 2000, 2010). Shinoda et al. (2009) developed primers for the detection of heat-treated cetacean parts in porcine meat and bone meals.

Fish meal, as a type of meat and bone meal, is often added to animal feed to boost growth and

productivity (Hendriks et al., 2002). According to the European Union (EU) (2002) regulation, meat and bone meals are classified as animal byproducts not intended for human consumption since they usually originate from slaughterhouse waste and animal carcasses (e.g., pigs, poultry, ruminants, and fish) that are thermally treated and then ground. However, fish meal can be contaminated with the remains of farm animals, sea mammals (e.g., seals, manatees [family Trichechidae], and sea otters [*Enhydra lutris*]), and sea birds (Fumière et al., 2009; Shinoda et al., 2009; Boncheva, 2011; van Raamsdonk et al., 2012). It can also be contaminated with whale meal, presumably due to illegal whaling or whale catch byproducts (Shinoda et al., 2009). Fish meal contaminated with whale meat and/or meat and bone meal coming from farm animals can cause health problems in feeding animals. For example, whale tissues usually have high mercury and methyl mercury concentrations, and animals fed on fish meal contaminated with whale meal can suffer from neurological disorders (Fielding & Evans, 2014; Krey et al., 2015; Squadrone et al., 2015).

In 1980, a significant amount of evidence revealed the connection between the incidence of bovine spongiform encephalopathy (BSE) and animal feeding on prion-infected meat and bone meals originating from farm animals (Bradley, 1994), which can also contaminate a fish meal. In response to the above situation, the EU (2011) banned the use of meat and bone meal originating from farm animals for animal feeding purposes, and developed and introduced a microscopic method for the detection of meat and bone meal in feed (EU, 1998, 2013; van Raamsdonk et al., 2007) to be able to fight against the spread of the disease across the EU member states. This ban led to the significant decrease in BSE incidence (Budka et al., 2008) but also to the increase in representation of fish meal in feedstuffs. Since 2013, poultry meat, pig meat, and bone meal have been allowed to be present in fish feed used in the EU (2013) so that nowadays mixing poultry meat and bone meal with fish meal is a common practice (Hernández et al., 2014).

The microscopic method developed by the EU is based on subjective impressions on the shape of the analysed bone fragments; the shape, density, and distribution of the osteocyte lacunae; and the orientation and density of the canaliculae. Osteocyte lacunae are small cavities within the bone matrix containing an osteocyte from which tiny canaliculae radiate and penetrate into the adjacent lamellae to anastomose with the canaliculae of the neighbouring lacunae, thus forming a system of cavities interconnected by minute

canals. The morphotype of a lacuna depends on its location in the bone tissue, the type of bone (cortical or cancellous bone), and the type of lamellae (osteon or interstitial) (Cadena & Schweitzer, 2012).

Microscopic analysis of feed is subject to human error. Its accuracy strongly depends on the experience of an analyst, while the estimations of dimensions and densities are approximately rather than precisely determined (Pinotti et al., 2004). Nevertheless, the method is simple, inexpensive, and reliable as long as it is entrusted with an experienced analyst (the limit of detection below 0.1% w/w).

Numerous investigators have produced some reliable visual markers of bone fragments of certain fish, poultry, and terrestrial mammalian species (Pinotti et al., 2004; Fumière et al., 2009; Shinoda et al., 2010, 2011; van Raamsdonk et al., 2012). A qualitative and quantitative visual analysis of the tissue microstructure in the ground bone sections can allow for the differentiation of the source (Martiniaková et al., 2007; Zedda et al., 2008; Cao et al., 2011). For example, avian and mammalian osteocyte lacunae are usually differentiated based on canalicular arrangement. In birds, canaliculae are uniformly spread throughout the bone and are seen as extensively branching channels diverging at large angles from the parental canaliculus. In contrast, in terrestrial mammals, the canaliculae are oriented radially, with branches running almost in parallel with the parental canaliculae (Rensberger & Watabe, 2000). However, morphological analyses that may work well when ground bone sections are targeted are often ineffective should milled bone fragments be analysed as is the case with meat and bone meals since, in the latter case, the bones are milled in different directions, not just one as with grinding.

To date, this is the first study describing the morphology of bottlenose dolphin (*Tursiops truncatus*) bone fragments present in fish meal. The methodology of this study and thereafter the measurements of the lacunae may facilitate the detection of cetacean bone fragments in fish meal and could be used to alert the Marine Mammal Commission to the possibility of illegal whaling activities. Our aim is to describe the microscopic characteristics of bone fragments of a globally spread cetacean (the bottlenose dolphin) present in simulated fish meal and to provide a comparison with the reference bone fragments of fish, pigs, ruminants, and poultry. We had the humeri of bottlenose dolphins of various ages and both genders at our disposal; thus, we were able to establish age- and gender-based differences in size and shape of the osteocyte lacunae.

Methods

To establish whether bottlenose dolphin bone fragments can be used as a suitable and representative whale model, mixed parts of the scapula, skull, vertebra, ribs, and humeri of other cetaceans were analysed to look for any qualitative differences in osteocyte lacunae within and between the cetacean suborders. The cetacean species examined included the Risso's dolphin (*Grampus griseus*) and striped dolphin (*Stenella coeruleoalba*), falling into the odontocetes suborder (to which the bottlenose dolphin belongs), and fin whale, falling into the mysticetes suborder. These bones were retrieved from our archival bone collection, processed, and qualitatively analysed in the same manner as the bottlenose dolphin bone samples.

Bottlenose Dolphin Bone Processing

For the purpose of this study, the right humerus was retrieved from 10 female and 10 male bottlenose dolphins aged from under 1 y old to 21 y old and divided into five age groups: 1st age group: under 1 y; 2nd age group: 1 to 3 y; 3rd age group: 4 to 7 y; 4th age group: 8 to 14 y; and 5th age group: over 14 y. Each age group included two animals of each gender. Age estimation was based on the counts of dentinal growth layer groups (GLGs) found in teeth sections (Hohn et al., 1989; Slooten, 1991). According to this method, it is assumed that one GLG found in the dentine corresponds to a calendar year. The age groups were established based on the growth curve published for bottlenose dolphins inhabiting the Adriatic Sea (Đuras et al., 2016). All of the dolphins under study had been found dead in the Croatian part of the Adriatic Sea between 1990 and 2011, and had undergone *postmortem* examinations according to the annual permits issued by the Croatian authorities. The skeletons had been archived in the Department of Anatomy, Histology, and Embryology of the Faculty of Veterinary Medicine at the University of Zagreb, Croatia, together with the necropsy reports. To prepare the skeletons for storage, the soft tissue covering the humerus and other pectoral flipper bones were removed using a sharp knife, while the tissue between the bones was left intact to preserve the structure and the form of the flipper as a whole. The processed skeletons were stored in separate boxes until examined for this study.

Each humerus was cut at its neck (*collum humeri*) using an Ultimate 500 saw (NSK, Gunma, Japan) to obtain a triangular transversal 0.5-cm-thick sample composed of the compact and the cancellous bone tissue. Bone samples were then processed to replicate the manner in which meat and bone meals are treated as described under European Regulation 1774/2002/EC (EU, 2002).

In brief, the bones were heated to the core temperature of more than 133°C for at least 20 min under the (absolute) pressure of at least 3 bars produced by saturated steam. Bone samples were then crushed in a mortar to the particle size of < 0.5 mm, bleached in sodium hypochlorite for 10 min, and air-dried. A few bone particles were mixed with Norland Optical Adhesive 65 (Norland Products, Cranbury, NJ, USA), dripped onto a microscope slide, and covered. The slides were irradiated for 10 min with a UV light (320 to 400 nm) to create a sealed system (EU, 2013).

Characterization of Bone Fragments

We would like to reemphasise that microscopy is primarily a qualitative, analyst's impressions-based method. In the study by van Raamsdonk et al. (2012), the shape of the bone fragments was described and recorded, together with the analyst's impression on density (defined as low, medium, and high), dispersion (even or uneven), and shape of the osteocyte lacunae (elliptical, elongated elliptical, and/or roundish), as well as the impressions on orientation (mutually parallel or in all directions), density (sporadic or dense), and length of the canaliculae (short or long) (Figure 1). Furthermore, the impression of lacunar density and prevalence of different lacunar shapes depends on the image planes. By manipulating a microscope's focus adjuster to achieve a sharp picture, different bone layers can be observed and actually analysed since the thickness of the bone is not uniform.

Bone particles were analysed and photographed under 100 and 200× magnification using a Zeiss Axio Imager M2 microscope (Zeiss, Oberkochen, Germany). Morphometric measurements were taken from the photographs using Zeiss Axio Vision software.

Comparison with Other Species

Bone fragments of the bottlenose dolphin and the reference fish (herring, family Clupeidae; and cod, family Gadidae), poultry (order Galliformes), pig (*Sus scrofa*), and ruminant (suborder Ruminantia) bone material obtained within the frame of the proficiency testing and training procedure organized by the European Union Reference Laboratory for Animal Proteins in Feedstuffs (Walloon Agricultural Research Centre, Gembloux, Belgium) were first visualised under a microscope (each sort of bone fragment individually) to get a general impression on the type and distribution of the lacunae (100× magnification).

To determine whether it would be possible to distinguish whale from fish bone fragments, the above two fragments were mixed to obtain a sample that simulates a fish meal contaminated with whale meal.

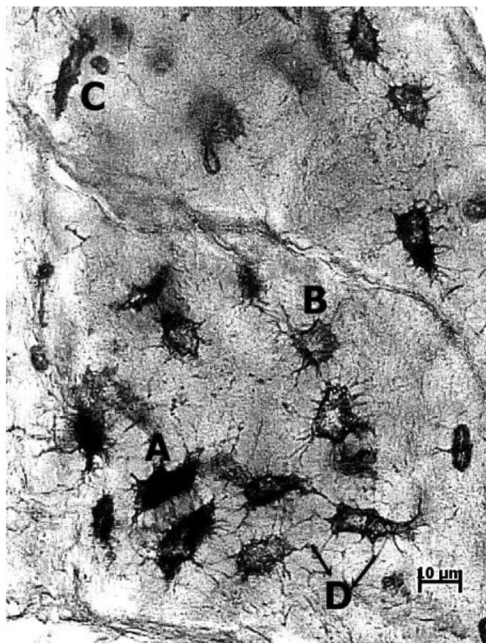


Figure 1. Representative photomicrographs of milled bottlenose dolphin (*Tursiops truncatus*) bone fragments showing (A) elliptical, (B) roundish, and (C) elongated elliptical osteocyte lacunae, as well as (D) lacunar canaliculae. A female specimen, 4th age group, bar 10 µm.

Mixed bone fragments were mounted onto Norland 65, dispersed, visualised, and analysed.

Analysis of Animal Age- and Sex-Specific Quantitative Characteristics

At the magnification of 200 $\times$, only clearly visible lacunae of the bottlenose dolphin bone fragments were chosen to be measured. Each type of the lacunae (i.e., elliptical, elongated elliptical, and roundish) was measured in five independent samples of each of the 20 animals. The length and the width of the lacunae were recorded (Figure 2). Irregularly shaped lacunae were measured at their widest point in a straight line. Given that the same quantitative data descriptive of fish, poultry, and terrestrial mammals' osteocyte lacunae already exist (Animal Remains Identification and Evaluation System [ARIES], 2004), measurements of these lacunae were not performed.

Quantitative data on the length and the weight of bottlenose dolphins were statistically processed using the IT programme *Stata 10.0* (StataCorp LLC, College Station, TX, USA). After testing for the normality of distribution (Kolmogorov-Smirnov test), the results were analysed using the analysis of variance (ANOVA), while the differences between animal groups were processed

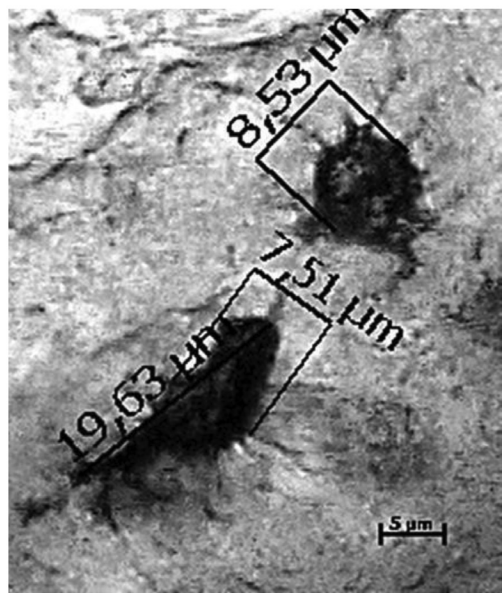


Figure 2. Osteocyte lacuna length and width measured in milled bottlenose dolphin bone fragments using light microscopy. A male specimen, 3rd age group, bar 5 µm.

using the post-hoc Tukey's Honestly Significant Difference Test in which $p < 0.05$ was accepted to indicate a statistically significant difference.

Results

Qualitative Characteristics

When comparing the osteocyte lacunae of bottlenose dolphin with those of Risso's dolphin, striped dolphin, and fin whale, no major noticeable qualitative differences in shape, density, and distribution of the lacunae and canaliculae of the analysed bone fragments were noticed (Figure 3A-3D).

The following descriptors came as a result of the comparison between the bottlenose dolphin bone fragments and the reference bone fragments coming from fish, poultry, pig, and ruminants (Figure 4). In agreement with the descriptor-related recommendations given by van Raamsdonk et al. (2012), bottlenose dolphin bone fragments had a smooth outline. The osteocyte lacunae were evenly dispersed and showed a moderately dense distribution (Figure 1) as compared to their dispersion in pig and poultry reference bone fragments. No significant visual differences were observed between bone particles retrieved from bottlenose dolphins of different sex or age. The osteocyte lacunae were elliptical, elongated elliptical, or roundish in shape, with the elliptical shape as the most frequent. Several

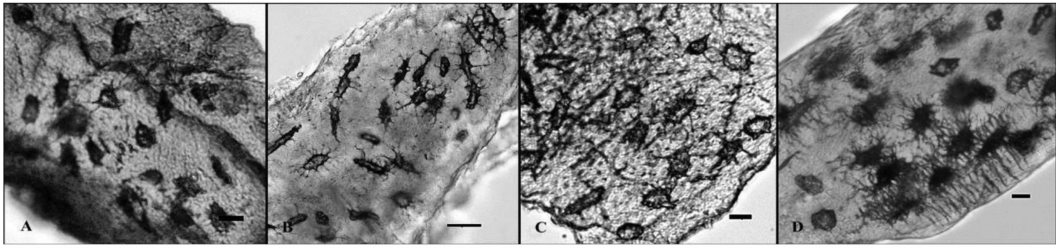


Figure 3. Reference photomicrographs of milled bone fragments of (A) Risso's dolphin (*Grampus griseus*), (B) fin whale (*Balaenoptera physalus*), (C) striped dolphin (*Stenella coeruleoalba*), and (D) bottlenose dolphin compared using light microscopy. Archival collection; bar 10 μ m.

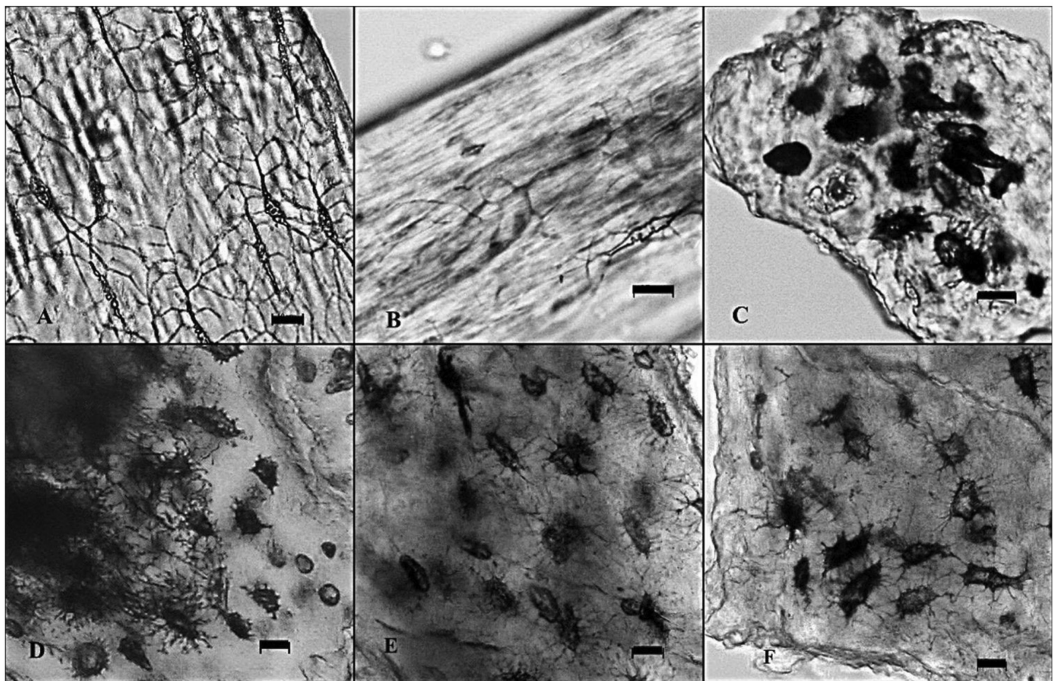


Figure 4. Reference photomicrographs of milled bone fragments of (A) herring (family Clupeidae), (B) cod (family Gadidae), (C) poultry (order Galliformes), (D) pigs (*Sus scrofa*), and (E) ruminants (suborder Ruminantia), as well as representative photomicrographs of (F) milled bottlenose dolphin bone fragments compared using light microscopy. Reference bone fragments were obtained from the European Union Reference Laboratory for Animal Proteins in Feed Stuffs. Bar 10 μ m.

differences were found when the bottlenose dolphins' bone fragments were compared to the reference bone fragments in terms of density and shape of the osteocyte lacunae, as well as visibility and arrangement of the canaliculae (Figure 4). In bottlenose dolphins, the canaliculae of the osteocyte lacunae were clearly visible and relatively long as compared to the canaliculae seen in pig, poultry, and cod bones. They radiated in all directions and showed a moderately dense distribution (Figure 1) as opposed to the canaliculae seen in pig and poultry bones.

Bottlenose dolphin bone fragments were easily distinguishable from those of herring (Figure 4A) and cod (Figure 4B) because in the latter two, the osteocyte lacunae were much more elongated, with longer and more branched canaliculae. The osteocyte lacunae were denser in poultry (Figure 4C) and pigs (Figure 4D) than in bottlenose dolphins, sometimes appearing in the form of a black area. Pig osteocyte lacunae were generally round-shaped and had short, thick canaliculae. Ruminant bone fragments were most similar to those of the bottlenose dolphins, both showing

mostly elliptically shaped, low- to medium-dense lacunae and long and thin canaliculae (Figure 4E & 4F).

Quantitative Characteristics

Morphometric analysis of the osteocyte lacunae of bottlenose dolphins showed their length to range from 5.08 to 33.33 μm , and their width to span from 1.44 to 14.56 μm , with the means of 15.68 and 6.29 μm , respectively. The median length was 15.10 μm , while the median width was 6.00 μm (Table 1). As for each type of lacunae, the mean length of elongated elliptical lacunae was 21.93 μm ; for elliptical lacunae, it was 16.09 μm ; and for roundish lacunae, it was 8.97 μm . The mean width of the above lacunar types (in the same order of appearance) was 4.13, 6.38, and 8.24 μm (Table 1).

Animal Age- and Gender-Based Differences

Length and width of the lacunae established in bone fragments of bottlenose dolphins differed significantly across the animals of different ages and genders, and also varied with the lacunar shape ($F = 2.250$, $p = 0.024$). Lacunar width was proven to be dependent on animal age and lacunar

shape in a statistically significant manner ($F = 3.823$, $p = 0.023$) (Table 2).

Statistically significant differences in the lacunae length that were dependent on animal age and lacunar shape (Figure 5) were established for elongated elliptical lacunae in female dolphins of different ages but not in male dolphins (Figure 6). Similarly, significant differences in width of elongated elliptical and elliptical lacunae were seen in males but not in female animals of different ages.

Data displayed in Figure 5 showed significant differences in length of differently shaped lacunae observed in male and female bottlenose dolphins of all ages. In all age groups and in both genders, elongated elliptical lacunae were proven to be the longest, and roundish lacunae were proven to be the shortest.

Statistical differences were also observed in the width of differently shaped lacunae of female animals of different ages, with roundish lacunae proving to be the widest and elongated elliptical lacunae proving to be the narrowest. An age-specific pattern of an increase or decrease in lacunae length failed to be established in either male or female bottlenose dolphins; however, elongated

Table 1. Quantitative descriptors of various types of osteocyte lacunae found in bottlenose dolphin bones

	Length (μm)					
	<i>n</i>	Mean	SE	Median	Minimal	Maximal
Elongated elliptical	100	21.93	0.46	21.57	11.65	33.33
Elliptical	100	16.09	0.33	15.99	9.70	26.62
Roundish	100	8.97	0.25	8.74	5.08	14.15
Overall length	300	15.68	0.64	15.10	5.08	33.33
	Width (μm)					
	<i>n</i>	Mean	SE	Median	Minimal	Maximal
Elongated elliptical	100	4.13	0.11	4.08	1.44	6.59
Elliptical	100	6.38	0.16	6.07	3.48	9.11
Roundish	100	8.24	0.22	8.19	4.02	14.56
Overall width	300	6.29	0.24	6.00	1.44	14.56

SE = standard error

Table 2. Statistical strength of association between the total lacunae length and width, and animal gender, animal age, and lacunar shape (ANOVA)

	Length ($n = 300$)		Width ($n = 300$)	
	F-ratio	<i>p</i> value	F-ratio	<i>p</i> value
Gender*age	1.179	0.320	0.730	0.572
Gender*shape	2.040	0.132	3.823	0.023*
Age*shape	2.250	0.024*	1.809	0.075
Gender*age*shape	1.055	0.395	1.133	0.341

*Statistically significant

elliptical lacunae found in the bones of female animals were proven to be longer than those found in the bones of male dolphins (Figures 5 & 6). Furthermore, the length of elongated elliptical

lacunae of female animals differed significantly between the animals of various age groups and reached its maximum in the 3rd age group. As for male animals, the width of elongated elliptical

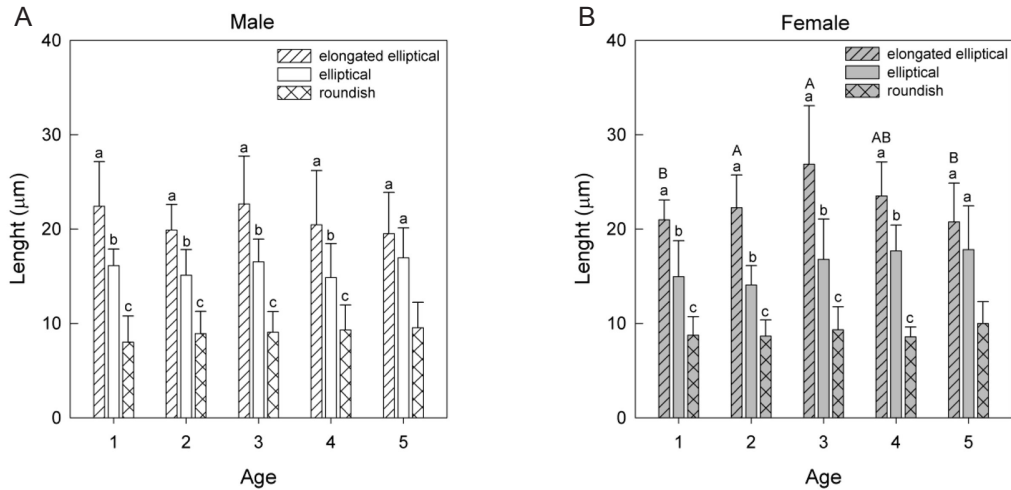


Figure 5. The average length of certain types of lacunae found in (A) male and (B) female specimens, and the statistical strength of its association with age and shape of the bottlenose dolphin. The data are represented by the means of independent analyses and standard deviations (SDs). For each lacunar type, 10 analyses were run in each age group, amounting to a total of 30 analyses per age group. A different letter within the same age group tags statistical significance ($p < 0.05$), and a different capital letter within the lacunar group tags statistical significance ($p < 0.05$).

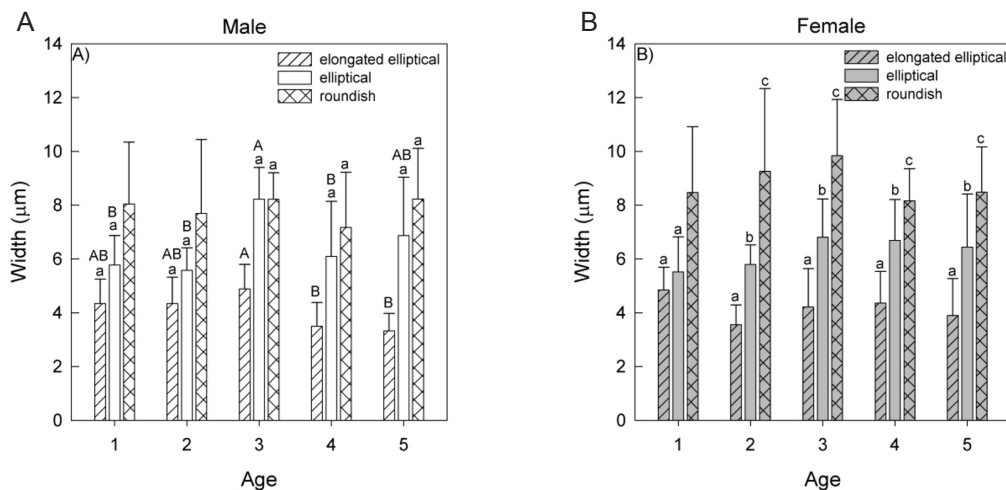


Figure 6. The average width of certain types of lacunae established in (A) male and (B) female specimens, and the statistical strength of its association with shape and gender of the bottlenose dolphin. The data are represented by the means of independent analyses and SDs. For each lacunar type, 10 analyses were run in each age group, amounting to a total of 30 analyses per age group. A different letter within the same age group tags statistical significance ($p < 0.05$), and a different capital letter within the lacunar group tags statistical significance ($p < 0.05$).

and elliptical lacunae significantly differed in animals of different ages, also reaching its peak in the 3rd age group.

Discussion

This study provides the first insight into the microscopic characteristics of bottlenose dolphin bone fragments milled in the same manner as animal bones intended for fish meal to mimic the appearance of osteocyte lacunae in dolphin bone fragments when present in such meals as contaminants. The study also presents the first measurements of length and width of these lacunae. No apparent qualitative differences in shape, density, and distribution of the lacunae and canaliculae were observed between bottlenose dolphin and Risso's dolphin, striped dolphin (falling into the same cetacean suborder), and fin whale (belonging to a different cetacean suborder); therefore, it is safe to assume that the bottlenose dolphin can be used as a whale model when the microscopic method described herein is in use. An experienced analyst can easily distinguish whale bone fragments from those of fish families. If a fish meal is contaminated with poultry, pig, or ruminant meat and bone meal, this task becomes much more challenging; however, we were able to visually distinguish whale bone fragments from those of poultry or pigs.

Poultry and pig bone fragments have sharp contours (van Raamsdonk et al., 2012), while cetacean bone fragments have smooth contours and are globular, similar to ruminant bone fragments (Gizzi et al., 2003). In contrast to uniformly distributed canaliculae in bottlenose dolphin bones, the canaliculae seen in poultry and pig bones are difficult or impossible to detect using the described microscopic method (Gizzi et al., 2003; Domenis et al., 2009; van Raamsdonk et al., 2012). Our results indicate that one can visually differentiate between whale and poultry or pig bone particles present in fish meal based on subjectively assessed distribution and density of the osteocyte lacunae, the lacunar density thereby being much higher in porcine and poultry bone fragments than in the bottlenose dolphin bone fragments (van Raamsdonk et al., 2012). Additionally, poor visibility of the canaliculae and a large lacunar surface area present in pig and poultry bone fragments as compared to bottlenose dolphin bone fragments can be useful in mutual distinction as well. However, differentiation between bottlenose dolphin and ruminant bone fragments that relies solely on qualitative descriptors may be challenging because of the similarity in lacunar shape, canalicular distribution, and lacunar density.

Quantitative results of this study demonstrated the average length of the osteocyte lacunae of

bottlenose dolphin (15.68 μm) to be within the range observed for fish, poultry, and terrestrial mammals (10 to 30 μm , 10 to 15 μm , and 10 to 20 μm , respectively). The average width of the osteocyte lacunae established in bottlenose dolphins (6.29 μm) was lower than in terrestrial mammals (8 to 15 μm), in the range observed for poultry (5 to 12 μm), and higher than in fish (1 to 3 μm) (ARIES, 2004).

No data have been found in the available literature concerning the association between the lacunar length and width on one hand and animal age, gender, and lacunar shape on the other. On top of that, to the best of our knowledge, no mixed-effect tests have been performed using general linear models. Our study findings show that in female dolphins, lacunar length is statistically significantly associated with gender and age, while the width of the lacunae was proven statistically significantly associated with animal gender and lacunar shape in male animals. Nevertheless, no specific animal age-related lacunar pattern was observed. It is to be expected that bone tissue changes during an animal's lifetime. In young specimens, the bone tissue is woven and less compact; while in older animals, the bone tissue is lamellar and more compact (Currey, 2002) so that the lacunae are expected to be more compressed and more elongated. Also, we presume that the significant differences in lacunar width dependent on animal age and gender seen in male animals are a consequence of bone development, bearing in mind the findings of Mulhern (2000) that female bone tissue has a slightly different developmental dynamic as compared to male animals, especially when it comes to the density of the osteocytes. To allow for a detailed comparison of bottlenose dolphin osteocyte lacunae with the osteocyte lacunae of poultry, pigs, and ruminants in any future studies, the lacunar surface area should be measured, and the number of canaliculae per bottlenose dolphin osteocyte lacuna should be determined (e.g., Pinotti et al., 2004).

The described microscopic method and the characterisation of bone fragments could be used in the fish meal industry or in feed factories as a screening method employed to check for undesirable admixtures, whale bones included. The method described herein is simple, inexpensive, and reliable, and it could help identify the class of the animal(s) responsible for contamination. If a fish meal screening that made use of the microscopic method discussed herein indicated the possibility of contamination of animal origin, PCR, a more expensive but also a more sophisticated technique, can be used to confirm or identify the contaminating species (Fumière et al., 2006; Shinoda et al., 2009). The findings of our study suggest that it is

possible, provided that trained and experienced analysts are engaged, to detect whale contamination in fish meal and pinpoint the illegal whale trade sites and activities.

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