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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Computed Tomography of the Mandibles of a Stranded Offshore Killer Whale (*Orcinus orca*)

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Summary

A mature, adult female, offshore killer whale (*Orcinus orca*) was stranded deceased in Portage Bay, Alaska, in October 2015. Full necropsy examination with histopathology was performed. Consistent with previous studies of offshore killer whales, and thought to be a result of their unique elasmobranch diet, all the teeth were significantly abraded and almost flush with the gingival margin. Age was estimated at 30–35 years based on annuli and growth arrest lines in a remaining tooth. The dentate portion of the mandibles were excised *en bloc* and frozen until imaging could be completed. Radiography and computed tomography revealed lesions consistent with severe abrasion, pulp exposure and evidence of endodontic and/or periodontal disease in nine of the 15 mandibular teeth present (60.0%). Only five (33.3%) teeth were suspected to have been vital at the time of death based on imaging. Lesions were more severe rostrally, with the caudal teeth less affected. Autolysis precluded gingival histopathology and no teeth were analyzed histologically. Necropsy examination revealed a likely multifactorial cause of death, with most significant lesions including the severe chronic periodontal/endodontic disease with abrasion, inanition and emaciation with possible cardiovascular disease. This case highlights the importance of imaging in evaluating periodontal and endodontic status, especially *post mortem* when other tissues are no longer available, and demonstrates that periodontal and endodontic disease occur naturally in this species and can be a significant cause of morbidity in mature free-ranging killer whales of the offshore ecotype.

Keywords: killer whale; offshore ecotype; *Orcinus orca*; periodontal disease

Killer whales (*Orcinus orca*) are the largest member of Delphinidae and are distributed throughout the world's oceans (Reeves *et al.*, 2002; Ford and Ellis, 2014). While currently considered one species, it is widely understood that there are three ecotypes of killer whales in the northeast Pacific Ocean: resident, transient and offshore (Morin *et al.*, 2010; Ford and Ellis, 2014). All three groups are genetically, behaviourally and morphologically distinct, and do not interbreed (Ford *et al.*, 2011). Resident killer whales live in large groups and predate fish, mainly salmonids (Herman *et al.*, 2005; Krahn *et al.*, 2007). Transient killer whales live in smaller groups, travel over larger distances and primarily consume marine mammals (Herman *et al.*, 2005; Krahn *et al.*, 2007). Offshore killer whales, the most elusive and thus least studied, travel the longest distances and eat fish, particularly elasmobranchs (Herman *et al.*, 2005; Dalheim *et al.*, 2008; Ford *et al.*, 2011). Individual identification of offshore

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killer whales is possible, but sightings and strandings occur less frequently than with the other two ecotypes, making any opportunity to examine them of high value.

Dental anatomy and disease in free-ranging killer whales have been described, although gross examination and imaging is only possible with post-mortem specimens. One study on dental pathology in post-mortem delphinid specimens found that all three killer whales examined had evidence of superficial dentine erosion (Loch *et al.*, 2011). Ford *et al.* (2011) described older resident and transient individuals as having significant wear on mesial and distal surfaces of crowns, but overall wear to the tooth crown was graded as negligible to moderate. In comparison, most of the offshore specimens displayed tooth wear that was categorized as extreme, with pulp exposure in many of the rostral teeth (Ford *et al.*, 2011). This contrast is posited to be due largely to the difference in diet between the ecotypes, as elasmobranchs have placoid scales that may abrade whale teeth over time (Ford *et al.*, 2011). Although gross examination is available for these cases, advanced imaging has not been used to further characterize dental pathology.

In October 2015, a mature, adult female offshore killer whale was found deceased on a beach in Portage Bay, Alaska (570′26.46″ N/13320′10.19″ W), and a full necropsy examination with histopathology was performed. Results specific to the animal's dental pathology are described in this manuscript. Via morphological characteristics, the animal was identified as O059, an offshore killer whale first identified in the early 1990s, making her age estimate at the time of death to be at least 21 years. One of the teeth, which was grossly most intact, was removed for ageing and sampling. The tooth was sectioned longitudinally and acid-etched for an estimate of age-at-death in accordance with previously described methods (Pierce and Kajmura, 1980). The dentate portions of the mandibles were excised and frozen until imaging could be completed. Two-dimensional radiography (SoundSmart DR unit with a 1717G plate, Sound, Carlsbad, California, USA) and computed tomography (CT) (General Electric HiSpeed CT, Waukesha, Wisconsin, USA) using standard and bone technique with 3 mm slice thickness were performed. Killer whales are homodonts, meaning their teeth do not vary in shape; so teeth were labelled by side (L, left mandible; R, right mandible) and numbered 1 to 12, from rostral to caudal (Fig. 1). Empty alveoli indicated missing teeth and were described based on the level of remodelling of the alveolar bony socket (Figs. 2 and 3). There is a paucity of diagnostic imaging on normal specimens to serve as baseline comparison; however, the results from O059 were compared with CT images (standard and bone technique, 3 mm slices) available from an immature 3-year-old free-ranging deceased resident killer whale. After imaging was completed, the mandibles and teeth were defleshed in a dermestid beetle (*Dermestes maculatus*) colony and were accessioned at the Burke Museum

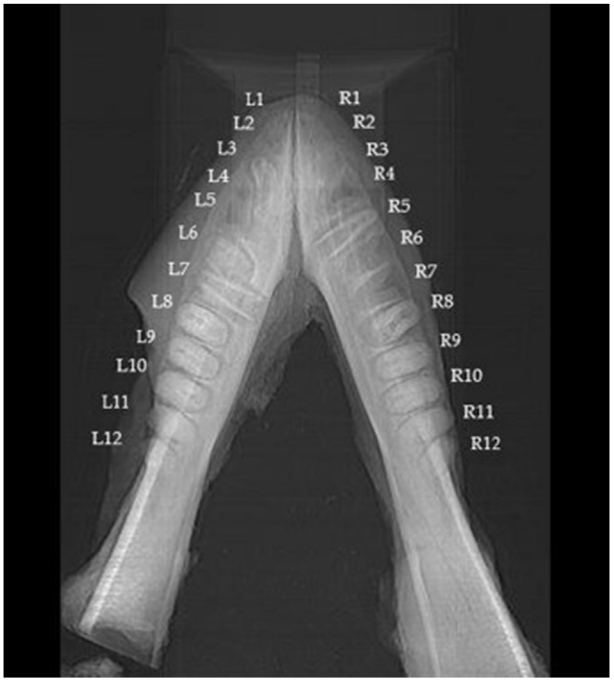

Fig. 1. Labelling of teeth and empty alveoli was established from this CT scan of the left and right mandibles. Failure to narrow of the pulp cavity in teeth L3, L6, L7, R5 and R8 indicates tooth death at an immature age. Relatively narrower pulp cavities in teeth L10 and R10 indicates tooth death at a more mature age. Viable teeth with subjectively adequate narrowing of the pulp cavity include L1, L11, L12, R11 and R12.

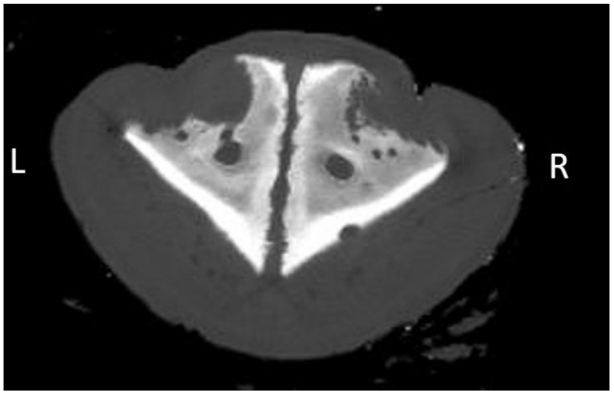

Fig. 2. Transverse CT image showing empty alveoli L2 (left), with mild remodelling, and R2 (right), with moderate remodelling.

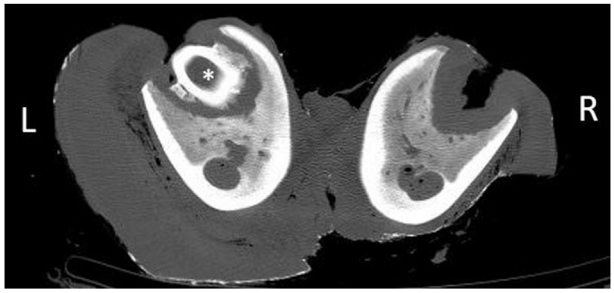

Fig. 3. Transverse CT image showing tooth L7 (L) and the empty alveolus of tooth R7 (R), which demonstrates no evidence of remodelling. Tooth L7 shows failure of the pulp cavity to narrow (consistent with tooth death at a relatively immature age), generalized widening of the periodontal space with evidence of pulp necrosis or irreversible pulpitis, irregular radicular proliferation (white asterisk) and embedding/impaction (as evidenced by lack of protrusion of the intact crown past the gingival margin).

of Natural History (Seattle, Washington, USA; specimen number UWBM 82688).

Through analysis of annuli and growth arrest lines, this animal was estimated to be 30–35 years old, which agrees with first observations of the animal as an adult in the early 1990s (Supplementary Fig. 1). A cartilaginous vertebral column was found in the stomach, further confirming a diet that includes elasmobranchs. As is consistent with previous reports of offshore killer whales, all the teeth were significantly abraded and nearly flush with the gingival margin (Ford *et al.*, 2011, 2014) (Supplementary Fig. 2). Nine teeth were missing and there was pulp exposure in many of those remaining. Overall, the more significant lesions, as interpreted on radiographs and CT, were seen in the more rostral potions of the mandibles. The cross-sectional CT images offered better image quality, resolution and visualization of structures when compared with two-dimensional radiography, so all the analysis described was performed from CT images. Described lesions and their clinical significance, together with the teeth affected, are listed in Table 1. There were nine empty alveoli with varying degrees of remodelling, leaving 15 teeth present for evaluation. More remodelling would suggest earlier, pre-mortem tooth loss, while the absence of remodelling would suggest either perimortem or post-mortem tooth loss. The majority of tooth loss in this specimen is assumed to have occurred shortly before death or *post mortem*.

Loss of crown integrity can be due to fracture or wear, with sharper edges of the remaining dental tissues more consistent with fracture and blunted edges more indicative of wear. In killer whales, however, sharper edges have also been hypothesized to be a result of long-term tooth-to-tooth contact (Ford *et al.*, 2011). The three types of dental wear consist of abrasion (produced by tooth contact with foreign materials, such as food or sand), attrition (produced by tooth-to-tooth contact) and erosion (dissolution of the tooth by chemical substances, such as regurgitation of acidic stomach contents) (Shafer *et al.*, 1983). Given the spacing of the teeth, smoothly domed occlusal surfaces and the elasmobranch diet in this killer whale ecotype, wear in this case was hypothesized to be due to abrasion. Nine of the 15 (60.0%) teeth present showed evidence of severe abrasion, with the crowns almost or completely flush with the surrounding alveolar margin (Fig. 4, Supplementary Fig. 3).

Five of the 15 (33.3%) teeth present showed generalized widening of the periodontal ligament space with evidence of pulp necrosis (failure to narrow of the pulp cavity) or at least irreversible pulpitis (pulp exposure) (Fig. 3, Supplementary Fig. 3). The combination of these findings supports a diagnosis of periodontal–endodontic lesion type I, meaning that these teeth most likely suffered endodontic disease (i.e. pulp exposure leading to pulp necrosis) first, followed by progression of apical infection that spread coronally and caused secondary periodontal disease (i.e. destruction of the periodontium manifested as a widened periodontal ligament space) (Wolf *et al.*, 2005). It is possible, but less likely, that these teeth developed periodontal–endodontic lesion type III, meaning that these teeth suffered concurrent, independent endodontic disease (i.e. pulp exposure leading to pulp necrosis) and simultaneous periodontal disease (i.e. attachment loss) (Wolf *et al.*, 2005). Four of the 15 (26.7%) teeth present showed buccally-widened periodontal ligament spaces with alveolar bone loss, consistent with a form of periodontitis classified as vertical bone loss (Fig. 4).

The width of the pulp cavity reveals valuable information on the age of the tooth or the age of the tooth at the time of its death, allowing for the approximate description of chronology (Fiani and Arzi, 2010). The pulp cavity is at its widest when the tooth is most immature and narrows as secondary dentine is deposited by odontoblasts. In a mature animal, like O059, a relatively wide pulp cavity is consistent with death of a tooth when the tooth/animal was immature. Five of the 15 teeth present (33.3%) showed failure of the pulp cavity to narrow, consistent with tooth death at a relatively immature age (Fig. 3, Supplementary Fig. 3). Two teeth (13.3%) had a narrower pulp cavity than the five obviously non-vital teeth, but this was still wider than for the adjacent teeth, likely indicating a non-viable tooth with tooth death occurring at a more mature age. Three of 15 teeth (20.0%) had pulp cavities that appeared obliterated, indicating

Table 1
Pathological dental changes found via CT described by the mandibular teeth affected, the appearance of the lesion on imaging and the most likely clinical significance of the lesion

Lesion	*Clinical significance*	*Teeth affected*
Empty alveoli with no remodelling	Perimortem or post-mortem tooth loss	R3, R4, R6, R7, L5
Empty alveoli with mild remodelling	More recent ante-mortem tooth loss	R1, L2
Empty alveoli with moderate remodelling	More chronic ante-mortem tooth loss	R2, L4
Severe abrasion	Chronic tooth contact with foreign materials	R5, R8, R9, R11, L3, L6, L8, L9, L11
Widening of periodontal ligament space with evidence of pulp necrosis or pulpitis	Periodontal–endodontic lesion type I (less likely type III)	R5, R8, L3, L6, L7
Buccally widened periodontal ligament spaces with alveolar bone loss	Periodontitis with vertical bone loss	R9, R11, L9, L11
Failure to narrow of the pulp cavity	Tooth death at a relatively immature age	R5, R8, L3, L6, L7
Moderate narrowing of the pulp cavity	Tooth death at a more mature age	R10, L10
Obliterated pulp cavities	Reparative dentine formation or foreign material in the pulp canal	R9, L8, L9
Vital teeth with a narrowed pulp cavity	No identified pathology	R11, R12, L1, L11, L12
Unerupted (embedded/impacted) teeth	Lack of eruptive force or physical obstruction of eruption	R5, L7
Mineral attenuating, irregular proliferation surrounding the root	Hypercementosis	R10, L6, L7, L10
Dense material within the pulp cavity	Foreign material or pulp calcification	L6
Uneven apex	External inflammatory root resorption	R8, L8
Exuberant soft tissue resembling gingival enlargement	Post-mortem degradation, fibrous hyperplasia, odontogenic tumour, other neoplasm	L9, L10, L11, L12

either reparative dentine formation or, potentially, foreign material in the pulp canal, which is often indistinguishable without histopathology (Supplementary Fig. 3). Unfortunately, the tissue was not a candidate for histopathology by the time imaging was performed. The remaining five teeth (33.3%) were presumed vital (at the time of death) based on a uniformly narrowed pulp cavity (Fig. 4). Variable pulp cavity widths in the teeth grossly is shown in Supplementary Fig. 4.

Two teeth (13.3%) appeared not to have erupted appropriately and were either embedded or impacted. Embedded teeth are properly positioned teeth that lack sufficient force for eruption, while impacted teeth have a physical obstruction blocking eruption (Haghanifar *et al.*, 2014) (Fig. 3).

Additional lesions were encountered on CT images, for which it was harder to determine a diagnosis or pathogenesis. For example, four teeth (26.7%) showed what appeared to be a mineral attenuating, periosteal-like, irregular proliferation surrounding their root (Fig. 3). This is potentially indicative of hypercementosis, the presence of idiopathic, excessive cementum (Lewis *et al.*, 2008). Two teeth (13.3%) had an uneven apex, possibly indicative of external inflammatory root resorption (Peralta *et al.*, 2010) (Supplementary Fig. 3). These same two teeth also had a thin rim of mineral attenuation lining the pulp cavity that was even more attenuating than the surrounding tooth substance. Finally, there was an area of exuberant soft tissue resembling gingival enlargement adjacent to L9–L12, although post-mortem degradation may have confounded this finding. Differential diagnoses for this lesion include focal fibrous hyperplasia, an odontogenic tumour or a different benign or malignant neoplasm. Gingival tissue autolysis precluded histopathology of this or other oral lesions.

The CT images from O059 were compared with those from a deceased 3-year-old resident killer whale. In that specimen, all the teeth displayed a relatively wide pulp cavity and open apices, consistent with immaturity. The teeth maintained their coronal cusps, with a lack of evidence of abrasion. As no younger offshore killer whale specimens are available, a direct comparison within the same ecotype could not be made.

The comprehensive necropsy report of O059 revealed a likely multifactorial cause of death, with the most significant lesions including inanition, arteriosclerosis and severe chronic dental disease. Imaging of the dentate mandibles confirms the presence of severe periodontal and endodontic disease, which caused morbidity in this individual, if not contributed to mortality. Odontocete cetaceans rely on their teeth for prehension of prey (Loch *et al.*, 2011), so without them, or with severely abraded crowns, it is reasonable to assume that this animal may have had a difficult time catching prey. Further research into this ecotype is needed to determine whether this level of

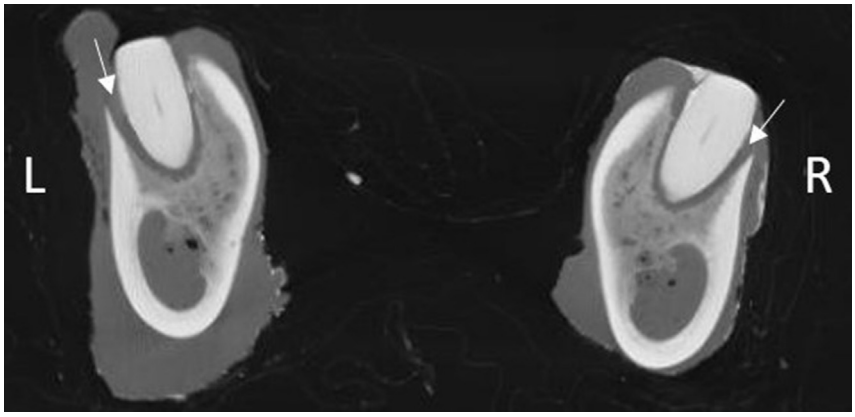

Fig. 4. Transverse CT image showing teeth L11 (L) and R11 (R), both of which were presumed vital (at the time of death) based on their uniformly narrowed pulp cavity. Both show evidence of severe abrasion and have buccally-widened periodontal ligament spaces (white arrows) with alveolar bone loss, consistent with a form of periodontitis classified as vertical bone loss.

wear is expected for an animal of this age or if this individual displayed excessive, pathological wear.

The severe abrasion seen grossly in this individual is consistent with what has been previously described in other offshore specimens and is most likely diet-induced (Ford *et al*., 2011). Via advanced imaging, we were able to describe periodontal and endodontic lesions in a free-ranging offshore killer whale in more detail than was previously possible. CT is time efficient and optimizes analysis of lesions as compared with standard radiography, with higher resolution and the ability to visualize the specimen in multiple planes. A larger sample size with individuals from all three ecotypes would be valuable. When possible, dentate mandibles with associated teeth, as well as gingival and dental samples for histopathology, should be obtained in deceased stranded killer whales for further classification of dental disease occurring in free-ranging populations.

Conflict of interest statement

The authors declare no conflicts of interest with respect to the publication of this manuscript.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcpa.2019.03.001.

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