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Cranial crassicaudiasis in two coastal dolphin species from South Africa is predominantly a disease of immature individuals

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ABSTRACT: *Crassicauda* spp. (Nematoda) infest the cranial sinuses of several odontocetes, causing diagnostic trabecular osteolytic lesions. We examined skulls of 77 Indian Ocean humpback dolphins *Sousa plumbea* and 69 Indo-Pacific bottlenose dolphins *Tursiops aduncus*, caught in bather-protecting nets off KwaZulu-Natal (KZN) from 1970–2017, and skulls of 6 *S. plumbea* stranded along the southern Cape coast in South Africa from 1963–2002. Prevalence of cranial crassicaudiasis was evaluated according to sex and cranial maturity. Overall, prevalence in *S. plumbea* and *T. aduncus* taken off KZN was 13 and 31.9%, respectively. Parasitosis variably affected 1 or more cranial bones (frontal, pterygoid, maxillary and sphenoid). No significant difference was found by gender for either species, allowing sexes to be pooled. However, there was a significant difference in lesion prevalence by age, with immature *T. aduncus* 4.6 times more likely affected than adults, while for *S. plumbea*, the difference was 6.5-fold. As severe osteolytic lesions are unlikely to heal without trace, we propose that infection is more likely to have a fatal outcome for immature dolphins, possibly because of incomplete bone development, lower immune competence in clearing parasites or an over-exuberant inflammatory response in concert with parasitic enzymatic erosion. Cranial osteolysis was not observed in mature males (18 *S. plumbea*, 21 *T. aduncus*), suggesting potential cohort-linked immune-mediated resistance to infestation. *Crassicauda* spp. may play a role in the natural mortality of *S. plumbea* and *T. aduncus*, but the pathogenesis and population level impact remain unknown.

KEY WORDS: *Sousa plumbea* · *Tursiops aduncus* · South Africa · *Crassicauda* spp. · Cranial osteolysis · Parasitosis

1. INTRODUCTION

Off the coast of South Africa, sympatric, nearshore Indian Ocean humpback dolphins *Sousa plumbea* and Indo-Pacific bottlenose dolphins *Tursiops aduncus* face several anthropogenic threats, including bycatch in bather protection nets (BPN) deployed off the coast of KwaZulu-Natal (KZN) to catch sharks and reduce the risk of attack (Dudley & Simpfendorfer 2006, Atkins et al. 2013a,b, Plön et al. 2015, 2016,

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Cockcroft et al. 2016). An average of 8 *S. plumbea* and 37 *T. aduncus* have been incidentally caught annually in BPN off KZN since 1980, likely having a negative impact on populations (Atkins et al. 2013a, Cockcroft et al. 2016, Plön et al. 2016). According to the IUCN Red List of Threatened Species *S. plumbea* and *T. aduncus* are considered 'Endangered' and 'Near Threatened', respectively (Cockcroft et al. 2016, Plön et al. 2016, Braulik et al. 2017, 2019).

Although several diseases, including parasitic pneumonia, endometritis and cutaneous lobomycosis, have been documented in these populations (Lane et al. 2014, Van Bressem et al. 2015), nothing is known about their cranial pathology. *Crassicauda* spp. (Nematoda), including *C. grampicola*, populate the cranial sinuses, among other organs, of several odontocete species, causing granulomatous sinusitis and osteolysis (Perrin & Powers 1980, Raga et al. 1982, Van Waerebeek et al. 1993, López et al. 1998, Van Bressem et al. 2006, 2007). The latter is observed as bone lysis with a characteristic 'basket-like' or trabecular appearance in de-fleshed crania (Robineau 1975, Dailey & Walker 1978, Walker & Cowan 1981) and likely results from erosion of the carbon-rich bone fraction by glycosaminoglycan-degrading enzymes secreted by the cephalic end of the nematodes (Raga et al. 1982, Pascual et al. 2000). While there is no direct evidence for the parasites causing death, it has been proposed that extensive bone lesions may lead to severe morbidity and mortality, especially in immature dolphins (Robineau 1975, Perrin & Powers 1980, Raga et al. 1982, Walker et al. 1984, Zucca et al. 2004, Balbuena & Simpkin 2014).

The objective of this study was to examine museum specimens of skulls from dolphins that were either bycaught in nets or stranded along the South African coastline for evidence of cranial crassicaudiasis to determine its prevalence and significance for 2 threatened sympatric species.

2. MATERIALS AND METHODS

2.1. Dolphins

We macroscopically examined skulls of 152 dolphins, comprising 69 *Tursiops aduncus* and 77 *Sousa plumbea*, caught in BPN off KZN between 1970 and 2017 and of 6 *S. plumbea* that stranded along the coastlines of KZN and the Eastern and Western Capes of South Africa between 1963 and 2002 (Fig. 1, Table 1). All skulls are deposited in the Graham Ross marine mammal collection at the Port Elizabeth Museum (PEM). With the exception

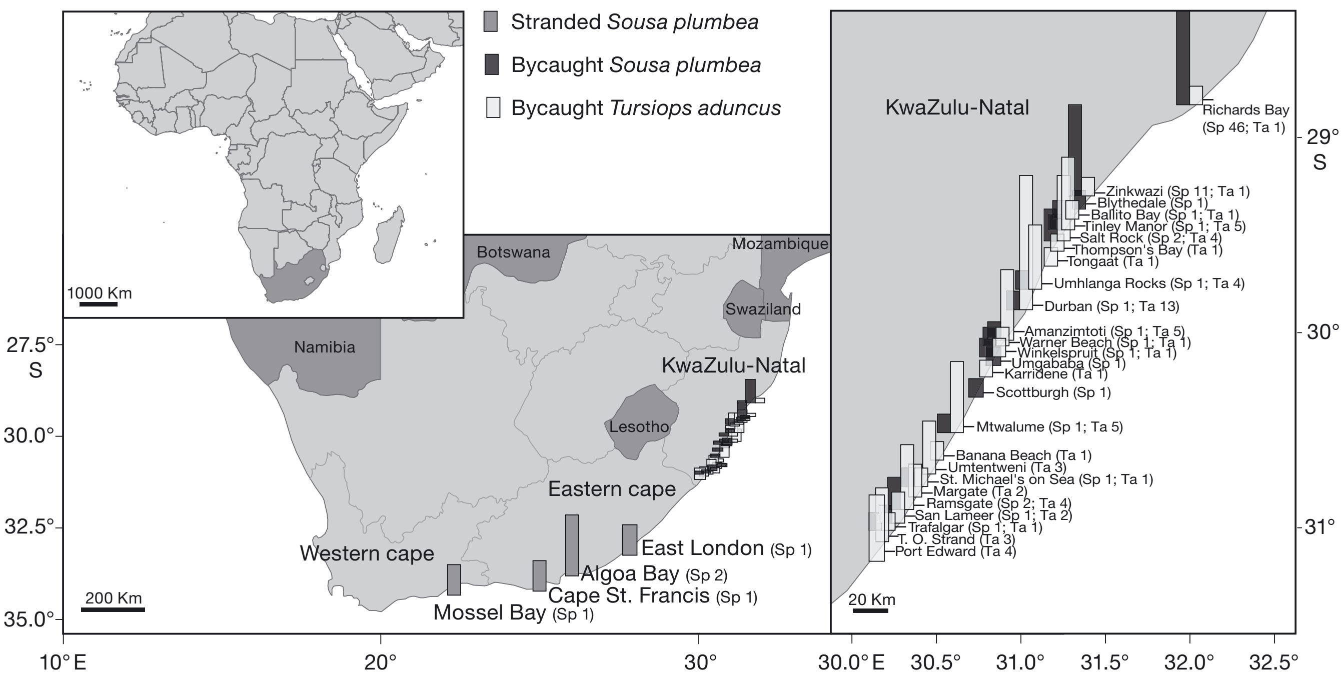

Fig. 1. Locations where the Indian Ocean humpback dolphins *Sousa plumbea* (Sp) and Indo-Pacific bottlenose dolphins *Tursiops aduncus* (Ta) examined during the present study were caught in nets or stranded along the coast of South Africa in the period 1963–2017

Table 1. Number of specimens of Indian Ocean humpback dolphins *Sousa plumbea* and Indo-Pacific bottlenose dolphins *Tursiops aduncus* examined for the presence of *Crassicauda* spp. cranial lesions during this study. Subsamples reflect the cause of death, age determination and specific examination of cranial sinuses at necropsy for the presence of *Crassicauda* spp. nematodes. GLGs: growth layer groups

	Sousa plumbea	*Tursiops aduncus*
Bycaught		
All specimens	77	69
Specimens with known sexual maturity	32	11
Specimens with age determination (GLGs)	59	0
Dolphins examined for *Crassicauda* spp. nematodes	2	3
Stranded		
All	6	0
Total	83	69

of 3 specimens (1 *S. plumbea*, 2 *T. aduncus*), data on sex and standard body length (SL) were available for all bycaught dolphins. In *S. plumbea*, sexual maturity and/or age were determined for 26 females and 42 males caught in nets (Plön et al. 2015; Table 1). Females were classified as sexually mature if at least 1 corpus luteum or corpus albicans was present in the ovaries or if lactation or pregnancy was evident (Cockcroft & Ross 1990a, Van Waerebeek & Read 1994). Males were considered sexually mature if semen was detected in the epididymis at necropsy (Plön et al. 2015). Age was determined from the number of growth layer groups (GLGs; sensu Perrin & Myrick 1980) counted in the dentine and cementum of longitudinal thin sections of teeth (Cockcroft & Ross 1990a, Plön et al. 2015). When no data on sexual maturity were available, humpback dolphins younger than 7.5 yr or smaller than 220 cm (females) and 230 cm (males) were regarded as immature (Atkins et al. 2013a, Plön et al. 2015, S. Plön unpubl. data). Data on sexual maturity were only available for 5 female and 6 male *T. aduncus* (Table 1), so SL was used as a proxy for maturity for the other specimens, with females and males larger than 213 and 240 cm, respectively, considered sexually mature (Cockcroft & Ross 1990a, Best 2007). Bycaught dolphins were necropsied at the KZN Sharks Board laboratory following a standard protocol (Atkins et al. 2013a, Lane et al. 2014). Stranded specimens were necropsied at the Port Elizabeth Museum. After flensing, remaining soft tissue was removed by maceration in fresh water over several months, and skulls were cleaned and stored in the PEM collection. The presence of nematodes in the cranial sinuses was only examined directly in 2 male (1 immature, 1 mature) *S. plumbea* and in 3 *T. aduncus* (1 mature female, 1 immature and 1 mature male) caught in 2017 (Table 1).

2.2. Cranial maturity

Host maturity is an important factor in the pathogenesis and epidemiology of infectious diseases. Cranial maturity in *S. plumbea* skulls was determined based on criteria described by Jefferson & Van Waerebeek (2004), as fusion in the maxillary-premaxillary and other cranial sutures develops at a later developmental stage in *Sousa* spp. than in other Delphinidae. Crania that showed a relative degree of bone ossification and fusion, an overall massiveness and a high degree of closure of the tooth pulp cavity were considered 'mature' (Jefferson & Van Waerebeek 2004). *T. aduncus* were considered cranially mature when an advanced degree of fusion was present in the maxillary-premaxillary suture and/or in the frontal-supraoccipital suture (Ross & Cockcroft 1990, Van Waerebeek 1993). Deep, well-defined dental alveoli on the maxillaries and mandibles were considered as an additional indication of cranial maturity. All other specimens were considered cranially 'immature'. Whenever possible, cranial maturity classification was further corroborated with data on SL, sexual maturity and/or age (see Section 2.1). The number of cranially immature and mature specimens by sex for each species is provided in Table 2.

Table 2. Sample composition (number, percentage) of cranially immature and mature Indian Ocean humpback dolphins *Sousa plumbea* and Indo-Pacific bottlenose dolphins *Tursiops aduncus* that died in bather protection nets off KwaZulu-Natal, 1970–2017, and were examined for the presence of *Crassicauda* spp. cranial lesions

	Immature	Mature	Total
Sousa plumbea			
Females	14 (18.2%)	15 (19.5%)	29 (37.7%)
Males	30 (38.9%)	18 (23.4%)	48 (62.3%)
Total	44 (57.1%)	33 (42.9%)	77
Tursiops aduncus			
Females	12 (17.4%)	13 (18.8%)	25 (36.2%)
Males	22 (31.9%)	22 (31.9%)	44 (63.8%)
Total	34 (49.3%)	35 (50.7%)	69

2.3. Characteristics of *Crassicauda* spp. lesions

Crassicauda spp. cause multifocal, circular to ovoid, lytic lesions in cranial bones arranged in trabecular or basket-like clusters (Robineau 1975, Dailey & Walker 1978, Walker & Cowan 1981, Pascual et al. 2000). These lesions are quite distinct from artefacts, such as bone perforations, fenestrations or defects that may have occurred during the maceration process. They were measured with a Vernier caliper (Mitutoyo B40986), or the percentage area of bone affected was estimated, enabling a classification as small, medium or large. Small lesions had a diameter less than 20 mm or affected less than 25% of the cranial bone, medium lesions measured between 20 and 25 mm or altered between 25 and 50% of the bone, while large lesions were over 25 mm or involved over 50% of the bone. Clinical consequences of infestation were deemed severe when at least 2 large lesions were present in 1 or more cranial bones or when infestation had resulted in the perforation or partial destruction of at least 1 bone. They were considered benign when the lesions were small and affected only 1 cranial bone, and intermediate when small lesions affected 2 bones or when medium or large lesions were seen on at least 1 bone.

2.4. Prevalence

Prevalence is defined as the number of skulls positive for cranial crassicaudiasis divided by the total number of individuals checked for this condition. We examined whether lesion prevalence varied with sex and cranial maturity in each species and with age in a subsample of 59 *S. plumbea* (Table 1). To avoid bias from potentially diseased animals, the 6 stranded *S. plumbea* were not included in the analysis of prevalence. We further evaluated whether the prevalence of *Crassicauda* spp. cranial pathology significantly varied between the 2 species in the period between 1970 and 2017. Significance of differences ($\alpha = 0.05$) was verified with chi-square contingency tests (Swinscow 1981). Confidence intervals (95% CI) for proportions were computed exactly using the Wilson method (Brown et al. 2001).

3. RESULTS

Crassicauda spp.-associated osteolysis was observed in the skulls of 10 of the 77 (13%) *Sousa plumbea* and 22 of the 69 (31.9%) *Tursiops aduncus* caught in BPN off KZN between 1970 and 2017, affecting the nasal (pterygoids), pre-orbitary (lacrimal-maxillary) and orbitary (frontal-sphenoid) regions surrounding the accessory sinus system (Figs. 2 & 3, Table 3).

3.1. *S. plumbea*

The 36 cranially immature individuals were between 1 and 7.2 yr old (mean ± SD; 4.5 ± 1.8 yr), while the age of the cranially mature specimens varied between 7.9 and 17.7 yr (13 ± 3 yr) in 11 females and be-

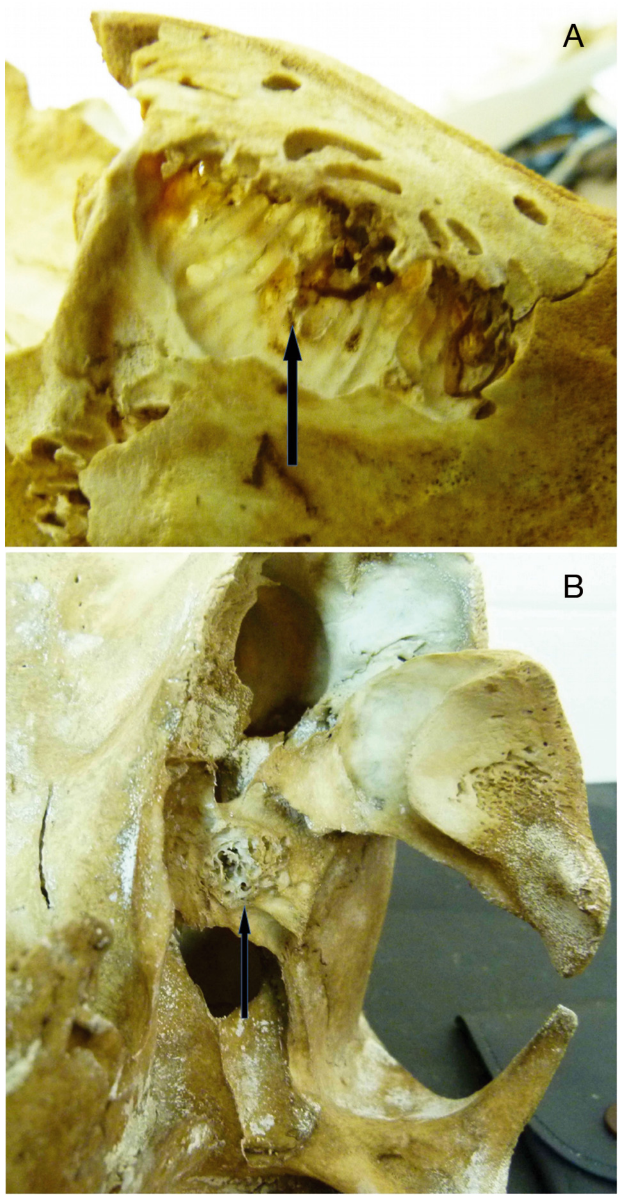

Fig. 2. *Sousa plumbea. Crassicauda* spp. multifocal osteolysis (A) in the left pterygoid of immature male PEMN1036 and (B) in the right sphenoid of immature male PEMN2060

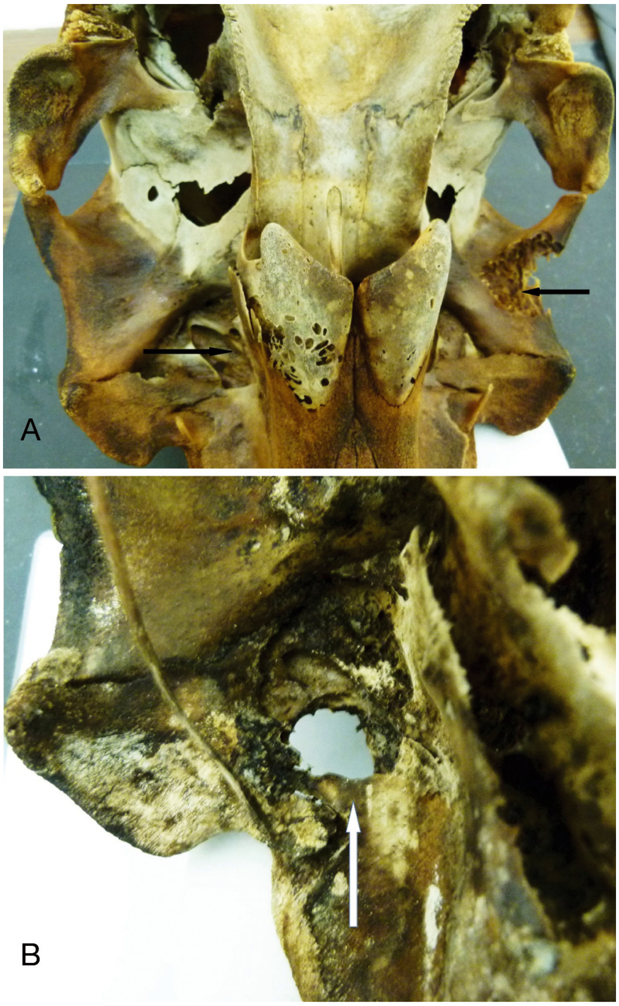

Fig. 3. *Tursiops aduncus. Crassicauda* spp. multifocal osteolysis (A) in the right frontal and left pterygoid of immature female PEMN1110 and (B) in the left maxillary of immature male PEMN2463

tween 7 and 23.5 yr (15.5 ± 4.8 yr) in 12 males. Cranial crassicaudiasis was observed in 9 (4 males, 5 females) cranially immature individuals and in 1 mature female, but not in any of the 18 mature males. The age of 7 positive immature *S. plumbea* ranged between 3 and 7 yr (5.1 ± 1.3 yr; Fig. 4, Table 3). The 2 males examined for the presence of parasites in the cranial sinuses at necropsy were negative for *Crassicauda* spp. nematodes but were infested by the trematode *Nasitrema* sp. Their skulls were also negative for crassicaudid osteolysis.

The pterygoid bones were affected in 6 of the 9 immature specimens (Fig. 2, Table 3). The maxillary, sphenoid and palatine bones were occasionally altered (Table 3). Lesions were severe in the pterygoids of 3 immature individuals and in the sphenoid and palatine of the mature female (Table 3). In the latter, bone pathology was less clearly defined, possibly because of remodelling after clearance of the parasites. In 2 females, *Crassicauda* spp. osteolysis affected more than 1 bone (Table 3).

Prevalence of cranial crassicaudiasis in the 77 bycaught specimens was 13% (95% CI 7.2–22.3%). It was only marginally higher in females (20.7%, CI 9.9–38.4%, n = 29) than in males (8.3%, CI 3.3–19.6%, n = 48, χ^2 = 2.44, df = 1, p = 0.12), so sexes were grouped for further analysis. Lesion prevalence was significantly (χ^2 = 5.1, df = 1, p = 0.024) higher in cranially immature (20.5%, CI 11.2–34.5%, n = 44) than in mature (3%, CI 0.54–15.3%, n = 33) individuals. It also significantly (χ^2 = 4.504, df = 1, p = 0.033) varied with age in the 59 dolphins for which the age (GLGs) was known, reaching 18.9% (CI 9.5–34.2%) in 37 individuals younger than 7.5 yr and was absent (CI 0–15.5%) in 22 older individuals (Fig. 4).

Table 3. Characteristics of *Crassicauda* spp. skull lesions in Indian Ocean humpback dolphins *Sousa plumbea* and Indo-Pacific bottlenose dolphins *Tursiops aduncus*. SL: standard body length; Imm: immature; Mat: mature; NA: not available; Orb: orbitosphenoid; Fro: frontal; Max: maxillary. Dates are given as dd.mm.yy. Parentheses indicate that sexual maturity and/or age were inferred from the standard body length

Specimen	Date	Sex	Sexual maturity	Age	SL (cm)	Cranial maturity	Characteristics of *Crassicauda* spp. skull lesions: Affected bones	Size (mm)	Severity
S. plumbea									
PEMN1045	22.12.83	F	Imm	4.5	188	Imm	Right pterygoid	Medium	Intermediate
PEMN2058	04.07.93	F	(Imm)	6	197.5	Imm	Right pterygoid	Large	Severe
PEMN2609	22.02.96	F	(Imm)	5.3	201.5	Imm	Left maxillary	Small (18 × 15)	Benign
PEMN2611	07.02.96	M	(Imm)	7.1	206.5	Imm	Both pterygoids	Large	Severe
PEMN487	09.10.80	F	(Imm)	6	210	Imm	Left maxillary	Medium	Intermediate
PEMN1036	10.01.84	M	(Imm)	4	210	Imm	Left pterygoid	Large	Severe
PEMN3052	13.12.02	F	Imm	NA	210	Imm	Left pterygoid & sphenoid	Medium to large	Intermediate
PEMN2060	12.02.93	M	(Imm)	NA	214	Imm	Right basisphenoid & presphenoid	Medium (20 × 12)	Intermediate

Table continues on next page

Table 3 (continued)

Specimen	Date	Sex	Sexual maturity	Age	SL (cm)	Cranial maturity	Characteristics of *Crassicauda* spp. skull lesions: Affected bones	Size (mm)	Severity
PEMN825	25.01.82	M	Imm	3	215	Imm	Right pterygoid	Medium	Intermediate
PEMN4731	18.04.13	F	(Mat)	NA	223.8	Mat	Right orbitosphenoid, presphenoid & palatine	Large (Orb: 34 × 22)	Severe
T. aduncus									
PEMN1105	11.10.84	M	Imm	(2)	184	Imm	Right maxillary	Large (26 × 25)	Intermediate
PEMN1103	24.08.84	F	(Imm)	(2)	188.5	Imm	Left frontal, orbitosphenoid	Large (Fro: 40 × 26) & small (Orb: 18 × 15)	Intermediate
PEMN1110	25.09.84	F	Imm	(2)	191	Imm	Right frontal (perforating lesion) & maxillary; left pterygoid	Large (Fro: 33 × 35)	Severe
PEMN1125	14.11.84	F	(Imm)	(3)	204	Imm	Left maxillary & pterygoid; right frontal, maxillary, alisphenoid & palatine	Large	Severe
PEMN1953	16.04.92	F	(Imm)	(3.5)	206.5	Imm	Left maxillary (perforating lesion)	Large (28 × 24)	Severe
PEMN2688	14.07.97	F	(Mat)	(4.5)	214	Imm	Right frontal	Large	Intermediate
PEMN1505	21.04.88	M	(Imm)	(5.5)	216	Imm	Left maxillary, pterygoid, basisphenoid, presphenoid palatine; both frontals, alisphenoids & orbitosphenoids	Small, medium & large	Severe
PEMN2445	17.08.95	M	(Imm)	(7)	220	Imm	Both pterygoids; right frontal & maxillary	Large	Severe
PEMN2444	17.08.95	M	(Imm)	(7.5)	221	Imm	Right maxillary (perforation); left orbitosphenoid; both pterygoids & alisphenoids	Medium & large	Severe
PEMN2434	02.07.95	M	(Imm)	(7.5)	222	Imm	Both pterygoids; right maxillary	Large	Severe
PEMN1557	26.09.88	M	(Imm)	(8)	223	Imm	Left pterygoid; right frontal	Large	Severe
PEMN1129	14.11.84	M	(Imm)	(8)	223	Imm	Left frontal; right pterygoid	Large (Fro: 35 × 32)	Intermediate
PEMN1303	04.08.86	F	(Mat)	(8)	224	Imm	Left frontal	Large (36 × 31)	Intermediate
PEMN1757	01.07.90	M	(Imm)	(8)	224	Imm	Right frontal	Large (26 × 22)	Intermediate
PEMN2331	01.09.94	M	(Imm)	(9)	225	Imm	Left pterygoid	Large	Intermediate
PEMN1555	12.10.94	M	(Imm)	(9)	226	Imm	Right maxillary, presphenoid, alisphenoid, orbitosphenoid, palatine & pterygoid; both frontals	Small, medium & large	Severe
PEMN2463	27.06.95	M	(Imm)	(9)	226	Imm	Right pterygoid; left maxillary (perforating lesion)	Large	Severe
PEMN857 (1)	30.07.82	M	Imm	NA	NA	Imm	Right frontal	Large (26 × 25)	Intermediate
PEMN2227	24.06.94	F	(Mat)	(6)	223.5	Mat	Left frontal; right orbitosphenoid; both pterygoids	Large (Orb: 24 × 18)	Severe
PEMN1000	28.09.83	F	(Mat)	(9)	228	Mat	Right maxillary (perforating osteolysis); left pterygoid & lacrimale	Large	Severe
PEMN1464	09.09.87	F	(Mat)	(10)	230	Mat	Left frontal	Large	Intermediate
PEMN1107	21.08.84	F	(Mat)	(20+)	243	Mat	Right maxillary; left frontal	Large (Max: 31 × 30), (Fro: 32 × 33)	Severe

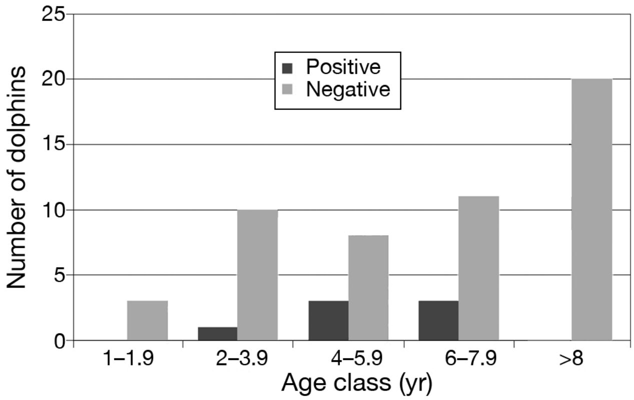

Fig. 4. Number of individuals positive and negative for *Crassicauda* spp. cranial bone lesions according to 2 yr age classes in 59 *Sousa plumbea* caught in nets off KwaZulu-Natal, South Africa

3.2. *Tursiops aduncus*

The SL of the individuals examined for cranial osteolysis varied between 188.5 and 254 cm in 26 females (mean ± SD; 221.2 ± 16.2 cm) and between 184 and 260 cm (229.6 ± 14.9 cm) in 42 males. In the period 1970–2017, cranial crassicaudiasis was detected in 18 (6 females, 12 males) immature specimens and in 4 mature females, but not in any of the 21 mature males. The SL for affected dolphins ranged from 184 cm in 1 immature animal to 243 cm for an adult. SL of the 10 positive females was 215.3 ± 16.7 cm, indicating an approximate mean age of 5 yr (Cockcroft & Ross 1990a). SL of the 12 affected males was 220.6 ± 12 cm, or an estimated 7 yr old (Cockcroft & Ross 1990a). The frontal was the most frequently affected bone (n = 15), followed by the pterygoids (n = 13), maxillaries (n = 12) and sphenoid bones (n = 6). The lesions affected more than 1 cranial bone in 14 dolphins and were considered severe in 10 immature and 3 mature specimens (Table 3). The cranial sinuses of 3 bottlenose dolphin carcasses examined for parasites in 2017 were negative for *Crassicauda* spp. nematodes but were infested with *Nasitrema* sp. Indeed, none of these skulls showed crassicaudid osteolysis.

Prevalence of *Crassicauda* osteolysis was 31.9% (CI 22.1–43.6%) in 69 bottlenose dolphin skulls in the period 1970–2017. There was no significant difference ($\chi^2 = 0.8$, df = 1, p = 0.36) in prevalence between males (27.9%, CI 16.8–42.7%, n = 43) and females (38.5%, CI 22.4–57.5%, n = 26) and sexes were grouped for further analysis. Prevalence was significantly higher ($\chi^2 = 13.7$, df = 1, p = 0.0002) in immature dolphins (52.9%, CI 36.7–68.65%, n = 34) than in mature individuals (11.4%, CI 4.5–2.6%, n = 35). Severe lesions were seen in 50% of the affected cranially immature (n = 18) and mature (n = 4) specimens.

3.3. Interspecific variation

The proportion of cranially immature and mature males and females examined for *Crassicauda* spp. lesions that had been collected from 1970 to 2017 was similar, and thus comparable, in both species (Table 2). The prevalence of *Crassicauda* osteolysis was significantly higher ($\chi^2 = 7.6$, df = 1, p = 0.006) in *T. aduncus* (31.9%, n = 69) than in *S. plumbea* (13%, n = 77) during that period. This difference stems from the higher prevalence ($\chi^2 = 8.9$, df = 1, p = 0.003) of infestation in the immature subsample of *T. aduncus* (52.9%, n = 34) than in the equivalent *S. plumbea* subsample (20.5%, n = 44). While prevalence was also higher in *T. aduncus* (11.4%, n = 35) than in *S. plumbea* (3%, n= 33) in the cranially mature specimens, this difference was not significant ($\chi^2 = 1.8$, df = 1, p = 0.18). Severe bone lysis occurred frequently in both species (*T. aduncus*: 59%, n = 22; *S. plumbea*: 40%, n = 10) (Figs. 2 & 3; Table 3).

4. DISCUSSION

Cranial osteolysis has been commonly encountered in the crania of delphinids worldwide (e.g. Robineau 1975, Dailey & Walker 1978, Walker et al. 1984, Van Waerebeek et al. 1990, 1993, López et al. 1998, Pascual et al. 2000, Van Bressem et al. 2006, 2007). During necropsies, *Crassicauda* spp. nematodes have been found embedded in bone and periosteum, coiled beneath the mucosa in the pterygoid and the supraorbital areas of Risso's dolphin *Grampus griseus*, pantropical spotted dolphin *Stenella attenuata*, common bottlenose dolphin *Tursiops truncatus* and (rarely) dusky dolphin *Lagenorhynchus obscurus* (Walker & Cowan 1981, Raga et al. 1982, Van Waerebeek et al. 1990, 1993, Zucca et al. 2004). Though infestation caused no or only local tissue reaction in some specimens, in others it was associated with purulent, necrotic and/or haemorrhagic sinusitis that may have contributed to stranding or death (Dailey & Stroud 1978, Raga et al. 1982, Resendes et al. 2002, Zucca et al. 2004). *Crassicauda* spp. sinus infestation may also affect respiration and echolocation (Dhermain et al. 2002) and increase susceptibility to bacterial and viral pathogens (Pascual et al. 2000, Balbuena & Simpkin 2014). Super-infection of the perforating skull lesions with bacterial pathogens could conceivably lead to severe sinusitis, osteomyelitis, meningitis, encephalomyelitis, sepsis and mortality in both *Sousa plumbea* and *Tursiops aduncus*. Several bacteria have been detected in the lungs of these species, including the opportunistic *Aeromonas media* and *Photobacterium damselae* (Lane et al. 2014).

This study found the prevalence of basket-like bone lesions to be significantly higher in *T. aduncus* than in *S. plumbea*, possibly because the diet of Indo-Pacific bottlenose dolphins includes more cephalopods (Cockcroft & Ross 1990b, Plön et al. 2015) which are likely intermediate or paratenic hosts in the life cycle of *Crassicauda* spp. Because of the taxonomic position of Tetrameridae within the Habronematoidea, Balbuena & Simpkin (2014) suggested that arthropods may be the intermediate host for the infective larval stage. As cephalopods and fish feed on marine arthropods such as copepods and krill, they could serve as paratenic hosts, acting as reservoir for the different larval stages of *Crassicauda* spp. Similarly, the almost complete absence of *Crassicauda* nematodes in 3 coastal-dwelling small cetacean species in Peru compared to the 69% prevalence in the Peruvian offshore population of common bottlenose dolphins led Van Waerebeek et al. (1993) to conclude that the massive infestation was probably related to some pelagic element in their diet. A prime suspect, because of its significance in the pelagic diet, was jumbo flying squid *Dosidiscus gigas*.

Our study found that *Crassicauda* spp. osteolysis affected significantly more immature than mature dolphins in both *T. aduncus* and *S. plumbea*. For immature *S. plumbea*, the age determined for 7 animals ranged between 3 and 7 yr with a mean of 5.1 yr. This is comparable with data on *Stenella attenuata* from the eastern tropical Pacific (ETP), where the peak age of infestation was also 5 yr (Perrin & Powers 1980). While it is conceivable that mild bone lysis resolves by remodelling, it seems unlikely that the more severe cases with massive bone loss and perforation would heal without trace. The absence of remodelled bone in older dolphins suggests that severe infestation may be a cause of mortality for some individuals. Alternatively, given that the immature dolphins with severe cranial lesions in this study had died following bycatch in BPN, it is possible that severe sinusitis or parasitic auditory neuritis impeded their ability to detect and avoid the nets. A similar conclusion of parasite-associated mortality was made by Perrin & Powers (1980) who observed a higher prevalence of *Crassicauda* spp. cranial lesions in immature *S. attenuata* and *D. delphis* bycaught in tuna fisheries in the ETP. A statistical reassessment of their data supported the hypothesis of parasite-induced mortality between 6.5 and 9 yr and at roughly 12 yr (Balbuena & Simpkin 2014). It is conceivable that in some species of Delphinidae, heavy crassicaudiasis render young individuals more prone to entanglement in both gillnets and purse-seines. Sonar-impaired dolphins may have difficulty locating the escape route window created during the purse-seine back-down procedure meant to let trapped dolphins escape. By contrast, the prevalence of crassicaudid osteolysis did not vary with cranial maturity in *T. truncatus* and long-beaked common dolphins *Delphinus cf. capensis* caught off central Peru in 1986–2000 (Montes et al. 2004, Van Bressem et al. 2006), suggesting that *Crassicauda* spp. pathogenesis and associated mortality may differ between odontocete species and regions. In South Africa, *Crassicauda* spp. osteolysis was not observed in mature males suggesting potential resistance to infestation in this cohort that may be immune-mediated, but the pathogenesis remains to be elucidated. Likewise, Walker & Cowan (1981) observed that the presence of intact nematodes was not always associated with bone lesions, especially in adult dolphins, and suggested that osteomyelitis leading to bone lysis occurs more frequently in immature dolphins, possibly because of the incomplete bone development. However, it is also likely that the younger animals have either less immune competence in clearing the parasites, or that they mount an over-exuberant inflammatory response progressing to extensive bone lysis, possibly in concert with enzyme-mediated erosion caused by the nematodes (Raga et al. 1982, Pascual et al. 2000). Further studies on the immune response of cetaceans to endemic parasites are warranted.

Altogether these data suggest that *Crassicauda* spp. may play a role in the natural mortality of *S. plumbea* and *T. aduncus*, but the extent and pathogenesis are currently unknown, as is the impact on population dynamics. In *S. attenuata* from the ETP, maximum mortality due to cranial osteolysis was estimated to represent between 2 and 4% of the natural mortality in dolphins aged 6 to 8 yr old (Balbuena & Simpkin 2014). Given the conservation status of *S. plumbea* and *T. aduncus* globally as well as in southern African waters (Cockcroft et al. 2016, Plön et al. 2016, Braulik et al. 2017, 2019), the role of *Crassicauda* spp. as a potential source of mortality and recruitment failure should be further investigated. Researchers performing necropsies are encouraged to carefully examine cranial sinuses for helminth parasites, identify, quantify and sample these, and document associated pathologic conditions.

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