Hawai‘i and Mariana Islands Stranding Analyses

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ABSTRACT

The University of Hawai‘i Health and Stranding Lab, located at Marine Corps Base Hawai‘i (MCBH), is the only entity in the Pacific Islands region that responds to strandings, conducts necropsy and cause of death investigations, archives tissues and performs research to identify and evaluate threats to Pacific Island cetaceans. The purpose of this project is to conduct analyses of historical stranding patterns and causes of mortality that incorporate quantitative estimates of stranding dates, genetic identification of species when necessary, and advanced laboratory diagnostics. This report focuses on conducting genetic species identification for stranding events where an initial species determination was not possible. This data will be used to increase the robustness of stranding data in a historical analysis of temporal and spatial stranding patterns in the Pacific Islands. We also report on the screening of archived tissues for the presence of *Brucella* in stranding cases where infectious disease is suspected. This advanced diagnostic information provides the ability to more thoroughly evaluate potential causes of mortality in stranded animals from the Hawaiian and Mariana Islands. We report on cause of death examinations by species over the time period of 2006 to 2022 in the Pacific Islands region, with major pathological findings in stranded cetaceans described.

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INTRODUCTION

Background: Stranded species identification using genetic tools

The examination of stranding patterns in the Pacific Islands region and how this relates to trends over time and the probability of species specific strandings by location is limited. Published stranding data involves an examination of approximately 200 records of odontocete strandings occurring between 1937 and 2002 in the main Hawaiian Islands (Maldini et al. 2009). Stranding data that has been obtained since 2002 includes well over 200 additional stranding records and stranding data from the Mariana Islands. In other regions of the world, historical stranding patterns have been assessed to describe the probability by location of species specific strandings and the relationship between stranding events and environmental factors (i.e., Norman et al. 2004; Coombs et al. 2019). A similar examination of historical stranding patterns in the Pacific Islands region could be strengthened by conducting species identification using genetic tools where determination of species identification was not possible at the time of stranding. It is possible to apply genetic techniques to distinguish between the pygmy sperm whale (*Kogia breviceps*) and dwarf sperm whale (*Kogia sima*), two species that are very difficult to distinguish from one another when size range overlaps and/or carcass condition precludes measurements of the dorsal fin. Other potential species that have historically stranded in the Pacific Islands region and are difficult to distinguish include sperm whales (*Physeter macrocephalus)* and humpback whales (*Megaptera novaeangliae*), whose carcasses may wash ashore or be found floating in advanced states of decomposition without any species identifying characteristics. Additional records also exist of unidentified odontocete carcasses being discovered in the Pacific Islands region that may represent beaked whales or pilot whales.

Background: Increasing our understanding of causes of mortality through disease screening

Infectious disease is becoming well recognized as a significant threat to Pacific Island cetaceans. *Brucella ceti* infections are common among cetaceans and lead to neurological disease, respiratory disease, bone disease, and reproductive failures. *Brucella ceti* is the only *Brucella* species recognized in cetaceans, and sequence types 23, 26, and 27 are now recognized to be associated with specific lesions. Early molecular analysis suggests the *Brucella ceti* sequence type infecting at least some of the animals stranded in Hawai'i may differ from *Brucella ceti* sequence types in cetaceans in the Eastern Pacific. Further research is needed to improve the diagnostic test for this infection. Morbillivirus was first identified in the Pacific Islands region from a Longman's beaked whale (*Indopacetus pacificus*) that stranded in 2010 (West et al. 2013), and we have since reported on morbillivirus findings from archived Hawaiian stranding samples (West et al. 2015; Jacob et al. 2016), were involved in new method validation for global surveillance of morbillivirus (Yang et al. 2016), and have contributed to whole genome sequencing in order to describe the unique beaked whale morbillivirus strain (Landrau-Giovanetti et al. 2019). We have most recently discovered an additional novel strain of morbillivirus in a Fraser's dolphin (*Lagenodelphis hosei*) that stranded in 2018 that is distinct from both the beaked whale morbillivirus and the Southern hemisphere strains (West et al. 2021). We have reported other pathogens that threaten Hawaiian cetaceans and have historically caused mortality, including the presence of *Cryptococcus gatti* in a Hawaiian spinner dolphin (*Stenella longirostris*) (Rotstein et al. 2010), *Brucella* cases that involve a neonate sperm whale co-infected with morbillivirus (Chernov 2010; West et al. 2015), and fatal disseminated toxoplasmosis in three stranded spinner dolphin deaths, where we project based on low carcass recovery rates that this could be representative of the deaths of 60 spinner dolphins (Landrau-Giovanetti et al. 2022). We have recently characterized the full genome of the first known cetacean circovirus that was identified from a stranded

Hawaiian whale and subsequently identified this pathogen in 10 new host species (Landrau-Giovanetti et al. 2020; Clifton et al. 2023) as well as novel cetacean herpes viruses from this region (West et al. 2013; West and Waltzek, unpublished data). However, much remains unknown about the prevalence of identified diseases among Hawaiian and Mariana Island cetaceans.

Despite describing numerous pathogens that contribute to mortality in the Pacific Islands region, it is difficult to quantify the contribution of each identified pathogen to overall causes of mortality in the region without applying advanced diagnostic techniques. This project supports the polymerase chain reaction (PCR) analysis of archived tissues for 35 stranding cases where infectious disease is suspected. Specifically, PCR screening focuses on testing for the presence of the pathogen *Brucella* and the comparison of testing results with necropsy and histopathology findings. Disease screening results contribute to developing an integrated causes of mortality database as part of this overall project, and directly support a quantitative examination of causes of mortality among cetaceans in the Pacific Islands.

Background: Causes of mortality and pathologic findings in Pacific Island cetaceans

The Pacific Islands region spans over 4 million square miles across the central, Western, and South Pacific, and stranding investigations are conducted for cetaceans stranding in the Hawaiian Islands, the Mariana Islands, and American Samoa. At least twenty species of cetaceans regularly inhabit the Hawaiian archipelago (Baird 2016), with 13 species of cetaceans described from the Mariana Islands (Fulling et al. 2011) and 13 species of cetaceans identified to date from American Samoa.

Natural causes of death include infectious disease, which is a significant threat to Pacific Island cetaceans that has become well recognized through stranding investigations as described above. Anthropogenic impacts on cetaceans determined from stranding investigations are well described in the scientific literature from other regions. These include marine debris ingestion and entanglements with mortalities due to gastric blockages reported in sperm whales, Cuvier's beaked whales (*Ziphius cavirostris*), and Risso's dolphins (*Grampus griseus*) (Jacobsen et al. 2010; Alexiadou et al. 2019). Sperm and fin whales (*Balaenoptera physalus*), with diverse foraging strategies, have been suggested as potential indicators of global marine litter (Fossi et al. 2020). National disentanglement programs highlight the impact of entanglements on mortality and morbidity of cetaceans world-wide, with this threat included as one of the initiatives of the International Whaling Commission. Cetacean mortalities due to by-catch and lethal fishing hook penetration have also been described (Adimey et al. 2014; Byard et al. 2020; Cuvertoret-Sanz et al. 2020). Vessel strikes provide another example of anthropogenic impacts on cetaceans, with mortalities described from several regions of the world (Diaz-Delgado et al. 2018; Peel et al. 2018; Peltier et al. 2019; Pennino et al. 2022). Naval sonar and echosounders can also result in mass strandings, with beaked whales believed to be especially vulnerable to anthropogenic noise (Southall et al. 2013; Bernado de Quiros et al. 2019).

Pathological findings in stranded cetaceans have been described according to natural and anthropogenic causes of mortality from other regions (i.e., Arbelo et al. 2013; Delgado-Diaz et al. 2018; Alvorado-Rybak et al. 2020; Cuvertoret-Sanz et al. 2020; Burek-Huntington et al. 2022). In the Pacific Islands region, pathological findings from individual stranding investigations have been reported and important disease threats identified (West et al. 2013; West et al. 2015; West et al. 2021). However, this report describes progress towards the first effort to examine pathological findings and quantify causes of mortality observed in stranded cetaceans between 2006 and 2022 in the Pacific Islands region.

METHODS

Targeted stranded species identification using molecular tools

Kogia:

Distinguishing between species identification of pygmy sperm whales (*Kogia breviceps*) and dwarf sperm whales (*Kogia sima*) is notoriously difficult. Identification of *Kogia* species typically relies on morphological measurements that differ between the two species. If teeth are present in the upper jaw, this is diagnostic of *K. sima*, but the absence of teeth in the upper jaw could indicate either *K. breviceps* or *K. sima*. The first measurement that can be used diagnostically is total body length (TBL), as *K. breviceps* reach a larger total length than *K. sima*. Individuals with a total body length beyond the maximum reported for *K. sima* (270 cm) are identified as *K. breviceps* when stranding events occur. However, when total body length is less than the maximum for *K. sima*, this single measurement cannot be used to distinguish between the two species. The other useful measurement that is applied when diagnosing species is the dorsal fin height (DFH). *K. sima* typically have a dorsal fin height that is larger in comparison to its total body length, whereas *K. breviceps* typically have a dorsal fin that is smaller compared to the body length. Generally, if the dorsal fin height is less than 5% of the total body length, the animal is considered to represent *K. breviceps*, and greater than 5% represents *K. sima*. Due to variation in or a lack of morphological measurements available because of carcass condition at the time of carcass recovery, individuals with borderline measurements could not be identified on morphometrics alone. Borderline measurements included a DFH:TBL ratio between 4 and 6% where total body length was less than the maximum reported for *K. sima*, which indicated a potential overlap in body size. Ten animals were initially selected based on potential overlap in total body length measurements and/or a DFH:TBL ratio that was borderline or considered not definitive as a species diagnostic indicator. Two of the animals had no dorsal fin measurements available. The initial species identification using molecular methods included 10 animals with indistinguishable measurements and 14 animals as confirmation of identification using morphological methods. In 2022, this effort expanded the 24 animals to 27 to include recently stranded *Kogia* in Hawai‘i to either confirm identification using body morphometrics or determine species when morphometrics are not useful (Results, Table 1).

From the selected cases, tissues for genetic analysis were chosen based on availability and included the kidney, spleen, gingiva, testis, skin, brain, heart, muscle, and lung. Only one tissue is required for genetic identification, but multiple tissues were used as needed to increase the likelihood of PCR and sequencing success.

Other Cetaceans:

Several individual stranded cetaceans (both historically and currently) have resulted in inconclusive identification at the time of examination. This is typically due to advanced decomposition that results in incomplete remains being available and/or the loss of identifiable characteristics. From our archival and currently stranded animal tissues, we classified individual stranding cases into one of three categories: individuals where species identification is known (controls), individuals where a specific species is suspected but in need of confirmation, and individuals that represent unknown species. Controls were selected from cases where species identification is known and where animals were freshly dead and in good body condition to facilitate morphological identification. As cases involving advanced decomposition led to fewer archived tissues and decreased DNA concentration, all available tissues from unknown individuals were used to maximize attempts at successful genetic identification.

Control Animals:
One known individual from each of the previously stranded Hawaiian species was selected for validation of primer efficacy. This included 19 different animals that represent 19 of the 20 species in the Health and Stranding Lab tissue archive. Previously identified *Kogia* using *Kogia*-specific primers were included to ensure the efficacy of these primers (Results, Table 1).

Suspect or Undetermined Animals:
In addition to the focus on genetic identification of *Kogia* spp. that have previously stranded in the Pacific Islands, 13 cases where the specific species could not be determined morphologically were selected for identification by molecular analysis (Results, Table 2). Cases either had characteristics that suggested a small subset of possible species (ex., large whale: sperm or humpback; medium whale: pilot or beaked; small dolphin: spinner or striped) or could not suggest any species beyond cetacean or vertebrate.

Determining species identification by PCR and genetic sequencing

Kogia:
DNA was extracted from each tissue using Qiagen DNeasy Blood and Tissue Kits (Qiagen, Germantown, Maryland) according to the manufacturer's protocol. To confirm the success of the DNA extraction, the DNA concentration of each extract was determined using Qubit dsDNA Broad-Range Assay Kits and a Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts).

PCR was conducted for each tissue extract using *Kogia*-specific primers and thermocycler conditions based on Wang et al. (2013). Four primers were used in total: two for PCR amplification and two for Sanger sequencing of the amplified products. PCR primers included Kogia A DLP1.5 forward primer 5' TGTAAAACGGCCACTTCACCCAAAGCTGRARTT 3' and Kogia A DLP4H-fast reverse primer 5' AGCGGGWTRYTGRTTTCACGCGGCATG 3'. Thermocycler conditions included an initial incubation at 98ºC for 50s followed by 35 cycles of denaturation at 95ºC for 10s, annealing at 65ºC for 10s, and extension at 65ºC for 10s, with a final extension at 68ºC for 3 minutes. Amplified products were visualized using gel electrophoresis. Amplified DNA fragments measuring approximately 420bp in size were cut from the gel and further purified using QIAquick PCR and Gel Cleanup Kits (Qiagen, Germantown, Maryland). The DNA from a known *Kogia sima* was used as the positive control throughout the entire PCR screening to ensure the amplification and sequencing were successful.

Cleaned samples were submitted to the University of Hawai'i Advanced Studies in Genomics, Proteomics, and Bioinformatics (ASGPB) lab for genetic sequencing. These samples were sequenced once with each primer: Kogia B DLP1.5 5' ACGACGGCCAGTTCACCCAAAGCTG 3' and Kogia B DLP4H-fast 5' AGCGGGTTGCTGGTTTCACGCGGGATG 3'. Sequences were analyzed using NCBI Nucleotide BLAST and match percentages to *K. breviceps* and *K. sima* sequences were used to diagnostically identify specimens to species. BLAST results are interpreted using how similar the sample sequence is to sequences in the BLAST database. Results are filtered according to percent identity (how similar the sample sequence aligns with a database sequence), which will order sequence matches from high to low relatedness. Multiple quality metrics are used to support whether the percentage is valid, where the highest validated percentage is used for identification. The percentage may vary depending on the length and quality of the sample sequence.

Other Cetaceans:
DNA was extracted from each tissue using Qiagen DNeasy Blood and Tissue Kits (Qiagen, Germantown,

Maryland) according to the manufacturer's protocol. To confirm the success of the DNA extraction, the DNA concentration of each extract was determined using Qubit dsDNA Broad-Range Assay Kits and a Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts).

PCR was performed with two sets of universal vertebrate primers utilized by Dalebout et al. (2004). Primer set 1 included GLUDG-L 5' TGACTTGAARAACCAYCGTTG 3' and CB2-H 5' CCCTCAGAATGATATTTGTCCTCA 3' (Palumbi 1996). These primers were designed for the amplification of the cytochrome b gene in mitochondrial DNA (mtDNA). mtDNA is conserved among eukaryotes and experiences high rates of evolution, leading to significant variation in genetic sequences between closely related species, making it a useful tool for species identification (Yang et al. 2014). Thermocycler conditions were as follows: initial denaturation at 94ºC for 3 minutes, 35 cycles of 94ºC for 30 seconds, 53ºC for 30 seconds, and 72ºC for 60 seconds, followed by a final extension at 72ºC for 7 minutes. Conditions were modified from Lorenz (2012) to match primer properties.

Primer set 2 was used following unsuccessful amplification from primer set 1 (Results, Table 2), which included primers Dlp10-L 5' CCACAGTACTATGTCCGTATT 3' (Baker et al. 1993) and Dlp4-H 5' GCGGGWTRYTGRTTTCACG 3' (Baker CS, Unpublished data). These primers were designed for the amplification of the control region of mtDNA (Dalebout et al. 2004). Thermocycler conditions were as follows: initial denaturation at 94ºC for 30 seconds, 35 cycles of 94ºC for 30 seconds, 47ºC for 30 seconds, and 72ºC for 60 seconds, followed by a final extension at 72ºC for 5 minutes. Conditions were modified from Lorenz (2012) to match primer properties.

Amplified products were prepared for sequencing using QIAquick PCR and Gel Cleanup Kits (Qiagen) and sequenced at the Advanced Studies in Genomics, Proteomics, and Bioinformatics lab at the University of Hawai'i. Each sample was sequenced twice using both primers used in amplification. Both sequences from each sample were compared to reference sequences from NCBI Nucleotide BLAST. BLAST results from the control animals were used to ensure the top species match to the sample was accurate.

Increasing our understanding of causes of mortality through disease screening

Brucella: Animal and Tissue Selection:

We selected 35 individual cases from our tissue archive for *Brucella* screening efforts, with a suite of five tissues targeted from each animal (Table 3). Many of these cases were selected based on the examination of necropsies and histopathology findings that suggested disease presence. These also included animals involved in a mass stranding of short-finned pilot whales (*Globicephala macrorhynchus*) in 2017 and pygmy killer whales (*Feresa attenuata*) stranded in 2019, as well as multiple striped dolphins (*Stenella coeruleoalba*) that stranded over a timescale of less than a month. Additionally, a full-term humpback whale fetus that stranded on Moloka'i in 2020 was selected for testing as several strains of *Brucella* have been linked to abortions and other reproductive abnormalities. The remaining animals that were selected for *Brucella* screening included several species of dolphins and dwarf and pygmy sperm whales. The initially targeted tissues for testing included the brain, lung, kidney, liver, and lymph nodes. Potential substitutions of tissue types depended on sample availability and histopathology findings available for individual animals. In-house tissue samples from cases previously confirmed at outside diagnostic laboratories were used as both positive and negative controls for all amplifications.

Primer Selection and Brucella Detection:

Multiple genes from the *Brucella* genome were initially selected for assessment in order to develop a

testing protocol with high specificity for detecting infections, while reducing the likelihood of false positives. Primers from the published literature and in-house primer design were tested for BCSP-31, OMP2b, and IS711 gene sequences, in both singular and nested formats. A final selection was made of primers from published literature that targeted a 150bp sequence from the IS711 gene: Forward – 5' TACCGCTGCGAATAAAGCCAAC 3' and Reverse – 5' TGAGATTGCTGGCAATGAAGGC 3' (Wu et al. 2014).

DNA was extracted from each tissue using Qiagen DNeasy Blood and Tissue Kits (Qiagen, Germantown, Maryland) according to the manufacturer's protocol. To confirm the success of the DNA extraction, the DNA concentration of each extract was determined using Qubit dsDNA Broad-Range Assay Kits and a Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts). Final extracts were frozen at -20°C prior to amplification. PCR protocol steps were also adapted from Wu et al. (2014), which in brief involved an initial denaturation of 10 minutes at 95°C and then 40 cycles of denaturing for 10 seconds at 95°C and 30 seconds of annealing at 60°C.

All amplified products were visualized using gel electrophoresis to determine suspected positive cases. Bands were cut from suspect positive gels and cleaned using Qiagen QIAquick PCR and Gel Cleanup Kits (Qiagen, Germantown, Maryland). These samples were then submitted to the University of Hawai'i Advanced Studies in Genomics, Proteomics, and Bioinformatics Lab for final genetic sequencing. These sequences were then analyzed using the NCBI database's Nucleotide BLAST online tool for potential matches with *Brucella ceti* and *Brucella pinnipedialis*.

Causes of mortality and pathologic findings in Pacific Island cetaceans

The Health and Stranding Lab, formerly operating at Hawai'i Pacific University, was involved in 250 stranding investigations representing 20 different species of whales and dolphins between 2006 and 2022. These include all stranded specimens where either minimal soft tissue was sampled, such as skin, muscle, and blubber, or where partial or full necropsies were conducted. Partial and/or full necropsies were conducted in almost all cases of smaller odontocetes where size did not limit the ability to conduct a necropsy. For example, in the case of pygmy killer whales, full necropsy examinations were conducted for each of the 10 stranded individuals where cause of death investigations were attempted (Table 4). Partial or full necropsies were not as likely in the case of mid to large sized odontocete and mysticete strandings where size, remote stranding locations, and/or access to floating carcasses limited the ability to conduct necropsies. These limitations were most extreme in the examination of sperm whale strandings, where 27 individuals were confirmed stranded and at least minimal tissue samples were collected, but where partial or full necropsies were only conducted for five of these cases (18.5%). Of the total 250 stranding investigations conducted, 140 of the total cases had carcass condition codes of 1 and 2, which represent fresh dead animals (Geraci and Lounsbury 2005). Codes 3, 4, and 5 represent moderate to advanced decomposition, and 107 specimens were represented by these condition codes (Geraci and Lounsbury 2005). Carcass condition was not determined in three cases. In addition to the 250 cases, we also examined samples from seven cases collected from live animals (e.g., skin, feces, and placenta), but these were not included in the total of stranded cases.

Of the 250 cases examined, 54 of the cases (22%) involved tissue sampling of 1-4 tissues and did not constitute partial or full necropsies. Partial or full necropsies are defined by at least a partial examination of internal organs within body cavities and the collection of a minimum of five different tissue types. Full

or partial necropsies were conducted for 196 of the 250 cases examined (78%). This annual report focuses on findings from 64% of the stranding cases (125 of 196) where partial or full necropsies were conducted and where one or several significant diagnoses were obtained from gross necropsies, histopathological evaluation, and/or ancillary tests. The Health and Stranding Lab has expanded diagnostic capacity to include in-house PCR diagnosis of common pathogens and in-house histopathological examination of formalin-fixed tissue samples processed at the Histology and Imaging Core Facility at the University of Hawai'i, John A. Burns School of Medicine.

RESULTS

Stranded species identification using genetic tools

Kogia:

Using genetic sequencing, species diagnostics were confirmed for 24 of the 27 total individuals analyzed (Table 1). Our sequencing results demonstrated genetic confirmation that 18 of the 24 specimens (75%) identified using morphological characteristics were correct when differentiating between pygmy sperm whales and dwarf sperm whales. A single specimen (4%) was genetically identified as the opposite species than that indicated by species diagnostics that were based on morphological information. Five cases (21%) were identified to species using molecular techniques where it had not been previously possible to assign a species identification beyond the taxonomic level of genus. The species with the higher valid percent identity was understood to be the identity of the stranded animal.

We had difficulty conducting species identification through genetic sequencing efforts for three of the 27 cases (11%) examined, and species identification remains unknown for these three individuals (Table 1). One individual has a body length measurement just over the *K. sima* maximum and an overlapping Dorsal Fin Height:Total Body Length (DFH:TBL) value. This case had very few available tissues due to advanced decomposition, and the extracted tissues did not produce interpretable results. Another case has a total body length that is within the range of *K. breviceps* and *K. sima* and has no available measurements for the dorsal fin. This case similarly has few available tissues due to advanced decomposition, and sequencing attempts were unsuccessful. The last case consists of only bone samples, which did not produce results using DNA extraction procedures routinely utilized at the Health and Stranding Lab.

Table 1. Results from genetic sequencing of stranded *Kogia* spp. using *Kogia* specific primers. BLAST Result: Identified species of unconfirmed case based on Percent Identity. Top Match Percent Identity: Relatedness between sample sequence and database sequence, 100% representing exact match. More distantly related species have a lower percent identity. Each primer provides a sequence compared to the database and the identification is accepted if both primers have the same BLAST result species.

ID	BLAST Result		Tissue	Top Match Percent Identity	
	Species	Common		Primer 1	Primer 2
KS-3903*	*K. sima*	Dwarf sperm whale	Kidney	98.5% 98.77%[1]	95.11% 97.56%[1]
KB-1829	*K. breviceps*	Pygmy sperm whale	Kidney	64.29%	94.92%
KX-4319	NR	NR	Gingiva	NR	NR
KB-6831	*K. breviceps*	Pygmy sperm whale	Kidney	85.94%	78.39%
KB-9806	*K. breviceps*	Pygmy sperm whale	Testes	93.63%	90.27%
KX-8128	NR	NR	Gingiva	NR	NR
KX-7991	NR	NR	Bone	NR	NR

KS-7686	*K. sima*	Dwarf sperm whale	Muscle	92.38%	97.67%
KS-3452	*K. sima*	Dwarf sperm whale	Muscle	90.49%	97.39%
KB-1608	*K. breviceps*	Pygmy sperm whale	Cerebellum	98.2%	97.65%
KB-3606	*K. breviceps*	Pygmy sperm whale	R atrium	90.94%	93%
KB-8100	*K. breviceps*	Pygmy sperm whale	L kidney	69.11%	69.11%
KS-9835	*K. sima*	Dwarf sperm whale	Brain	95%	95.36%
KS-7593	*K. sima*	Dwarf sperm whale	L kidney	98.8%	98.4%
KS-8901	*K. sima*	Dwarf sperm whale	Spleen	98.79%	95.61%
KB-4630	*K. breviceps*	Pygmy sperm whale	L kidney	99.1%	97.36%
KB-5948	*K. breviceps*	Pygmy sperm whale	Muscle	92.13%	85.51%
KS-8283	*K. sima*	Dwarf sperm whale	Soft tissue	99.7%	98.73%
B-3756	*K. breviceps*	Pygmy sperm whale	L kidney	99.68%	92.38%
KB-4803	*K. breviceps*	Pygmy sperm whale	L kidney	98.79%	95.07%
KB-9355	*K. breviceps*	Pygmy sperm whale	L kidney	88.36%	79.12%
KS-7629	*K. sima*	Dwarf sperm whale	Lung	94.67%	98.34%
KS-8329	*K. sima*	Dwarf sperm whale	Muscle	97.68%	97.33%
KB-2757	*K. breviceps*	Pygmy sperm whale	Cerebrum	99.37%	99.43%
KB-9961	*K. breviceps*	Pygmy sperm whale	Muscle	99.95%	98.24%
KB-7347	*K. breviceps*	Pygmy sperm whale	Cerebrum	97.89%	94.74%
KB-5324	*K. breviceps*	Pygmy sperm whale	L kidney	99.07% 98.03%[1]	97.66% 96.96%[1]
KB-7354	*K. breviceps*	Pygmy sperm whale	L kidney	98.08%[1]	96.96%[1]

*Positive Control Animal
NR: No Results
[1]mtDNA Primer Set 1

Other Cetaceans – Control Animals:

Analysis of PCR products was 100% accurate for animals with a confident morphologic identification (n = 19). The percentage of similar base pairs (percent identity) between the control sample sequence and the top matching reference sequence was between 92-100% for all controls.

Suspect or Undetermined Animals:

Nine of the 13 cases where we attempted to determine species identification using genetic tools produced sequencing results (Table 2). The percent identity between unknown and reference sequences were between 93 and 100% for mtDNA primer set 1 and 72-99% for mtDNA primer set 2. For sequences obtained using primer set 2 (n = 14, Table 2), only one was below 95%. This suggests a lower-quality DNA sample; however, while this sequence has a low percent identity, there were no alternate species matches. Three of the unknown cases did not have results from primer set 1 as the available tissues were heavily degraded from carcasses representing advanced decomposition; however, results were obtained in these cases using primer set 2. Primer set 2 is less accurate than set 1 but is more effective with degraded tissues (which do not have complete mtDNA) as it targets smaller fragments of DNA (Dalebout et al. 2004). Successful sequencing of the control animals using mtDNA primer set 2 supports the interpretation of these results.

Four of the 13 identified cases where species identification is unavailable have not been successfully tested to date. One case involves a single large vertebra in which usable DNA cannot be extracted using routine extraction procedures at the Health and Stranding Lab and may require different procedures utilized in laboratories specializing in the extraction of ancient DNA. Samples were not collected from two cases (one of which was due to cultural sensitivities and the other due to a remote stranding location), where the available documentation was not sufficient for species identification. Samples have been collected from a suspected Cuvier's beaked whale in American Samoa and are awaiting shipment to the Health and Stranding Lab facility, where they will be genetically tested for confirmation of

species identification. Further efforts are planned for alternate methods of DNA extraction from hard tissues (bone/teeth), which may prove successful at identifying the remaining cetacean cases where these are the only available samples.

Table 2. Results from genetic sequencing of suspect or undetermined stranded Hawaiian cetaceans using two mtDNA primer sets. BLAST Result: Identified species of unconfirmed case based on percent identity. Top Match Percent Identity: Relatedness between sample sequence and database sequence, 100% representing exact match. More distantly related species have a lower percent identity. Each primer provides a sequence compared to the database and the identification is accepted if both primers have the same BLAST result species.

ID	BLAST Results		Tissue	Top Match Percent Identity			
	Species	Common		Set 1 Primer 1	Set 1 Primer 2	Set 2 Primer 1	Set 2 Primer 2
MN-8613	*M. novaeangliae*	Humpback whale	Blubber	99.28%	95.77%	98.49%	97.78%
PM-5410	*P. macrocephalus*	Sperm whale	Soft tissue	NR	NR	99.61%	96.64%
U-6039[1]	NT	NT	NT	NT	NT	NT	NT
PM-2107	*P. macrocephalus*	Sperm whale	Blubber	NR	NR	99.61%	98.15%
PM-9621	*P. macrocephalus*	Sperm whale	Soft tissue	NR	NR	72.00%	97.93%
MD-3902	*M. densirostris*	Blainville's beaked whale	Lymph node	99.26%	99.75%	NT	NT
MN-6525	*M. novaeangliae*	Humpback whale	Amniotic fluid	99.51%	95.56%	96.97%	95.57%
U-4150[2]	NT	NT	NT	NT	NT	NT	NT
SL-8345	*S. longirostris*	Spinner dolphin	Soft tissue	100.00%	94.36%	98.89%	98.08%
U-2846[1]	NT	NT	NT	NT	NT	NT	NT
U-1390[3]	NT	NT	NT	NT	NT	NT	NT
MNi-1232	*M. nigricans*	Blue marlin	Soft tissue	93.35%	95.12%	NT	NT
SL-5710	*S. longirostris*	Spinner dolphin	Skin	99.51%	97.41%	98.88%	96.93%

NT: Not Tested
NR: No Results
[1]No Samples Collected
[2]Bone Only
[3]Awaiting Sample Shipment

Increasing our understanding of causes of mortality through disease screening

Brucella Disease Screening Results:
The complete screening effort for the presence of *Brucella spp.* included 175 tissues from 35 different animals (Table 3). Of the animals tested, results indicated that 15 of these individuals (43%) are positive for a marine mammal strain of *Brucella* in one or more tissues. These animals include a dwarf sperm whale (n = 1), a rough-toothed dolphin (*Steno bredanensis*) (n = 1), a Longman's beaked whale (n = 1), a spinner dolphin (n = 1), a Fraser's dolphin (n = 1), and a humpback whale fetus (n = 1). Additionally, pygmy killer whales (n = 5) from a mass stranding event and multiple striped dolphins (n = 4) were also found to be positive for *Brucella*.

The tissue type that tested positive for *Brucella* most frequently was the brain, with a 60% positivity rate among the positive individuals identified through this screening effort. The second most likely tissue type screened to test positive is lung tissue, at 47%. Genetic sequencing has been conducted for seven of the suspected positive cases to date (Table 3). Infection was detected in tissues dating back to 2000 in a stranded rough-toothed dolphin, indicating the presence of *Brucella* in stranded cetaceans from Hawaiian waters over a time span that exceeds 20 years.

Table 3. Results of tissue analyses conducted for the presence of *Brucella spp.* from 35 stranded Pacific cetaceans.

Species	Common Name	Detected Tissues	Not Detected
K. sima	Pygmy sperm whale	Brain, liver	Kidney, lung, spleen
S. bredanensis	Rough-toothed dolphin	L Adrenal1, lung, spleen	Heart, kidney
S. longirostris	Spinner dolphin	Brain	Kidney, liver, lung, spleen
S. longirostris	Spinner dolphin		Brain, liver, muscle, R kidney, tb LN
S. attenuata	Pantropical spotted dolphin		Blubber, brain, liver, muscle, R kidney
I. pacificus	Longman's beaked whale	Cerebellum1	Liver, mediastinal LN, R lung, R ventricle
M. densirostris	Blainville's beaked whale		Cerebellum, liver, LN, lung, spleen
P. electra	Melon-headed whale		Cerebrum, cerebellum, L lung, mediastinal LN, R lung
P. electra	Melon-headed whale		Cerebrum, bronchial LN, L lung, R lung, spleen
G. macrorhynchus	Short-finned pilot whale		Cerebrum, L lung, R lung, spleen, tracheal LN
P. electra	Melon-headed whale		Cerebrum, cerebellum, L lung, R lung, L marginal LN
P. crassidens	False killer whale		Cerebrum, L lung, R lung, L marginal LN, spleen
P. electra	Melon-headed whale		Cerebrum, cerebellum, L lung, R lung, L marginal LN
P. electra	Melon-headed whale		Cerebrum, L lung, R lung, L marginal LN, spleen
P. electra	Melon-headed whale		Cerebrum, L lung, R lung, L marginal LN, spleen
G. macrorhynchus	Short-finned pilot whale		Cerebrum, L lung, L marginal, liver, spleen
G. macrorhynchus	Short-finned pilot whale		Cerebrum, L lung, L marginal, liver, spleen
L. hoseii	Fraser's dolphin	Cerebrum, lung, liver, mesenteric LN	Spleen
F. attenuata	Pygmy killer whale	Cerebrum1	Lung, liver, R marginal LN, spleen
F. attenuata	Pygmy killer whale		Cerebrum, lung, liver, L marginal LN, spleen
F. attenuata	Pygmy killer whale	L marginal LN1	Cerebrum, lung, liver, spleen
F. attenuata	Pygmy killer whale	Marginal LN	Brain, lung, liver, spleen
F. attenuata	Pygmy killer whale	Lung	Brain, liver, marginal LN, spleen
F. attenuata	Pygmy killer whale	Brain	Lung, liver, marginal LN, spleen
P. crassidens	False killer whale		Cerebrum, lung, liver, mediastinal LN
M. novaeangliae	Humpback whale	Lung, liver, aortic LN1	Kidney, mesenteric LN
S. coeruleoalba	Striped dolphin	R marginal LN	Cerebrum, R lung, hilar LN, meninges

Z. cavirostris	Cuvier's beaked whale		Cerebrum, L lung, liver, L hilar LN, spleen
K. breviceps	Pygmy sperm whale		L adrenal, cerebrum, L lung, liver, diaphragmatic LN
L. hoseii	Fraser's dolphin		Cerebrum, lung, liver, hilar LN, spleen
K. breviceps	Pygmy sperm whale		Cerebrum, R lung, liver, hilar LN, spleen
S. coeruleoalba	Striped dolphin	Cerebrum1, R lung, spleen	Liver, L marginal LN
S. coeruleoalba	Striped dolphin	Cerebrum, lung	Liver, L marginal, LN, spleen
S. coeruleoalba	Striped dolphin	Cerebrum1, L lung, spleen	Liver, L marginal LN
S. coeruleoalba	Striped dolphin		Cerebrum, cerebellum, L lung, R lung, L prescapular LN

[1]Positive sequencing results for *Brucella spp.*

Causes of mortality and pathologic findings in Pacific Island cetaceans

Our pathological findings from 125 cases had a significant diagnosis or diagnoses (Table 4). Natural disease was significant in 60% of stranded animals with a diagnosis across all species, and half of the individuals where significant natural disease was identified were in poor body condition due to chronic illness. We also identified that approximately 12% of stranded individuals across all species examined were in the perinatal/neonatal age group, with three cases of dystocia where the mother and calf died. Trauma was observed in 28% of strandings across all species of known cause, and 15 of these cases involved fishery interactions (bycatch, hook injury, and entanglement). Blunt trauma was responsible for mortality in 16 cases among all species examined, which may include extreme intraspecies and interspecies aggression, boat strikes, and blast injuries. Significant changes suggesting decompression sickness were noted in four cases when all species were examined collectively. Species specific observations associated with causes of mortality and pathological findings observed between 2006 and 2022 are summarized below. Table 5 summarizes cases with significant trauma. Significant findings in animals in the perinatal/neonatal category are summarized in Table 6. Table 7 lists pathological findings and diseases in animals in poor nutritional status, and Table 8 lists natural diseases detected in animals in good nutritional status. Nutritional status was assessed by examination of body shape, the length/weight relationship, and evaluation of blubber metrics.

Table 4. Summary of pathological findings. Undetermined cases include both stranding investigations where only minimal samples were collected as well as partial and fully necropsied individuals without significant pathological findings and where cause of death was not determined.

Species	Pathological Categories									
	Natural - Good Condition	**Natural - Poor Condition**	**Perinatal and Neonatal**	**Blunt and Blast Trauma**	**Fishery Bycatch**	**Trauma Fishery Injury (hooks, ingestion of nets, gunshot, etc.)**	**Entrapment and Entanglement**	**Decompression**	**Undetermined***	**Total**
B. brydei[1]									2	2
F. attenuata	3	5							2	10
G. macrorhynchus	5		1	1		3		1	6	17
G. griseus		1				1			1	3
I. pacificus	1									1
K. breviceps	5	2		5				1	6	19
K. sima	1	1					1		5	8
L. hosei	1	2		3		1				7
M. novaeangliae	1		5	1					19	26
M. densirostris		1							4	5
O. orca		1							1	2
P. electra	3	6	2						9	20
P. macrocephalus			1						26	27
P. crassidens	3	1			1				3	8
S. attenuata	2	1	1				1	1		6
S. coeruleoalba	7	8		1				1	9	26
S. longirostris	2	8	5	1		1	2		23	42
S. bredanensis		1			2	1				4
T. truncatus	1					1			3	5
Z. cavirostris	2			4					6	12
Total	**37**	**38**	**15**	**16**	**3**	**8**	**4**	**4**	**125**	**250**

*Includes minimal tissues

[1]Two cases (Guam 2014, Wake Island 2021) represent the pelagic Bryde's whale (*Balaenoptera edeni brydei*) but offered no pathological findings due to minimal sampling.

Pygmy killer whales (Feresa attenuata)

Full necropsies were conducted for ten pygmy killer whales, which represent each of the specimens of this species that were reported dead stranded between 2006 and 2022. Seven of these individuals were part of a prolonged mass stranding event in Māʻalaea Bay, Maui, in 2019. During this prolonged mass stranding event, five individuals were initially necropsied in good body condition (Table 8), and two additional individuals were monitored out of habitat for a several weeklong period where body condition declined prior to eventually stranding (Currie et al. 2021). Two of the other strandings were solitary stranding events off Oʻahu and Hawaiʻi Island. The other stranding of a single individual in 2009 also occurred in Māʻalaea Bay, Maui, that was the result of a group of 4-6 animals initially out of habitat that were monitored over time while the group size steadily declined until a single individual stranded. Pneumonia was the cause of death in the individual that stranded in 2009, where a larger group size gradually declined while the animals were out of habitat and was also the cause of death in a calf that washed ashore dead the day of the initial stranding associated with the prolonged mass stranding in 2019 off of Maui. Respiratory disease was the significant pathological finding in a calf that stranded in 2015. In 40% of the total cases examined (4/10), pneumonia secondary to bacterial and viral infection caused death. Animals in poor body condition had neurobrucellosis in two cases and metabolic disease in one case. In addition to *Brucella*, morbillivirus RNA was identified by PCR analysis of a lymph node in one case, and sequencing of this pathogen indicated close genetic similarity to the recently described and novel

Fraser's dolphin morbillivirus. Scoliosis was also documented in a solitary pygmy killer whale stranding event off Oʻahu but was not believed to be a contributing factor to the stranding. Prior scars along the mouth and in the esophagus of this same individual are believed to represent areas of healing associated with a prior fishery interaction.

Pilot whales (Globicephala macrorhynchus)

Short-finned pilot whale strandings in the Pacific Islands region between 2006 and 2022 are represented by both solitary strandings and a mass stranding event that occurred off the island of Kauaʻi in 2017. Partial or full necropsies were conducted for 11 of the 17 pilot whales that were stranded during this time period. In the 2017 mass stranding event, five individuals that had died were necropsied by the Health and Stranding Lab, with histopathology and disease screening conducted. The cause of the stranding was not determined despite describing 12–15 pounds (lbs) of marine debris from the stomach of one of the individuals and pathological changes in the abdominal cavity of an older adult female that may have been associated with trauma during the stranding event. Significant marine debris ingestion (>10lbs) was noted in two additional solitary stranded individuals but did not cause fatal intestinal blockages. Pterygoid verminous sinusitis and intestinal nematodiasis have also been noted as common in stranded pilot whales in the main Hawaiian Islands. A recently stranded calf in 2022 died of verminous bronchopneumonia (*Halocercus* sp.). Limited samples were obtained from six cases due to advanced decomposition and/or remote location of stranding.

Risso's dolphins (Grampus griseus)

Risso's dolphins are a pelagic species that rarely strand in the Pacific Islands region. Between 2006 and 2022, three carcasses were examined. One of these was in an advanced state of decomposition and was identified from the skull and tooth counts and it was not possible to conduct a partial or full necropsy in this case. The other two individuals were fresh, dead carcasses. One was recovered as by-catch in a known

fishery interaction. The other was in poor nutritional status, and changes in the heart associated with dilated cardiomyopathy were noted. This individual also had heavy parasitosis of the liver and stomach, and an incidental finding included the recovery of a plastic bag from the esophagus of the animal.

Pygmy sperm whales (Kogia breviceps)

Pygmy sperm whales are deep-diving small whales with a world-wide distribution. Pathological changes were documented in 13 out of 19 pygmy sperm whale stranding investigations occurring between 2006 and 2022 in the Pacific Islands region. Six pygmy sperm whale deaths were associated with acute death, secondary to trauma, including five cases of blunt trauma. In one case, the animal had a fractured cervical vertebrae and severe decompression sickness. It is likely the animal surfaced, suffered from decompression sickness, and then experienced a vessel collision leading to instant death. Cardiac disease has been described in pygmy sperm whales from other regions of the world. Two cases of cardiomyopathy, including cardiac tamponade leading to sudden death in a pregnant female, were documented during cause of death investigations. Necropsied animals had moderate to severe endoparasitism regardless of body condition, consisting of gastrointestinal nematodes and subcutaneous phyllobothria. Screening for infectious pathogens by PCR is ongoing.

Dwarf sperm whales (Kogia sima)

Dwarf sperm are often difficult to distinguish from pygmy sperm whales, and part of this overall project included genetic testing to either identify or confirm the species identification of eight dwarf sperm whales examined during stranding investigations. Significant pathologic changes were documented in three of the dwarf sperm whale cases, including one case of entrapment. Cardiac hypertrophy and multifocal myocardial fibrosis were found in a pregnant animal in good body condition, and another pregnant animal died from bronchopneumonia and septicemia. It is possible that the septicemia that resulted in death was secondary to another pathogen.

Longman's beaked whale (Indopacetus pacificus)

Longman's beaked whales are one of the world's most poorly known beaked whale species and rarely strand. There is only one confirmed case of a Longman's beaked whale stranding in the Pacific Islands that occurred in Hana, Maui, in 2010. The animal had fractures of mandible and maxilla, determined to be peri-mortem and likely were associated with trauma occurring to the individual during the stranding. Multiple fresh cookie cutter shark bites were also noted on the abdomen. Histopathological examination revealed multi-organ inflammatory disease, and this represents the first known animal from the central Pacific to test positive throughout all organ systems with a novel beaked whale morbillivirus (West et al. 2013). Tissues also tested positive for an alpha herpes virus (West et al. 2013). Later examination of tissues from this animal also revealed the first circovirus in a marine mammal world-wide (Landrau-Giovanetti et al. 2020), and more recently, this animal also tested positive for *Brucella* during in-house testing at the Health and Stranding Lab.

Fraser's dolphins (Lagenodelphis hosei)

Fraser's dolphins are pelagic pantropical dolphins, and stranding events of this species are rare. However, despite a historical infrequency of stranding in the Pacific Islands, the Health and Stranding Lab has been involved in conducting full necropsies for seven Fraser's dolphins that have stranded since 2018 and documented significant findings in all cases (Table 4). A dolphin stranded in 2018 off Maui represented

the novel finding of a Fraser's dolphin morbillivirus in the central Pacific (West et al. 2021). Since publishing this finding in the scientific literature, additional pathogens have been identified in the tissues of this individual, which represents a tri-infection by *Brucella*, circovirus, and morbillivirus. Another adult female animal with signs of infectious disease and positive for circovirus stranded in 2021 on Hawai'i Island. A male stranded in 2022 off Maui had a skin lesion that was caused by the fungus *Paracoccidioidis braziliensis*, the pathogen that causes lobomycosis-like disease. This is the first time that this fungus has been reported in the central Pacific, with prior cases of lobomycosis-like disease in dolphins known from the Atlantic coast and from central America. The mass stranding of three Fraser's dolphins represents an unusual mortality event occurring over two days in 2021 off the island of O'ahu where each of the three individuals were in good nutritional condition. These necropsied individuals had multiple hemorrhages across organ systems and evidence of ear and mandibular fractures, consistent with blast trauma. Another Fraser's dolphin that stranded dead in Guam and was necropsied had an entry and exit wound consistent with a gunshot wound.

Humpback whales (Megaptera novaeangliae)

Similar circumstances that limit cause of death investigations for sperm whales also apply to humpback whales and include advanced decomposition, floating carcasses, and large size, with strandings in remote areas or along shorelines that are inaccessible with heavy equipment. Out of 26 stranding events where at least one tissue sample was collected from stranded humpback whales, full necropsies were possible in six cases (23%) and partial necropsies in ten cases (38%) between 2006 and 2022. Of the 26 stranding investigations, five of these involved neonates. One of these cases represented a breech birth resulting in dystocia, which is the likely cause of deaths of both the mother and calf. In this same event, calf tissues tested positive for the pathogen *Brucella*, representing vertical transmission from the mother that was in a moderate to advanced state of decomposition. Another case involving a neonate in 2022 off Hawai'i Kai, O'ahu, indicated cerebral hemorrhage and evidence of a concussive force that led to the death of this neonate, which was most likely caused by a vessel strike. Another case of a humpback whale calf indicated a cause of death due to septicemia, where the calf also had a costal rib fracture. It is possible that the septicemia was secondary to immunosuppression in the calf that was caused by another pathogen.

Blainville's beaked whale (Mesoplodon densirostris)

Blainville's beaked whales only occasionally strand in the Pacific Islands region, and a total of five strandings have been recorded between 2006 and 2022. Partial necropsies were conducted for two individuals stranded at Midway and in American Samoa, and another necropsy was attempted, but only minimal samples were collected from an individual in an advanced state of decomposition in American Samoa. Full necropsies were conducted on two individual Blainville's beaked whale strandings in the main Hawaiian Islands. Cause of death could not be determined in four of the five cases where full or partial necropsies were conducted due to moderate to advanced decomposition of the carcasses examined. Multi-system organ disease was responsible for the death of one of the Blainville's beaked whales, initially stranded alive on Maui in 2010, where rehabilitation was attempted. Pyogranulomatous pneumonia perforating gastritis, and acute nephropathy were found during the cause of death investigation for this individual. Fungal organisms (*Aspergillus)* were noted in lesions. PCR tests indicated the presence of circovirus DNA in multiple organs and morbillivirus RNA in a small number of a suite of tissues tested (Jacob et al. 2016; Clifton et al. 2023).

Killer whales (Orcinus orca)

Hawai'i has a small population of killer whales that are infrequently sighted and even more infrequently strand. Between 2006 and 2022, one live killer whale stranding occurred in 2008 off Kaua'i, and this individual was euthanized and necropsied. A skull was also recovered during this period from a killer whale on Lāna'i, but is believed to have surfaced years after the burial of a 2004 killer whale stranding near the site of the recovered skull. The necropsied killer whale that stranded in 2008 was in poor body condition. The gross necropsy and histopathology findings were not conclusive but suggested metabolic or inflammatory disease.

Melon-headed whales (Peponocephala electra)

During the 2006 to 2022 time period, 20 melon-headed whale stranding investigations were conducted, with 18 of these animals stranded in the main Hawaiian Islands and one stranding occurred in Guam, and another in the Federated States of Micronesia. From these strandings, partial or full necropsies were conducted for 18 individuals. Cause of death was undetermined in nine of these cases. Two of these cases were associated with perinatal or neonatal mortality (Table 6). Natural disease associated with poor nutritional status was found in five cases (Table 7). A heavy parasitic load, especially nematodiasis in pterygoid sinuses, was observed in several individuals. PCR identification of nematode species is ongoing. Two animals in good nutritional status had infectious respiratory disease, and lymphadenopathy was noted in one case. Respiratory disease was found most frequently among animals dying of natural disease, and additional testing for viral and bacterial pathogens including morbillivirus, herpes virus, circovirus, *Brucella* and *Mycoplasma* is needed.

Sperm whales (Physeter macrocephalus)

Partial or full necropsies were conducted for five of a total of 27 stranded sperm whales because of a combination of advanced decomposition, strandings in remote locations or floating carcasses and/or logistical challenges, and a lack of heavy equipment access when carcasses are reported. Cases of large, decomposed sperm whales have also been reported from American Samoa and Guam, where very limited sampling was possible. Two of the five partial necropsies were carcasses in advanced states of decomposition, and cause of death is unknown. Two other partial necropsies represent a stranded calf where cultural practitioners requested limited sample collection and cause of death is unknown, the other was moderately decomposed, but analysis of the samples collected from this event are currently underway. A perinatal calf that was stranded in 2011 off O'ahu allowed for a full necropsy and comprehensive investigation. This individual was initially deemed to represent a *Brucella* and morbillivirus co-infection (West et al. 2015) where cause of death was attributed to brucellosis. Since this report, this same individual has also tested positive for a gamma herpes virus and circovirus, representing an immunosuppressed individual with multiple pathogens present (Clifton et al. 2023).

False killer whales (Pseudorca crassidens)

False killer whales in the Pacific Islands include an endangered insular main Hawaiian Islands population, where most recent abundance estimates suggest only 162 individuals remain (Bradford et al. 2018). Six individuals were examined and necropsied from the insular main Hawaiian island population between 2006 and 2022, and two individuals were necropsied that were not insular false killer whales. A pelagic false killer whale that died from its tail being wrapped in fishing line was recovered by the NOAA/NMFS Pacific Islands Region Fishery Observer Program and necropsied, and a partial necropsy was conducted

for a false killer whale that stranded in Rota, Commonwealth of the Northern Mariana Islands, during this period. All six individuals examined from the insular main Hawaiian population were adults, with only one of these not identified using the Cascadia Research Collective photo identification catalog. One of these individuals died from cardiac failure, and another was extremely emaciated with changes consistent with chronic heart valve disease, endocardiosis, pneumonia, and adrenalitis. Another died of a pulmonary embolism. Verminous pterygoid sinusitis was observed in two cases, and one had a severe intestinal infection with *Acantocephalus* sp. Incidental findings that were not associated with cause of death include the ingestion of fishing hooks in two of the animals from the insular main Hawaiian Islands population, other debris ingestion, including a laundry soap cap, and ingestion of a fishing hook and gear in the pelagic false killer whale that died from its tail being wrapped in line.

Spotted dolphins (Stenella attenuata)

Pantropical spotted dolphin strandings are rare throughout the Pacific Islands region. Cause of death investigations have been conducted for a total of six partial or fully necropsied spotted dolphins in the region that stranded between 2006 and 2022. Two of these cases represent strandings from Guam, one was a calf, and the other was an adult female and unborn calf that both died from dystocia. Two of the strandings from the main Hawaiian Islands represent animals whose cause of death is related to pneumonia, where one of these emaciated individuals also suffered from renal disease. One of the examined animals had significant bone disease. Two of the spotted dolphins examined had evidence of trauma, a calf had signs of blunt trauma to the side of the head, and decompression sickness was observed in multiple organ systems. Another spotted dolphin was recovered by the NOAA/MMFS Pacific Islands Fishery Observer program near the main Hawaiian Islands following a fatal fishery interaction.

Striped dolphins (Stenella coerulealba)

Between 2006 and 2022, 26 strandings of Pacific Island striped dolphins were recorded, and 57% (15/26) of these individuals suffered from natural disease. In eight cases, animals were in poor body condition, indicative of chronic disease. Neurobrucellosis and infectious bronchopneumonia were diagnosed in several of the cases. The Health and Stranding Lab has detected *Brucella* infection by PCR in eight different species to date, but striped dolphins have a high frequency of testing positive for this pathogen. This includes three positive striped dolphins stranded within a 26-day period in 2021 with pathological changes in the brain consistent with severe infection in two of the three animals that were fresh dead and allowed for histopathological examination. Additionally, fusion of the Atlanto-occipital joint has been observed in several striped dolphins, which is indicative of significant bone disease that may be a direct result of *Brucella* infection. We also report on a blunt trauma death with skull fractures consistent with a vessel strike in a stranded striped dolphin. Incidental findings in this species include healed lesions documented at the time of necropsy that are consistent with prior fishery interactions. Investigations into the nine cases that thus far are undetermined are ongoing and consist of additional histological examination of tissues and pathogen screening by PCR.

Spinner dolphins (Stenella longirostris)

Partial or full necropsies were conducted for 38 spinner dolphins out of a total of 42 stranded specimens of this species over the time period examined (2006-2022). Spinner dolphins are near-shore residents in Hawaiian waters, and therefore we anticipate that a greater number of strandings were publicly reported and observed when compared to pelagic cetacean species. Trauma led to death in four cases and included blunt trauma and abdominal injury likely due to a harpoon. Changes consistent with entrapment were

found in two cases. Infectious diseases identified in this species include fatally disseminated toxoplasmosis, brucellosis, herpes virus infection, and systemic mycosis. In isolated spinner dolphin stranding cases, we also observed a gastro-intestinal stricture that likely led to the death of one animal, dystocia that likely resulted in the deaths of mother and calf, an entanglement that led to drowning, and a lethal fishery interaction. We also report on a case of Atlanto-occipital fusion in a spinner dolphin that represents significant bone disease.

Rough-toothed dolphin (Steno bredanensis)

Examinations of rough-toothed dolphins between 2006 and 2022 in the Pacific Islands region were rare. Full necropsies were conducted on three individuals, and the head was examined, and samples collected from a fourth individual that was by-caught and obtained from the NOAA/NMFS Pacific Islands Fishery Observer program. Two of the full necropsies of rough-toothed dolphins were of by-caught animals obtained by the Fishery Observer program in American Samoa. The other full necropsy was of a fresh, dead stranded specimen that had orchitis and died of septicemia. Bacteria were confirmed in lesions examined. The septicemia that caused the death of this individual may have been secondary to immunosuppression by another pathogen.

Bottlenose dolphins (Tursiops truncatus)

During the period between 2006 and 2022, five stranded bottlenose dolphins were examined, with all of these individuals stranding in the main Hawaiian Islands. Bacterial disease and endotoxemia were diagnosed in a bottlenose dolphin in good body condition. An x-ray of a case in moderate decomposition in terms of carcass condition demonstrated an ingested fishhook lodged in the esophagus of the animal that was confirmed to have penetrated during necropsy. The fishhook ingestion in this case was believed to be lethal (Table 5).

Cuvier's beaked whales (Ziphius cavirostris)

Twelve Cuvier's beaked whale strandings were investigated in the Pacific Islands between 2006 and 2022. These stranding locations spanned the main Hawaiian Islands, American Samoa, Guam, and Saipan, and partial or full necropsies were conducted in nine of these cases. In four cases, cranial hemorrhage and microvascular hemorrhages were noted, but one of these animals had bronchopneumonia and was categorized as in good body condition, natural disease, while the other three were categorized as trauma. A cervical vertebral fracture caused death in another case that was categorized as trauma. Significant renal parasitism by *Crassicauda* sp. Was documented in three of the adult cases examined. The parasitism was so severe that it was initially thought to contribute to stranding. However, beaked whales taken in a Japanese fishery also exhibited extreme renal parasitism in almost all healthy individuals examined (Robert Brownell, Pers. Comm.).

We report on significant pathological changes found in 250 cause-of-death investigations of stranded cetaceans representing 20 species between 2006 and 2022. Carcass recovery of cetaceans is low as many animals die out at sea and do not strand. However, gross, and histopathological findings, plus ancillary diagnostic tests to detect infectious disease, provide insight into morbidity and mortality. Morbillivirus and *Brucella* infections that cause respiratory and neurological disease cause mortality in a number of species, including the striped dolphin, Longman's beaked whale, and sperm whale. *Brucella* infections may contribute to reproductive loss, as evidenced by dystocia in a humpback whale where *Brucella* was detected in the calf. *Toxoplasma* infection led to deaths of two spinner dolphins during the time period

examined, with an earlier spinner dolphin fatality also documented. Pygmy and dwarf sperm whale, as well as beaked whale and pilot whale carcasses, showed heavy parasitism by nematodes, cestodes, and trematodes that may indicate nutritional or environmental stress or underlying infections leading to immunosuppression. Blunt trauma was observed in *Kogia spp*., and beaked whales, both deep diving whales. Vertebral and skull fractures and brain hemorrhage, likely due to direct vessel strikes were observed in a pygmy sperm whale, humpback whale calf, and striped dolphin. Fishery interactions threaten dolphin species and false killer whales. Plastic debris, and/or fishery debris have been found in stomachs of many species, which may lead to obstruction and death. Notable is the general absence of neoplasia or tumors in stranded animals, though many examined animals were older individuals.

Table 5. Main morphological and etiologic diagnoses in animals included in 'pathology associated with fishery interaction or trauma' of all causes.

ID	Species	Morphological diagnosis	Etiological diagnosis
GG-9135	*G. griseus*	Integumentary scarring and penetrating, focal, incisive wound	Hooking injury, entanglement
GM-1721	*G. macrorhynchus*	Gastritis with foreign body, osteoporosis	Foreign material ingestion
GM-9927	*G. macrorhynchus*	Ulcerative gastritis with foreign body (20lbs)	Net and fishing gear ingestion
KB-4319	*K. breviceps*	Pulmonary edema, systemic parasitosis, verminous gastritis	Pulmonary edema (trauma), systemic parasitosis (Phyllobothriosis)
KB-9355	*K. breviceps*	Fractures, subcutaneous craniofacial hematoma, right ear, subcutaneous phyllobothriosis, verminous gastritis	Fractures and hematoma (trauma), verminous gastritis, pterygoid sinusitis
KB-2757	*K. breviceps*	Focal subcutaneous hemorrhage, systemic parasitosis, epicardial gas embolism	Subcutaneous hemorrhage (trauma), verminous gastritis, pterygoid sinusitis
KB-7347	*K. breviceps*	Multiple hemorrhages in musculature and internal organs, pulmonary edema	Hemorrhage (trauma)
KB-5324	*K. breviceps*	Bilateral mandibular comminuted fracture, comminuted axial fracture, systemic gas embolism with hemorrhage, dilated cardiomyopathy	Trauma, decompression sickness, dilated cardiomyopathy
KB-4803	*K. breviceps*	Pulmonary edema, verminous gastritis, multifocal granulomatous balanitis	Trauma, infectious balanitis, verminous gastritis
KB-7354	*K. breviceps*	Subcutaneous and muscular hemorrhage around right ear, spinal hemorrhage, incomplete squamosal fracture, unilateral pleurisy, ulcerative gastritis	Blunt trauma
KS-7686	*K. sima*	Pulmonary edema	Trauma (entrapment)
LH-2901	*L. hosei*	Compound mandibular and cervical vertebral fracture acute, with extensive hemorrhage, vascular gas embolism	Trauma, decompression sickness
LH-4931	*L. hosei*	Compound mandibular fracture with hemorrhage, internal diffuse hemorrhages	Trauma
LH-4050	*L. hosei*	Diffuse internal hemorrhage	Trauma
LH-7528	*L. hosei*	Penetrating abdominal wounds (2)	Trauma (gunshot)
MN-4272	*M. novaeangliae*	Costal fracture (rib 1), muscular trauma, fibrinous, necrotizing epicarditis	Costal fracture, septicemia with intralesional bacteria
MN-3534	*M. novaeangliae*	Pulmonary edema, cranial hemorrhage, cerebral hemorrhage	Trauma
PC-4290	*P. crassidens*	Fishhook ingestion	Trauma (fishery bycatch)
SA-	*S. attenuata*	Nonspecific	Trauma (entanglement)

3595			
SA-1729	*S. attenuata*	Vascular gas embolism, subcutaneous hemorrhage left ventral side	Trauma, decompression sickness
SC-6909	*S. coeruleoalba*	Occipital and mandibular fracture, hematoma	Trauma (boat)
SL-6047	*S. longirostris*	Subcutaneous hemorrhage and bruising ventrally, left laterally	Trauma
SL-6417	*S. longirostris*	Nonspecific	Trauma (entanglement)
SL-7045	*S. longirostris*	Abdominal wound	Trauma (fishery interaction)
SL-2466	*S. longirostris*	Nonspecific	Trauma (entanglement)
TT-2908	*T. truncatus*	Fishhook ingestion	Trauma (fishhook)
ZC-4990	*Z. cavirostris*	Left cerebral ventricular hemorrhage, L mandibular hematoma, nephropathy	Trauma (acoustic, blast)
ZC-2247	*Z. cavirostris*	Cranial hematoma, cerebral hemorrhage	Trauma (acoustic, blast)
ZC-8769	*Z. cavirostris*	Cervical vertebral fracture (C1)	Trauma
ZC-2875	*Z. cavirostris*	Microvascular hemorrhages, multiple organs	Barotrauma, Blast trauma

Table 6. Main morphological and etiologic diagnoses in animals included in 'perinatal, neonatal' category.

ID	Species	Morphological Diagnosis	Etiological Diagnosis
GM-5409	*G. macrorhynchus*	Acute bronchopneumonia	Parasitic bronchopneumonia (*Halocercus* sp.)
MN-4155	*M. novaeangliae*	Dermatitis, epidermal-dermal separation, focal granulomatous dermatitis and perirenal eosinophilic steatitis	Infectious dermatitis, parasitic migration, perirenal
MN-5816	*M. novaeangliae*	Pulmonary edema, cranial contusion	Trauma
MN-8248	*M novaeangliae*	Dystocia	Breech birth (Trauma)
PE-2938	*P. electra*	Lymphoid hypoplasia	Premature birth
PE-1572	*P. electra*	Mild lymphoid meningitis	Infectious meningitis
PE-5246	*P. electra*	Foul fetal membranes	Infection, fetal membranes
PM-3464	*P. macrocephalus*	Non suppurative meningitis	Infectious meningitis (viral, bacterial)
SA-4262	*S. attenuata*	Pulmonary atelectasis, still birth	Dystocia
SL-7063	*S. longirostris*	Birth trauma	Dystocia (umbilical cord obstruction)

Table 7. Main morphological and etiologic diagnoses in animals included in 'pathology associated with poor nutritional status'.

ID	Species	Morphologic diagnoses	Etiologic diagnoses
FA-7449	*F. attenuata*	Cachexia, hepatic lipidosis and glycogenosis	Undetermined
FA-2937	*F. attenuata*	Lymphocytic meningitis, focal granulomatous gastritis	Neurobrucellosis, parasitic gastritis
FA-7995	*F. attenuata*	Lymphocytic meningitis, interstitial pneumonia, myocarditis	Neurobrucellosis, bacterial pneumonia
GG-7370	*G. griseus*	Dilated cardiomyopathy, ulcerative gastritis	Hepatic and gastric parasitosis
KB-5948	*K. breviceps*	Dilated cardiomyopathy, mandibular fractures, bilateral	Dilated cardiomyopathy, perimortal mandibular fracture
KS-8901	*K. sima*	Bronchopneumonia, ulcerative gastritis	Bronchopneumonia, bacterial septicemia
LH-2283	*L. hosei*	Subcutaneous phyllobothriosis, hepatic telangiectasia, chronic lymphadenitis, microhemorrhages	Systemic viral infection, cachexia, phyllobothriosis
LH-1112	*L. hosei*	Focal granulomatous dermatitis, lymphadenopathy, encephalopathy with multifocal neuronal loss, microgliosis and lipofuscinosis	Fungal dermatitis (Lobomycosis), chronic encephalopathy
MD-2108	*M. densirostris*	Pyogranulomatous pneumonia, ulcerative, perforating gastritis, acute renal tubular necrosis, mandibular fracture associated with hemorrhage (peri mortal)	Fungal pneumonia (Aspergillosis), viral infection
PC-6644	*P. crassidens*	Endocardiosis (L AV valve), necrotizing adrenalitis, interstitial pneumonia,	Infectious pneumonia, endocardiosis

		renal pigmentary nephrosis, necro suppurative conjunctivitis	
PE-8824	*P. electra*	Granulomatous pneumonia	Fungal pneumonia (Aspergillosis)
PE-7757	*P. electra*	Interstitial pneumonia, chronic pancreatitis	Infectious pneumonia (bacteria, virus), pancreatic trematodiasis
PE-6423	*P. electra*	Non suppurative meningitis, meningeal fibrosis, pterygoid sinusitis	Verminous pterygoid sinusitis, infectious meningitis
PE-5132	*P. electra*	Lymphadenopathy, pulmonary fibrosis, pterygoid sinusitis	Verminous pterygoid sinusitis, systemic parasitosis, infectious pneumonia
PE-7548	*P. electra*	Pneumonia, sinusitis, septicemia - fetal membranes?	Verminous pterygoid sinusitis, septicemia
SA-9230	*S. attenuata*	Pneumonia, renal disease	Verminous pneumonia
SB-9078	*S. bredanensis*	Orchitis, septicemia	Septicemia
SC-4198	*S. coeruleoalba*	Meningitis	Infectious meningitis
SC-9044	*S. coeruleoalba*	Meningitis	Infectious meningitis
SC-6274	*S. coeruleoalba*	Cardiomegaly, atrial infarct	Cardiac arrhythmia secondary to infarct
SC-8788	*S. coeruleoalba*	Chronic pneumonia, meningoencephalitis, myocardial fibrosis	Verminous pneumonia, infectious meningoencephalitis
SC-4690	*S. coeruleoalba*	Non suppurative meningitis, fusion C-1- occipital bone	Neurobrucellosis
SC-1142	*S. coeruleoalba*	Non suppurative meningitis	Infectious meningitis
SC-1763	*S. coeruleoalba*	Non suppurative meningitis	Neurobrucellosis
SC-6259	*S. coeruleoalba*	Meningitis	Neurobrucellosis
SL-6326	*S. longirostris*	Mastitis, septicemia	Septicemia secondary to mastitis
SL-8088	*S. longirostris*	Non suppurative meningitis, severe, chronic	Infectious meningitis
SL-2287	*S. longirostris*	Systemic lymphadenitis, granulomatous pneumonia	Fungal pneumonia, systemic fungal infection
SL-6111	*S. longirostris*	Non suppurative meningoencephalitis	Infectious meningencephalitis
SL-2694	*S. longirostris*	Granulomatous myocarditis	Infectious myocarditis
SL-1047	*S. longirostris*	Bronchopneumonia	Bacterial bronchpneumonia
SL-4200	*S. longirostris*	Disseminated systemic necrotizing inflammation	Acute toxoplasmosis
SL-9678	*S. longirostris*	Disseminated systemic necrotizing inflammation	Acute toxoplasmosis

Table 8. Main morphological and etiologic diagnoses in animals included in 'pathology associated with good nutritional status'.

ID	*Species*	Morphologic diagnosis	Etiologic diagnosis
FA-9239	*F. attenuata*	Chronic-active pleuropneumonia, epicarditis, myocardial atrophy and degeneration, hepatic lipidosis	Bacterial septicemia and pleuropneumonia
FA-6436	*F. attenuata*	Lymphocytic meningitis, fibrinous, pyogranulomatous bronchopneumonia, chronic lymphadenitis	Infectious meningitis and bronchopneumonia, parasitic pyogranulomatous pneumonia
FA-9529	*F. attenuata*	Lymphadenopathy, fibrinous bronchopneumonia	Infectious lymphadenopathy, bacterial bronchopneumonia
FA-9519	*F. attenuata*	Acute pterygoid sinusitis	Parasitic pterygoid sinusitis
FA-8456	*F. attenuata*	Broncho interstitial pneumonia, lymphadenopathy	Infectious pneumonia
GM-3500	*G. macrorhynchus*	Systemic gas embolism, granulomatous gastritis, pericystitis, myocarditis	Gas embolism, systemic parasitosis
GM-1694	*G. macrorhynchus*	Non-suppurative meningoencephalitis, pterygoid sinusitis and facial contusion	Infectious meningoencephalitis, pterygoid and facial trauma
GM-8865	*G. macrorhynchus*	Pterygoid sinusitis, ulcerative gastritis, parasitic dermatitis	Verminous pterygoid sinusitis (*Stenurus* sp.? *Crassicauda*?), gastric nematodiasis, cutaneous phyllobothriosis
GM-5331	*G. macrorhynchus*	Pterygoid sinusitis and gastritis	Verminous pterygoid sinusitis, verminous gastritis
GM-5665	*G. macrorhynchus*	Ulcerative gastritis, mucometra, pterygoid sinusitis, retroperitoneal hematoma	Verminous pterygoid sinusitis (*Stenurus* sp., *Crassicauda*), verminous gastritis, traumatic retroperitoneal hematoma
GM-4462	*G. macrorhynchus*	Pterygoid sinusitis, pulmonary edema, focal glossitis	Traumatic and stress related pulmonary edema (stranding event), verminous pterygoid sinusitis
GM-3151	*G. macrorhynchus*	Pterygoid sinusitis, ulcerative gastritis, dermatosis	Verminous pterygoid sinusitis and gastritis, dermatitis by Xenobalanus
IP-4830	*I. pacificus*	Open mandibular and maxillary fracture, subacute encephalitis, fibrinous Pneumonia, periglomerulonephtritis, lymphoid depletion, multifocal non-suppurative myocarditis	Viral encephalitis, bacterial pneumonia, septicemia, maxillary and mandibular fracture (trauma), infectious multifocal myocarditis
KB-9806	*K. breviceps*	Bilateral mandibular and palatine fracture, verminous gastritis, glomerulopathy	Verminous gastritis, glomerulopathy, peri mortal mandibular and palatine fractures
KB-1608	*K. breviceps*	Chronic gastritis, dermatosis, pulmonary edema	Verminous gastritis, cutaneous phyllobothriosis, pulmonary edema (trauma)
KB-8100	*K. breviceps*	Chronic gastritis	Verminous gastritis
KB-3756	*K. breviceps*	Chronic gastritis, dilated cardiomyopathy, mandibular fracture (peri mortal)	Verminous gastritis, dilated cardiomyopathy, muscular endoparasitism (sarcocystis), peri mortal mandibular fracture (trauma)

KB-5110	*K. breviceps*	Cardiac tamponade, chronic gastritis	Cardiac tamponade (cardiomyopathy), verminous gastritis
KS-8329	*K. sima*	Multifocal myocardial fibrosis and hypertrophy	Cardiomyopathy of unknown origin
MN-6969	*M. novaeangliae*	Dystocia	Dystocia (breech birth)
PC-5516	*P. crassidens*	Pulmonary and cardiac fibrosis	Cardiac failure of unknown origin
PC-5206	*P. crassidens*	Gastric foreign bodies, pterygoid sinusitis	Verminous pterygoid sinusitis, intestinal acanthocephaliasis
PC-3118	*P. crassidens*	Pterygoid sinusitis	Verminous pterygoid sinusitis
PE-3796	*P. electra*	Interstitial pneumonia	Infectious pneumonia
PE-6633	*P. electra*	Lymphadenopathy	Infectious lymphadenopathy
PE-1540	*P. electra*	Interstitial pneumonia, non-suppurative meningitis, chronic gastritis	Infectious pneumonia and meningitis, parasitic gastritis and lymphadenitis
SA-3674	*S. attenuata*	Pneumonia	Verminous, bacterial pneumonia and septicemia
SA-8182	*S. attenuata*	Dystocia	Dystocia
SC-9107	*S. coeruleoalba*	Non-suppurative meningoencephalitis, granulomatous bronchopneumonia	Neurobrucellosis, infectious bronchopneumonia
SC-3597	*S. coeruleoalba*	Meningitis, pulmonary fibrosis, cervical vertebral fusion	Infectious meningitis
SC-1774	*S. coeruleoalba*	Meningitis, granulomatous lymphadenitis (eosinophilic)	Infectious meningitis, endoparasitism
SC-9761	*S. coeruleoalba*	Meningitis, lymphadenopathy	Neurobrucellosis
SL-7063	*S. longirostris*	Dystocia	Dystocia
TT-4860	*T. truncatus*	Hepatitis and pancreatitis	Infectious (bacterial) hepatitis, pancreatitis, endotoxemia
ZC-8534	*Z. cavirostris*	Vasculitis, nephropathy	Systemic parasitosis (vascular and renal), Crassicaudiasis
ZC-1099	*Z. cavirostris*	Suppurative bronchopneumonia, nephropathy	Infectious bronchopneumonia, renal Crassicaudiasis
ZC-4990	*Z. cavirostris*	Vasculitis, nephropathy	Renal Crassicaudiasis

FUTURE WORK

Immediately, next steps will focus on completion of a historical stranding analysis in the Pacific Islands that includes a spatial and temporal analysis with environmental factors. We will also further our interpretation of causes of death in Pacific Island cetaceans and finalize pathological findings according to species. Our cause of death examinations indicates a high percentage of stranded animals that were in poor body condition, with heavy parasitic burdens and/or suffering from multiple chronic infections suggestive of a poor immune status. Immunosuppression has been associated with viral infection in cetaceans, but also exposure to PCBs and other anthropogenic hazardous substances (Jepson et al. 2016). Future work could include an examination of immunosuppression using T-cell and B-cell markers to better understand the ability to respond to pathogens, which may be important in the continued monitoring of cetacean population health in the Pacific.

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