

## **Comprehensive Stranding Investigations for High Priority Species**

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### ***ABSTRACT***

This project provides the U.S. Navy with comprehensive cetacean stranding investigations that are essential for establishing and maintaining baseline information on marine mammal health across the Pacific Islands Region (PIR). Sustained baseline monitoring of marine mammal health, causes of injury, and causes of mortality within Navy training and testing areas is critical for meeting federal regulatory and permitting requirements. For stranded cetaceans, thorough cause-of-death investigations are the only direct means of assessing serious injury and mortality, including potential anthropogenic impacts. Necropsy examinations, sample collection, and diagnostic analyses also provide critical insight into sub-lethal stressors and other health threats that may have population-level consequences. In addition, rigorous investigations and evidence-based determinations help dispel misinformation, support accurate public understanding of stranding events, and ensure that management decisions are grounded in the best available science.

In the Pacific Islands Region (PIR), continued investment in cetacean stranding investigations is particularly important because the region poses distinct geographic, logistical, and cultural considerations that make this work both especially difficult and especially valuable. The PIR encompasses more than 4 million square miles across the North, South, and Western Pacific, including both the Mariana Islands Training and Testing area and the Hawaii-Southern California Training and Testing area. Responding to strandings across this vast and highly remote region requires substantial logistical effort, coordination, and sustained readiness, particularly in Hawai'i and other isolated Pacific Island locations. Recovery rates are also likely among the lowest in the world; in Hawai'i, data suggest that across 20 cetacean species, only approximately 1% of animals that die are recovered. This makes each recovered carcass an exceptionally important opportunity to investigate causes of mortality, assess health status, and collect archival samples that may provide some of the only biological insight into these poorly known populations. The region also requires sustained investment in coordination and relationship building with local partners and communities, particularly in Hawai'i, where marine mammals are of cultural significance. These place-based relationships are essential to ensuring that stranding investigations are conducted respectfully, effectively, and in a manner that supports both scientific and management needs.

Stranding response capacity for the PIR is centralized at a dedicated facility located at Marine Corps Base Hawaii (MCBH). This specialized center houses the University of Hawai'i (UH) Health and Stranding Lab, the only organization in the region equipped to conduct cause of death investigation for stranded dolphins and whales. This work relies on strong partnerships across the region and the ability to rapidly mobilize responses to each reported stranding event. The UH Health and Stranding Lab performs comprehensive cause of death investigations and analysis of

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| <b>14. ABSTRACT</b><br><p>This project provides the U.S. Navy with comprehensive cetacean stranding investigations that are essential for establishing and maintaining baseline information on marine mammal health across the Pacific Islands Region (PIR). Sustained baseline monitoring of marine mammal health, causes of injury, and causes of mortality within Navy training and testing areas is critical for meeting federal regulatory and permitting requirements. For stranded cetaceans, thorough cause-of-death investigations are the only direct means of assessing serious injury and mortality, including potential anthropogenic impacts. Necropsy examinations, sample collection, and diagnostic analyses also provide critical insight into sub-lethal stressors and other health threats that may have population-level consequences. In addition, rigorous investigations and evidence-based determinations help dispel misinformation, support accurate public understanding of stranding events, and ensure that management decisions are grounded in the best available science. In the Pacific Islands Region (PIR), continued investment in cetacean stranding investigations is particularly important because the region poses distinct geographic, logistical, and cultural considerations that make this work both especially difficult and especially valuable. The PIR encompasses more than 4 million square miles across the North, South, and Western Pacific, including both the Mariana Islands Training and Testing area and the Hawaii-Southern California Training and Testing area. Responding to strandings across this vast and highly remote region requires substantial logistical effort, coordination, and sustained readiness, particularly in Hawai'i and other isolated Pacific Island locations. Recovery rates</p> |  |                                                       |  |

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This work also provides the foundation for numerous research efforts aimed at identifying and evaluating threats to Pacific Island cetaceans. In-house advanced diagnostics include investigations into identified threats such as contaminant burdens and marine debris ingestion. This project also supports progress on an optional task to measure fecal stress and reproductive hormones in Pacific Island cetaceans that aims to advance capabilities for health monitoring of free-swimming cetaceans.

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archival stranding samples to assess anthropogenic impacts on Pacific Island cetaceans, with particular emphasis on potential effects associated with naval training and testing activities.

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### ***SUMMARY OF STRANDING CASES DURING CALENDAR YEAR 2025***

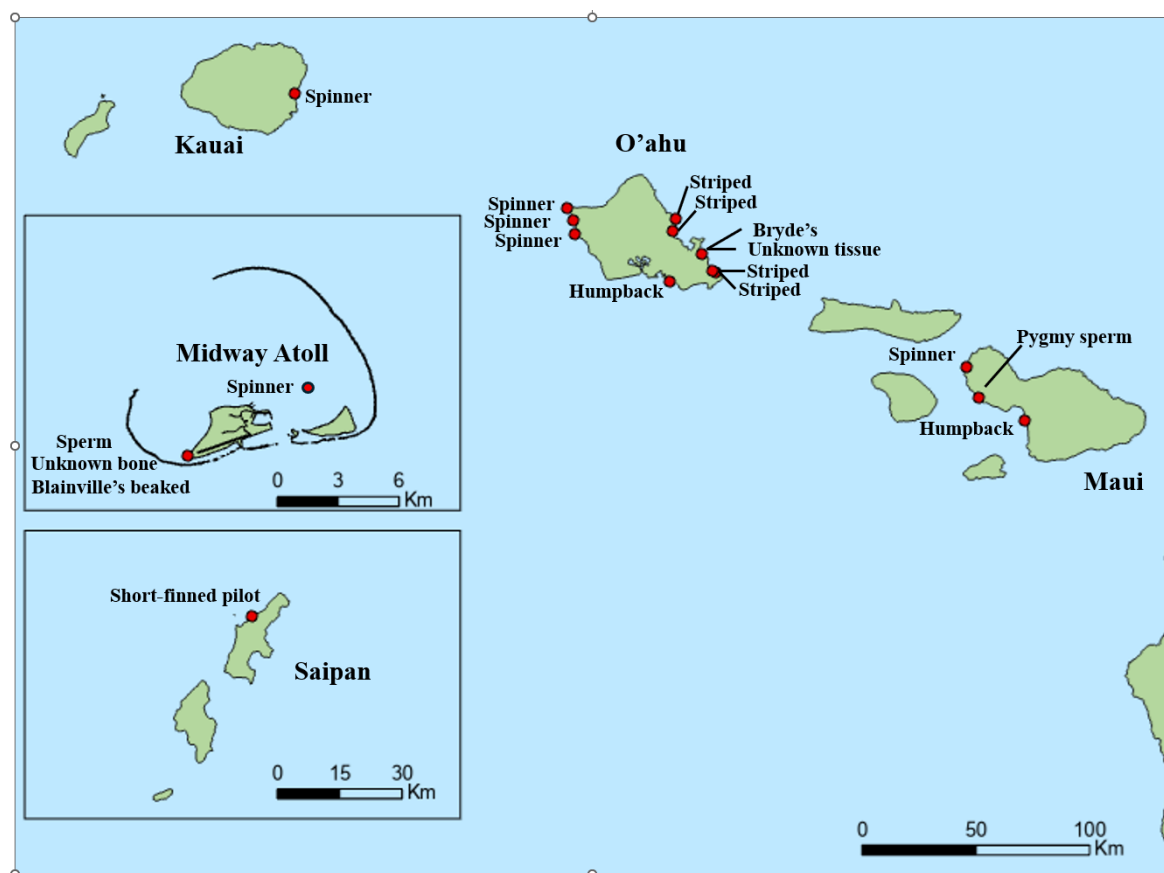
Stranding response, necropsy, and cause of death investigative summaries were provided for 19 stranding events in quarterly reports that occurred during the 2025 calendar year. In each of these cases, obtained specimen samples were given a unique specimen identification number that allows for long-term tracking of any analyses within the Health and Stranding Lab.

In addition to cetacean strandings or other rare events where cetacean specimen samples are obtained and tracked with a unique identifier, responses were mounted for a number of additional cetacean strandings that were confirmed but where limitations precluded necropsy and/or sample collection. In some cases, if a stranding response effort for a confirmed cetacean stranding event was significant, these events are included in the quarterly reports even if a unique specimen identified is not assigned. An example of one of these types of stranding events occurred on October 18th, 2025 when a deceased spinner dolphin was seen floating near Waianae on the island of O‘ahu. Photographs and locational data were available to confirm the report but the deceased calf was still with its assumed mother and could not be found when searching the area later that day nor the following day. No carcass or samples were recovered from this stranding response event. There were additional reports in the 2025 calendar year, but the images and reports obtained in those cases from the general public were not confirmed by first responders or were handled by NOAA’s Large Whale Entanglement Response.

During the 2025 calendar year, the UH Health and Stranding Lab coordinated or conducted stranding responses, necropsy, and sample collection from three of the main Hawaiian Islands including Kaua‘i (1), Maui (3), and O‘ahu (10), as well as Midway Atoll (4). We also report on a stranding event in Saipan (1) (Figure 1). Some of these responses were conducted on live animals that were distressed on O‘ahu. In 2025 the UH Health and Stranding Lab responded to a confirmed total of 7 species including humpback whale (*Megaptera novaeangliae*) (2), Blainville’s beaked whale (*Mesoplodon densirostris*) (1), sperm whales (*Physeter macrocephalus*) (1), striped dolphin (*Stenella coeruleoalba*) (4), Bryde’s whale (*Balaenoptera byrdei*) (1), pygmy sperm whale (*Kogia breviceps*) (1), spinner dolphins (*Stenella longirostris*) (6), unknown *Stenella* (1), and unknown cetacean (1) (Table 1). Collectively, across the stranding responses in calendar year 2025, the recovered specimens represented both male and female sexes, and various reproductive stages including newborn calves, juveniles and sexually mature adults.

**Table 1.** Cetacean stranding cases by species in 2025 where the Health and Stranding Lab was involved in mounting a stranding response, coordination, and sampling. Sex abbreviations are as follows: M = Male, F = Female and U = Unknown. Age classes are categorized as C = calf, SA = Sub-adult, A = Adult and U = Unknown.

| Common Name               | Species                 | Sex | Age Class | Stranding Date | Necropsy Date | Stranding Location |
|---------------------------|-------------------------|-----|-----------|----------------|---------------|--------------------|
| humpback whale            | <i>M. novaeangliae</i>  | M   | C         | 1/2/2025       | 1/3/2025      | Maui               |
| Blainville's beaked whale | <i>M. densirostris</i>  | F   | U         | 1/7/2025       | 1/8/2025      | Midway Atoll       |
| sperm whale               | <i>P. macrocephalus</i> | U   | U         | 2/11/2025      | na            | Midway Atoll       |
| humpback whale            | <i>M. novaeangliae</i>  | U   | C         | 4/12/2025      | na            | O'ahu              |
| striped dolphin           | <i>S. coeruleoalba</i>  | F   | A         | 4/22/2025      | 4/23/2025     | O'ahu              |
| striped dolphin           | <i>S. coeruleoalba</i>  | M   | A         | 6/7/2025       | 6/8/2025      | O'ahu              |
| striped dolphin           | <i>S. coeruleoalba</i>  | M   | SA        | 6/8/2025       | 6/8/2025      | O'ahu              |
| striped dolphin           | <i>S. coeruleoalba</i>  | F   | SA        | 6/14/2025      | 6/15/2025     | O'ahu              |
| Bryde's whale             | <i>B. brydei</i>        | U   | U         | 7/4/2025       | na            | O'ahu              |
| spinner dolphin           | <i>S. longirostris</i>  | U   | U         | 6/23/2025      | na            | Midway Atoll       |
| pygmy sperm whale         | <i>Kogia breviceps</i>  | U   | U         | 7/12/2025      | 7/13/2025     | Maui               |
| spinner dolphin           | <i>S. longirostris</i>  | M   | C         | 7/27/2025      | 7/27/2025     | Kaua'i             |
| spinner dolphin           | <i>S. longirostris</i>  | F   | C         | 7/30/2025      | 7/31/2025     | Maui               |
| unknown animal            | unknown cetacean        | U   | U         | 8/12/2025      | na            | O'ahu              |
| spinner dolphin           | <i>S. longirostris</i>  | M   | C         | 8/20/2025      | 8/28/2025     | O'ahu              |
| spinner dolphin           | <i>S. longirostris</i>  | F   | C         | 9/28/2025      | 9/28/2025     | O'ahu              |
| spinner dolphin           | <i>S. longirostris</i>  | U   | C         | 10/18/2025     | na            | O'ahu              |
| unknown bone              | <i>Stenella spp.</i>    | U   | A         | na             | na            | Midway Atoll       |
| short-finned pilot whale  | <i>G. macrorhynchus</i> | U   | SA        | 11/11/25       | na            | Saipan             |



**Figure 1.** Cetacean stranding locations by species in 2025 across the main Hawaiian Islands, Midway Atoll and Saipan.

Case highlights from quarterly reports submitted during the reporting period are included below. These include a rare Blainville's beaked whale on Midway Atoll, the last of four striped dolphins stranded on the island of O'ahu in a two-month period, documentation of the first Bryde's whale stranding in Hawai'i, a case of fatal acute infection in a spinner dolphin calf on the island of O'ahu and available details regarding the short-finned pilot whale stranding in Saipan.

***Blainville's Beaked whale, Mesoplodon densirostris, Midway Atoll, NWHI***

Stranding of a suspected Blainville's beaked whale reported to Dr. Kristi West at the Health and Stranding Lab on January 7th, 2025, by Caroline Pott, Midway Atoll National Wildlife Refuge, United States Fish and Wildlife Service,) at Midway Atoll (Figure 2). Stranding response was complicated by the lack of cell phone coverage at the site of the stranding. Guidance was provided by Dr. West over the phone prior to sample collection on the 7th, and a necropsy was conducted the following day. The length of the animal was recorded to be 3.7 m, and in a state of moderate decomposition. There was no externally obvious cause of death reported by the USFWS biologist who sampled the animal, and no evidence of entanglement. The head was removed from the body, and several organ samples were collected at necropsy. These were put into bags and frozen until they could be transported to the Health and Stranding Lab facility with the next supply vessel trip that occurred over a month later.

Because there was uncertainty regarding the species of the animal, a small skin sample was sent by plane the week following the stranding event for genetic analysis to determine the species identification. This sample allowed the Health and Stranding Lab to identify the animal genetically as a Blainville's beaked whale. Blainville's beaked whales are common in the Northwestern Hawaiian Islands and known to strand both at Midway atoll and other US-affiliated Pacific islands, including in the Hawaiian Islands (Brownell et al. 2024). Before it was identified using genetics,



**Figure 2.** Blainsville's beaked whale stranded at Midway Atoll.

it was suspected that this individual could represent another rare species of beaked whale that have been acoustically detected in the region. The coloration and anatomy visible in the images provided by the field biologists was not sufficient to confidently assign species and genetic confirmation was necessary in this case.

The head and internal organ samples that were collected at necropsy were shipped to O'ahu from Midway Atoll in February and arrived at the Health and Stranding Lab facility for examination on February 19th, 2025. Examination of the skull and sub-sampling of the tissues was led by veterinary pathologist, Dr. Ilse Silva-Krott, on February 20th, 2025. The whole heart was obtained at necropsy, and the gross examination of the heart at the lab indicated that the right ventricle formed the apex of the heart rather than the left ventricle. This was noted as abnormal, however, it is unknown whether this heart abnormality may have contributed to death. The gastrointestinal tract was examined and stomach contents were collected. There was evidence that the animal had recently fed prior to death as partially digested crustaceans, fish bones, eye lenses and squid beaks were present in the stomach. There was no indication that this animal had ingested any plastic debris or fishhooks. Most of the reproductive tract was also examined but the ovaries were not included in the reproductive tract that was collected at necropsy, and it was not possible to assess if past ovulation(s) had occurred.

Examination of the head indicated that the tip of the rostrum had a transverse fracture and the left mandible had multiple fractures typically indicative of blunt force or high impact trauma. The sound channel appeared to have evidence of hemorrhagic tissues, however, we cannot rule out the possibility that this represented blood that pooled after the animal had died. The rostrum and mandibles of these animals are relatively fragile, and because of the state of decomposition, it could not be determined if the fractures occurred peri- or post-mortem. In similar past cases, it is possible to detect fibrin at the site of the bone break, but both this method as well as other histological methods that could be potentially applied to determine if the fractures were peri- or post-mortem were not useful in this case because of state of decomposition. A limited number of internal organ tissues were collected at necropsy, and all tissues routinely screened for diseases that were collected were tested by PCR for toxoplasmosis, *Brucella* and morbillivirus. All tissues tested from this individual were negative by PCR.

### ***Striped Dolphin, *Stenella coeruleoalba*, Waiāhole, O'ahu***

At 2055h on June 14th, 2025, a dolphin was reported deceased near Waiāhole Beach Park on O'ahu. Video footage of the animal had been posted approximately three hours prior on social media and was estimated as a 6-foot striped dolphin. Due to the time of day and lack of information on the specific location, it was decided to wait until first light the following morning to search for the animal. The following morning, the Health and Stranding Lab team, led by Nicholas Hofmann, met at Waiāhole beach park at approximately 0545h and began walking the beach in search of the animal. After communicating with the person who posted the video to social media the animal was located at 0624h. The animal was covered during coordination between Health and Stranding Lab personnel and a cultural practitioner that assisted with this stranding event (Figure 3). It was decided that the cultural practitioner would meet the response team at a secondary location, so the animal was moved off the beach at 0750h and taken to the laboratory facility at MCBH. After arriving at the facility at 0825h, external morphometrics were conducted.



The animal was determined to be a 198 cm female. The individual was estimated at approximately 68 kg. Following morphometric measurements, the animal was put back into a vehicle on ice and transported to an appropriate location for a Hawaiian pule, a prayer or invocation, that was conducted by the practitioner. A ti leaf, considered sacred and used in some Hawaiian rituals for protection, was provided to Dr. West to be present in the room during the necropsy.

The necropsy began with an external examination at 1030h. It was noted that there was moderate bloating around the tongue and lower jaw, this



**Figure 3.** Health and Stranding Lab response team recovering the stranded striped dolphin.

bloating continued into the thoracic and abdominal cavities. Skin was missing on the left side from the ventral surface to the lateral left side of the body to the pectoral fin. Extensive bruising was also noted on the left mandible and ventral surface of the left side. A large mass measuring 12x20 cm was also noted on the left side of the body. There were multiple healed cookie-cutter bites along the body and mottled bruising was seen throughout the ventral surface to the anus. A lesion (Lesion B) was sampled and described as having a large amount of hemorrhaging beneath the skin surface. There was also a fresh superficial wound noted on the peduncle that measured 4 x 5 cm.

The internal examination began at 1102h. It was noted that rib 9 was externally detached from the sternum, however this may have occurred postmortem due to bloating. Cerebral spinal fluid (CSF) was collected and found to be blood with low viscosity, indicative of possible hydrocephalus. Both lungs were dark brown and mottled, necrotic from decomposition, and hemorrhaging was observed in the right lung. Several lymph nodes were enlarged and inflamed. The left prescapular lymph node was mottled and bruised on uncut surface and measured 6 x 7 cm. A lymph node chain near the right lung was collected, that measured 12 cm in length. The mesenteric lymph node was also a long chain of lymph nodes that measured 23 cm in total length. All three chambers of the stomach were empty, but a small amount of sand in one of the chambers suggested that the animal likely stranded alive. The animal has a miliary pattern on cut surface of the lung. Mesenteric hemorrhage was noted in the small intestine and there was a small amount of red-tinged, milky, viscous fluid observed throughout the gastrointestinal tract. Connective tissue adjacent to the colon contained hardened phyllobothria cysts. A section of gut about 18 cm proximal from the colon was distended, this measured approximately 7 cm width and included a sphincter valve. The mucosa was thickened, pale tan in color and formed irregular rugae suggestive of granulomatous inflammation. Approximately 10 ml of light brown fluid feces were collected. Perirenal connective tissue was edematous. No corpora lutea were observed on the ovaries of the reproductive tract suggesting that



this animal was an immature female. Also, significant amounts of phyllobothria were found in proximity to the uterus and anal lymph nodes. The cerebrum appeared flattened and meninges edematous. Upon sectioning, cloudy, white cerebral fluid was collected from lateral ventricles. The cortex of the brain appeared compressed compared to white matter and had expanded ventricular space suggestive of hydrocephalus.

Disease screening using PCR was conducted on various tissues from this case. *Brucella ceti* was detected in CSF, spinal cord, and cerebellar samples and confirmed with sequencing. Twelve other tissue samples that were collected from this same individual tested negative for *Brucella ceti*. Additional negative results included sample testing from 8 tissues that were tested for morbillivirus by PCR. Toxoplasmosis testing by PCR was also conducted, with all tested tissues negative.

A total of 19 Hemoxilyn and Eosin (H&E)-stained slides of formalin-preserved tissues that were collected at necropsy were processed for histopathological examination of tissues. Unfortunately, tissues were autolyzed due to decomposition state and cellular details could not be evaluated on the histological sections.

### **Concerning Mortality Event – Striped dolphins**

After *Brucella ceti* was identified in samples of three striped dolphins that stranded on East O‘ahu shores over seven days, a press release regarding these cases was prepared and released by University of Hawai‘i News on June 23, 2025. The press release included information about the importance of reporting stranded and distressed marine mammals to NOAA. The press release provided information about this potential zoonotic bacterial infection and the risk of disease transfer from a stranded dolphin to humans through direct contact or body fluids and tissues.

While the earlier stranding case of a striped dolphin which occurred in April on the island of O‘ahu was not included in the media coverage, samples of that animal also tested positive for *Brucella ceti*. There was concern that these four stranding cases could represent an outbreak of Brucellosis in Hawaiian striped dolphins. Due to the zoonotic potential of this bacterial pathogen, examination of stranded cetaceans, sampling and analysis of samples is critical for disease surveillance. Molecular analysis of *Brucella ceti* isolated from the April case revealed a bacterial strain that had not previously been identified in Hawai‘i. This newly detected strain in Hawaiian waters groups with the ST-26 strain of *Brucella ceti* that has previously been associated with meningitis and hydrocephalus in dolphins in the Atlantic (East Coast) and the Mediterranean Sea.

### ***Bryde’s Whale, Balaenoptera brydei, Kailua, O‘ahu***

On July 4<sup>th</sup> a member of the public picked up a 2 ft piece of tissue off the beach at Kailua Beach Park that they initially believed to be trash. Upon closer inspection they suspected it may be tissue from a whale and called the NOAA stranding hotline. Nicholas Hofmann responded from the Health and Stranding Lab and arrived at the residence of the caller at 0713h, 29 minutes after the initial call.

The tissue was confirmed to be blubber and connective tissue from a marine mammal (suspected large whale). Upon collection of the tissue Ocean Safety and NOAA were informed to be on alert

for a possible carcass in the area but no further pieces of tissue were recovered. Barnacle growth on the tissue suggested it had been floating in the water for some time. Genetic species identification was conducted in-house at the Health and Stranding Lab and confirmed the tissue to be from a Bryde's whale (*Balaenoptera brydei*). This stranding represents the first known case of a Bryde's whale stranding in Hawaiian waters. This finding was compared against the historical stranding database for the Pacific Islands that dates back to a first recorded event in the mid 1800's, with no prior records of a Bryde's whale stranding in the Hawaiian Islands.

***Spinner Dolphin, Stenella longirostris, Waianae, O'ahu***

At approximately 1310h on September 28, 2025, a dolphin was found floating dead and was pulled out of the water off West O'ahu. The removal from the water was done prior to contacting the NOAA hotline or Health and Stranding Lab personnel. The vessel captain had heard of other boats assisting with cetacean strandings and did not realize at the time that the stranding response team should be contacted prior to carcass recovery. Information regarding potential zoonotic disease exposure was shared with the vessel and their crew. Nicholas Hofmann and additional Health and Stranding Lab personnel met the boat at Waianae small boat harbor at 1430h. The captain and crew were informed about regulations regarding handling of distressed and dead marine mammals and safety concerns regarding zoonotic disease. The animal was retrieved from the harbor and transported to the Health and Stranding Lab facility at MCBH in Kaneohe.

Later in the day another report was made by personnel from a different Waianae tour boat regarding a spinner dolphin calf. The calf was observed swimming erratically and comparison of photographs confirmed that it was the collected deceased animal allowing for an estimate of time of death as late morning.

The necropsy examination of the spinner dolphin calf began at approximately 1800h the same day. The female calf measured 88.5 cm and weighted 8.95 kg. The body condition was classified as robust; to be confirmed by blubber histology analysis. The animal had fetal folds on both sides, and frills on the tongue. There were no erupted teeth on the bottom, but the animal did have around 40 teeth erupted from the maxilla on each side. There were 7 whisker holes on both sides of the rostrum, but only three whisker fragments were present. The umbilicus was fully healed, but the scar appeared fresh. A skin lesion was noted along the vertebral column on the right side 2 cm caudal to the anus. Samples of the lesion were collected for histology. Darker color suggestive of bruising was noted on the left side approximately 3 cm behind the eye and posterior to the dorsal edge along the midline. Potential markings from postmortem storage was seen in a "U" shape under the eyes to pec fins. Minimal feces were coming from the anus, and foam was coming from the blowhole.

The external exam began at approximately 1900h. The ribs and vertebrae were not yet ossified and were noted as being very brittle and fragile. Upon opening the thoracic cavity, abundant bright green, viscous fluid was noted. Close to 700 mL fluid was collected, identified grossly as an effusion. The organs in the thoracic cavity, including lungs and mediastinum were covered by a layer of white, stringy material that could be peeled from organs suggestive of fibrin. The lungs had nodules throughout that were sampled for histopathology. A small number of lung worms were observed and collected, but the lungs were otherwise unremarkable.

The left marginal lymph node was swollen and enlarged, but the inside was grossly normal. The



**Figure 4.** Pleuritis observed in a stranded spinner dolphin.

circulatory system (heart and large vessels) seemed grossly normal. The abdominal cavity contained close to 20 mL of amber fluid. The main chamber of the stomach had transparent walls and was full of fluid, as were the intestines. It was also noted that fat was present around both adrenal glands. The brain had congested meningeal and cerebral vessels but appeared otherwise grossly normal. Examination of the reproductive system revealed the animal as immature female. The cause of death was determined to be an acute bacterial infection of the pleura and lungs. Septicemia was suspected based on gross necropsy findings. There were no signs that direct human interaction contributed to death. Disease screening from this case is pending.

Microscopic examination of 34 H&E stained slides prepared from formalin fixed tissue samples revealed marked inflammatory changes of the pleura and pericardium, changes in the lung and multifocal midzonal necrosis of liver. While pleuritis and pulmonary changes indicate a sub-acute to chronic disease, hepatic necrosis is acute and contributed to death. Hepatic necrosis is likely due to septic shock, secondary to overwhelming infection.

### ***Short-finned Pilot Whale, Globicephala macrorhynchus, Saipan***

A short-finned pilot whale stranded on November 11, 2025. This was during the 2025 US government shutdown. Responders in Saipan notified NOAA but partners at the Division and Fish and Wildlife at the Commonwealth of the Northern Marian Islands did not receive a response and the animal was disposed of in a landfill. The Health and Stranding Lab learned of the stranding event after disposal. This was a missed opportunity to conduct both a cause of death investigation and to provide hands on necropsy training in Saipan during a real-time event. This animal stranded in the Tanapag village area along the beach and was found in about 1ft of water. No samples were taken and no examinations were conducted. The animal was fresh dead and no wounds or external scarring are apparent from the limited photographs. Using photos and the straight length of the animal that was measured at 293.6 cm, this likely represents a juvenile short-finned pilot whale. Photographs suggest that the animal was in poor body condition with visible ribs and other bone projections that are consistent with emaciation. Sex was not determined.



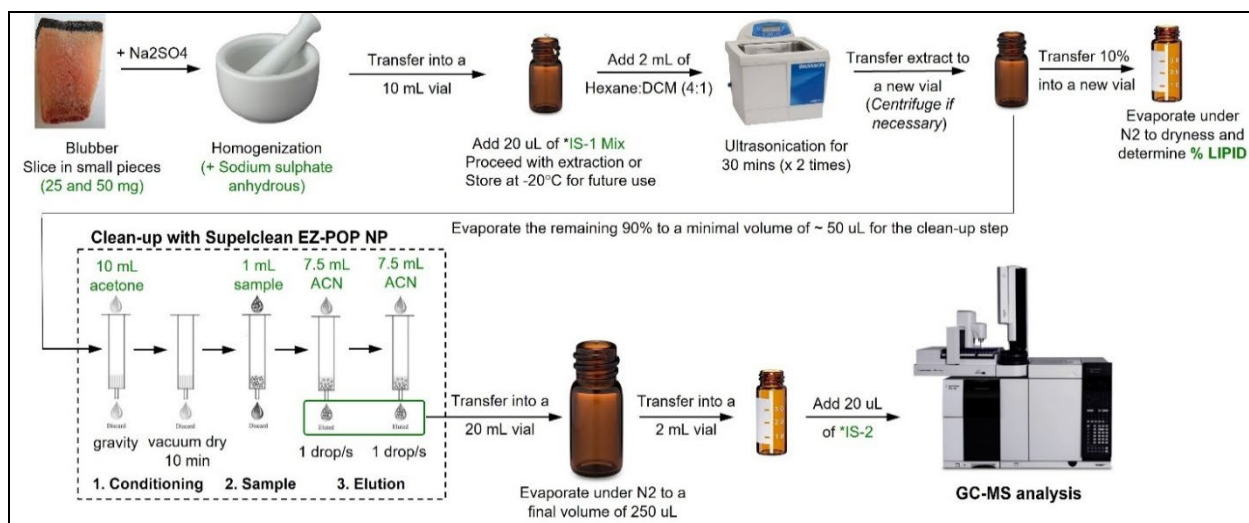
**Figure 5.** Juvenile short-finned pilot whale stranded in Saipan in November of 2025.

## ***CONTAMINANTS***

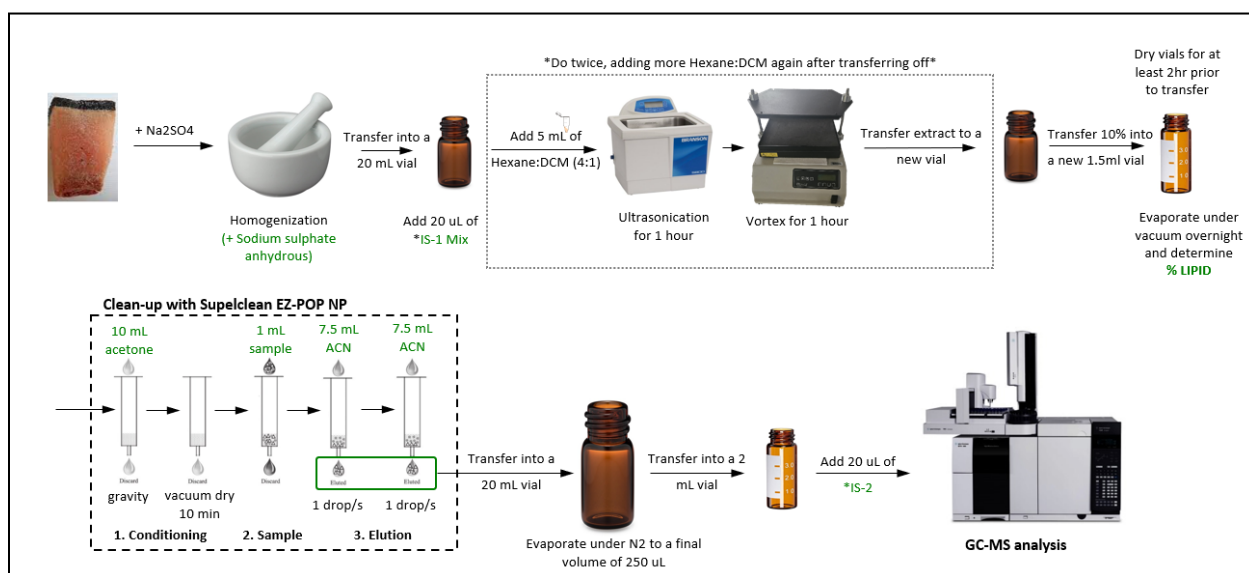
Persistent organic pollutants (POPs; e.g., DDT, PCBs) are synthetic contaminants that pose a well-documented threat to marine mammals. These compounds biomagnify through aquatic food webs and accumulate in cetacean blubber, where exposure has been linked to adverse effects on immune function, growth, development, reproduction, and population-level declines in some species. Phthalates, an emerging class of plasticizer-associated contaminants (e.g., DEHP, DBP, BBP), share similar hydrophobic and lipophilic properties with many POPs, resulting in deposition and retention in fatty tissues. Consequently, accurate measurement of tissue lipid content is essential for lipid-normalized reporting of contaminant concentrations when considering most POPs or phthalate compounds.

To support in-house contaminant analyses, the Health and Stranding Lab developed a modified ultrasonic extraction protocol based on Eze et al. (2025) to reliably quantify lipid percentage during contaminant extraction from stranded cetaceans (Figure 6). Method development and validation used humpback whale calf blubber samples where previously determined lipid percentages generated by an external commercial laboratory were available as part of a prior project on humpback whale energetics (Table 2; van Aswegen et al. 2025). Two female humpback whale calves that represented the highest and lowest known blubber lipid content from this prior project were selected for in-house method development. Blubber samples were processed using optimized methodology (Figure 7), that included frozen tissue sectioning to minimize lipid loss, freeze-drying to improve homogenization, and an added vortexing step to enhance lipid extraction. These methods produced reliable and repeatable lipid percentage estimates across blubber sample masses ranging from 50–200 mg (Table 3). Lipid values from the lowest-lipid sample (KW2019002) were





**Figure 6.** Cetacean blubber extraction workflow using the ultrasonic method in Eze et al. 2025.



**Figure 7.** Cetacean blubber extraction workflow that was modified during this project to ensure lipid extraction results in reliable lipid corrected data for contaminant concentrations. Blubber sample sizes ranged between 25 and 100 grams in developing the modified extraction workflow.

below detection or <1%, consistent with external laboratory results, while high-lipid samples yielded comparable values (~60%) across both laboratories and sample sizes.

To further ground-truth the in-house lipid percentage methodology, blubber extracts were prepared from ten pygmy killer whales, seven of these had mass stranded in Maui, Hawai'i, in 2021. Lipid percentage data from ten individuals, including a fetus, are presented in Table 4. The results demonstrate biologically meaningful variation across age and sex classes. The lowest lipid percentage was observed in the fetus, followed by a calf estimated at approximately six months of age. Reduced lipid percentages were also observed in two adult males that later stranded during a prolonged mass stranding event, during which photogrammetry documented declining body

condition over several weeks out of habitat (Currie et al. 2021). Collectively, results from humpback whale calves and pygmy killer whale blubber samples support the reliability of the modified ultrasonic extraction protocol for lipid quantification. This work strengthens the interpretation of in-house contaminant measurements that are obtained through sample extraction procedures conducted by the Health and Stranding Lab.

**Table 2.** Lipid percents by FQ Labs used to calibrate in-house methodology to measure lipid percentage in-house at the Health and Stranding Lab.

| Whale ID  | Species                | Body length (m) | Tissue Type | Age/Gender  | Lipid Concentration % |
|-----------|------------------------|-----------------|-------------|-------------|-----------------------|
| KW2008003 | <i>M. novaeangliae</i> | 4.1             | Blubber     | Calf Female | 36.1                  |
| KW2009002 | <i>M. novaeangliae</i> | 5.18            | Blubber     | Calf Female | 61.8                  |
| KW2013007 | <i>M. novaeangliae</i> | 4.8             | Blubber     | Calf Male   | 1.4                   |
| KW2019003 | <i>M. novaeangliae</i> | 4.12            | Blubber     | Calf Female | 0.3                   |
| KW2022002 | <i>M. novaeangliae</i> | 3.76            | Blubber     | Calf Male   | 5.2                   |
| KW2024006 | <i>M. novaeangliae</i> | 4.27            | Blubber     | Calf Male   | 1                     |

**Table 3.** Lipid extractions at varying sample sizes to determine the effect of sample size across known high and low blubber lipid percentage values in humpback whales. \* nd = not detected

| Animal ID | Species                | Age/Gender  | Blubber Weight (mg) | Lipid % (average) | CV      | Standard Deviation |
|-----------|------------------------|-------------|---------------------|-------------------|---------|--------------------|
| KW2009002 | <i>M. novaeangliae</i> | Calf Female | 50                  | 69.5              | 2.20%   | 0.02               |
| KW2009002 | <i>M. novaeangliae</i> | Calf Female | 100                 | 61.5              | 0.00%   | 0.00               |
| KW2009002 | <i>M. novaeangliae</i> | Calf Female | 150                 | 61.7              | 8.21%   | 0.05               |
| KW2009002 | <i>M. novaeangliae</i> | Calf Female | 200                 | 59.6              | 0.76%   | 0.00               |
| KW2019003 | <i>M. novaeangliae</i> | Calf Female | 50                  | nd                | -       | -                  |
| KW2019003 | <i>M. novaeangliae</i> | Calf Female | 100                 | nd                | -       | -                  |
| KW2019003 | <i>M. novaeangliae</i> | Calf Female | 150                 | nd                | -       | -                  |
| KW2019003 | <i>M. novaeangliae</i> | Calf Female | 200                 | 0.6               | 141.42% | 0.01               |

**Table 4.** Lipid percentages of pygmy killer whales (*F. attenuata*) previously investigated for contaminant concentrations.

| Animal ID  | Species             | Age/Gender      | Blubber Weight (mg) | Lipid % (average) | CV     | Standard Deviation |
|------------|---------------------|-----------------|---------------------|-------------------|--------|--------------------|
| KW2009006  | <i>F. attenuata</i> | Adult Male      | 100                 | 35.5              | 0.49%  | 0.17               |
| KW2015006  | <i>F. attenuata</i> | Calf Female     | 100                 | 14.0              | 2.68%  | 0.38               |
| KW2019016  | <i>F. attenuata</i> | Subadult Male   | 100                 | 54.5              | 1.58%  | 0.86               |
| KW2019017  | <i>F. attenuata</i> | Subadult Female | 100                 | 40.4              | 5.94%  | 2.40               |
| KW2019018  | <i>F. attenuata</i> | Adult Female    | 100                 | 42.4              | 2.99%  | 1.27               |
| KW2019018A | <i>F. attenuata</i> | Fetus           | 100                 | 3.3               | 37.31% | 1.23               |
| KW2019019  | <i>F. attenuata</i> | Adult Female    | 100                 | 47.9              | 8.62%  | 4.13               |
| KW2019020  | <i>F. attenuata</i> | Calf Male       | 100                 | 44.8              | 8.62%  | 3.86               |
| KW2019021  | <i>F. attenuata</i> | Adult Male      | 100                 | 28.4              | 1.60%  | 0.45               |
| KW2019022  | <i>F. attenuata</i> | Adult Male      | 100                 | 18.8              | 24.05% | 4.51               |



## ***TOOTH AGING***

Determining the age of an animal is a fundamental life history parameter and allows for information obtained on contaminant burdens and disease processes to be put into a broader context. We therefore include individual tooth aging, based on growth layer groups (GLG's) conducted in-house at the Health and Stranding Lab, as part of our associated laboratory analyses for high priority species cases selected for pathogen characterization/viral discovery work and/or determination of contaminant burdens associated with legacy compounds (POPs and PFAS).

Tooth aging has been conducted by preparation of teeth using two methods established by the South Australian Museum (Evans et al. 2011). One of these methods is based on acid etching and generally applied to medium to large sized teeth and the other follows decalcification and staining methods and is generally used for smaller odontocete teeth. In calendar year 2025 we focused on processing teeth using the acid etching method.

When applying the acid etching technique, one tooth from each individual will be sectioned using an isomet saw to reveal dentine layers. When using an isomet saw, the straightest and least worn tooth from each specimen will be selected for preparation. The tooth will be longitudinally sectioned to off-center allowing for the larger section to retain all of the surface necessary for the final aging process. This larger section will then be wet sanded using 400, 600, 1000, 1500 and 2000 grit sandpaper with a water coating until it is at the centerline of the formerly intact tooth's width. The tooth will then be alternated between exposure to dilute formic acid (15%) and acetone baths and rinsed with water for three-minute intervals between baths and drying post rinse. This process will be repeated to etch the dentine until the separate layers are visible. Once the etching is complete, a #1 soft-graphite pencil will be gently rubbed along the surface of the tooth to enhance the visibility of the dentine layers.

Following the tooth preparation, two readers independently age each specimen three times without knowledge of the data available for each specimen or previous GLGs counts. The final age for a specimen is determined as the mean of all readings rounded to the nearest whole year. In some cases, due to difficulty counting the dentine layers in the sectioned teeth and potentially high variability in the counts or the tooth is damaged, GLGs age estimates may be determined as minimum age from the lowest count.

Ten pygmy sperm whales (*Kogia breviceps*) and a goose-beaked whale (*Ziphius cavirostris*) of unknown age were prepared for counting of GLGs. The pygmy sperm whale teeth were sectioned with the isomet saw and acid etched in preparation for future aging. One of those animals, stranded in Molokai has a preliminary age of 13 years old (Figure 8). The goose-beaked whale was the first beaked whale that has been aged at the Health and Stranding Lab. Aging of this species was only possible because of the purchase of a new larger saw blade in support of this project that allowed for application of our routinely used methodology to age beaked whales and other odontocete species with larger teeth. Male beaked whales only develop zero, one, or two teeth, while female teeth do not erupt from the jaw. A goosebeaked whale that stranded in Guam in 2015 was aged to a minimum age of 35 years old. This number was a minimum age due to the wearing of the top of the tooth (Figure 9).



**Figure 8.** A thin cut section from the center of a pygmy sperm whale tooth that stranded on Molokai in 2006. The tooth was aged using the isomet saw and acid etching method with a preliminary age of 13 years old.



**Figure 9.** A thin cut section from the center of a from a goose beaked whale that stranded in Guam in 2015. The tooth was aged using the isomet saw and acid etching method. It was aged at a minimum of 35 years old, but a true age cannot be obtained because of the wearing of the top of the tooth. Left tooth shows the tooth marked with graphite and the right tooth is lit from beneath, highlighting the growth layer groups in a thinner section of the tooth (~1 mm thick).

### ***MARINE DEBRIS INGESTION***

During calendar year 2025, the Health and Stranding Lab advanced understanding of the threat of marine debris ingestion in Hawaiian cetacean through submission of a manuscript on macroplastic ingestion to the journal *Marine Pollution Bulletin*. This manuscript, currently under review, is entitled “Characterization of macroplastics ingested by 23% of stranded short-finned pilot whales (*Globicephala macrorhynchus*) in the main Hawaiian Islands (2010-2023).” The study characterizes the composition of ingested macroplastics in three dead-stranded pilot whales and reports that approximately 80% of the macroplastics, by both number and mass, consisted of abandoned, lost, or otherwise discarded fishing gear (ALDFG). These findings underscore the susceptibility of pilot whales in the Hawaiian Islands to ALDFG ingestion and identify marine debris as a significant threat to this species.

In 2025, we initiated investigations into the presence of microplastics in tissues of stranded cetaceans, with an initial focus on blubber and reproductive organs (testis). We selected matched tissue samples from the three adult male short-finned pilot whales with documented macroplastic ingestion and for which marine debris items had been previously characterized; findings from this work have been submitted for peer-reviewed publication (see above). In these cases, macroplastics recovered from the stomachs remain available for additional testing and comparison with microplastics that may be identified in corresponding blubber and reproductive tissues from the same individuals. This provides a unique opportunity to directly link known plastic ingestion with the presence of microplastics in internal tissues at the individual level. In addition, blubber and testis samples from adult male pilot whales with no evidence of plastic debris in the stomach are available for a comparative assessment of microplastic presence. The Health and Stranding Lab is actively collaborating with Dr. W. Rodriguez at the Institute for Biogenesis Research (IBR), John A. Burns School of Medicine, University of Hawai'i at Mānoa. Experimental trials are currently underway using pilot whale blubber and testis tissues to examine and identify microplastics via Raman spectroscopy. Frozen tissue sections are macerated and filtered prior to analysis. Raman spectroscopy assesses the scattering of monochromatic laser light by materials to characterize their chemical composition, enabling comparison of distinct chemical fingerprints associated with plastics of different origins. Preliminary results indicate the presence of microplastics in a macerated and filtered testis sample from KW2017005 (a pilot whale with substantial plastic debris in the stomach), suggesting that the experimental tissue digestion and analytical approach is effective.

### ***VIRAL DISCOVERY: HYBRID ENRICHMENT CAPTURE***

A component of advanced diagnostics carried out by the Health and Stranding Lab includes the application of new viral discovery techniques to the study of Pacific Island stranded cetaceans. Detection of viral pathogens by targeted PCR, followed by sequencing of amplicons, is the gold standard for viral diagnosis. Unfortunately, unknown and novel pathogens will be missed by this method, as PCR amplification relies on pathogen-specific primers. Hybrid capture-based enrichment is a novel next generation sequencing (NGS) method for viral discovery that has not previously been applied to the study of cetacean infectious diseases. Hybridization capture, followed by next generation sequencing, takes advantage of the ability to sequence multiple amplicons from a single sample and bioinformatic analysis of sequence data. The initial enrichment step in hybridization capture involves targeted labeling of sequences of interest in DNA/RNA extracted from samples by commercially available exploratory probes (Wylezich et al. 2021). Cleaned nucleic acid samples are then sequenced using NGS protocols. This approach promises detection of yet-unknown virus sequences in tissue samples of cetaceans for the first time and will lead to a greater understanding of cetacean infectious disease threats.

The Health and Stranding Lab postdoctoral fellow, Dr. Nelmarie Giovanetti (supported by the National Science Foundation), began hands-on training on hybrid capture-based enrichment protocols in 2025. This training was conducted in preparation for the application of these analyses to stranded cetaceans where infectious disease is suspected, but where individual animals have previously tested negative by PCR for viral, bacterial, fungal and parasitic diseases known to negatively impact marine mammals. Dr. Giovanetti's training involved studying commercially

available virus capture probe-based methodology and working with commercial representatives to understand the steps involved in probe design for cetacean infectious disease investigations. Dr. Giovanetti conducted hands-on advanced training in hybrid capture-base enrichment under the guidance of Drs. Thomas Waltzek and Azeza Falghoush at the Washington Animal Disease Diagnostic Laboratory at Washington State University in spring of 2025. Dr. Giovanetti's hands-on training was funded by travel support associated with her National Science Foundation fellowship. This specialized training was used to support informed decisions when selecting high-quality tissue samples and identifying strong viral sequence candidates for designing an effective custom panel in the probe design phase, ultimately improving the success of our targeted enrichment strategy for cetacean tissue samples.

Among the commercially available virus capture probe-based methods, the Twist Comprehensive Viral Research Panel (CVRP) (Twist Bioscience, San Francisco, USA) has been widely used in both human and animal research. CVRP contains approximately over 1M unique probes to screen for ~3,000 viral human and nonhuman pathogens. This panel has been used extensively for human respiratory diseases, most notably for SARS-CoV-2 (Rehn et al., 2021; Kapel et al., 2023; Ceballos-Garzon et al. 2024; Mourik et al., 2024; Tisza et al. 2024) and has been increasingly used for animal research in the last couple of years (Lwande et al. 2022; Haman et al., 2024; Bergner et al., 2025; Cronin et al., 2025; Karatas et al., 2025). In addition to the CVRP, Twist Bioscience offers Custom Target Enrichment Panels tailored to specific research needs. These custom-designed panels can also be used as an add-on to the CVRP. Incorporating this add-on in our study will enhance the detection and discovery of both RNA and DNA novel viruses in our archival cetacean tissue samples.

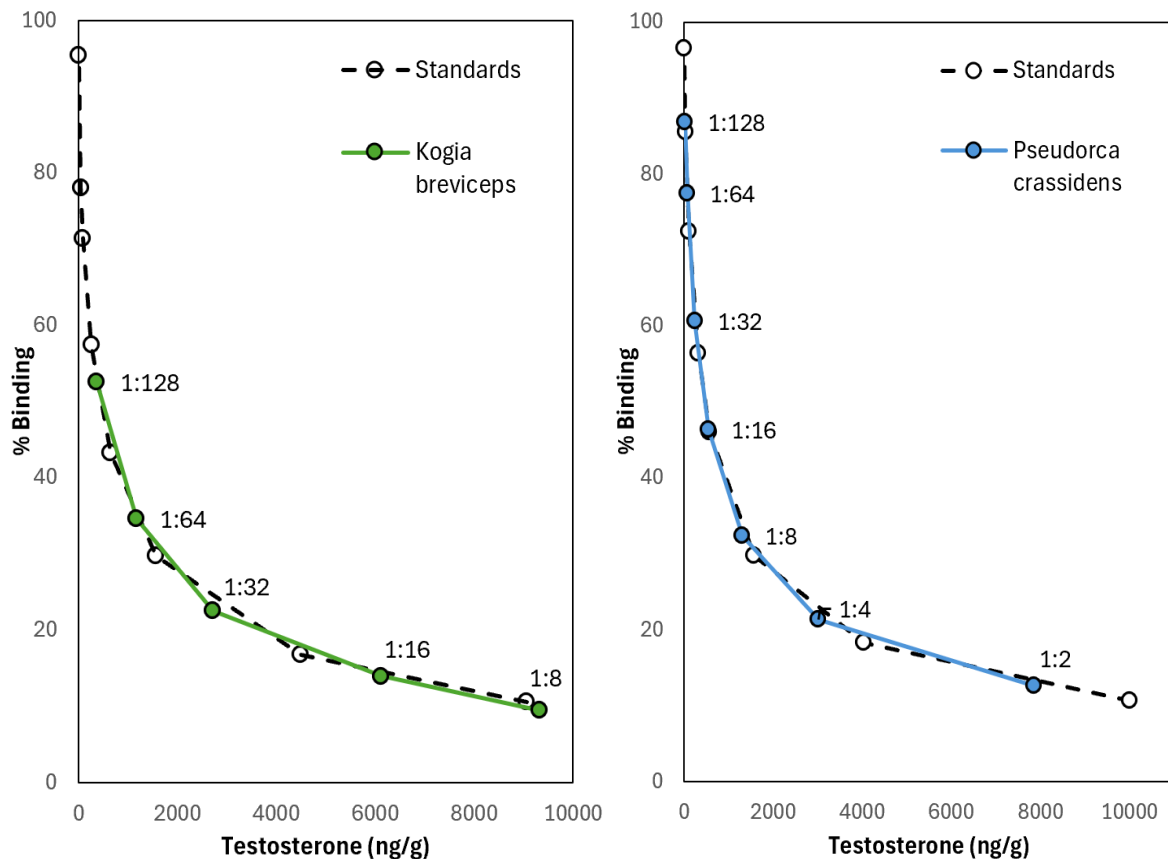
Drs. Giovanetti and West have worked closely with Twist sales representatives to use both the CVRP and design a custom add on panel based on viral discovery work that is specific to cetaceans on a world-wide scale. This has involved examination of all cetacean viral disease sequences available in Genbank. We have also identified key archival stranding cases, as well as current cases, that would most benefit from both the CVRP and custom designed add on panel to the CVRP. We anticipate results from this first effort to apply hybrid enrichment capture techniques for viral discovery in stranded cetaceans over the coming months.

### ***FECAL HORMONES OPTION***

Over the last quarter of 2025, we conducted experimental trials to further the development of laboratory research protocols to reliably measure fecal hormone concentrations in Hawaiian cetaceans. This effort focused on a number of important steps to be able to accurately measure fecal hormone concentrations across species and to provide the necessary foundation for meaningful interpretation of future measurements obtained from the feces of free-swimming cetaceans in Hawaiian waters and elsewhere.

In 2025 our laboratory efforts were focused on false killer whales and pygmy sperm whales. Specifically, we have focused on conducting technical validations for the reproductive hormone testosterone for these two species. Technical validation steps for each hormone of interest by species and sample matrix are required to test assay repeatability and reliability prior to the testing

of hormone concentrations from unknown samples. Parallelism trials are one of the necessary technical validations required to assess fecal matrix effects by generating serial dilutions of each animal's extracted hormones. We completed parallelism technical validations for testosterone from pooled fecal samples from false killer whale and pygmy sperm whales over the reporting period (Figure 10).



**Figure 10.** Testosterone serial dilution results from pooled pygmy sperm whale and false killer whale feces.

Fecal samples were directly collected from the intestines of both pygmy sperm whales and false killer whales that had previously stranded. Because samples were collected from the intestinal tract of stranded individuals, no filtration or centrifugation steps were necessary to remove saltwater contaminants. Approximately 10 g of total feces from multiple individuals of each species were collected in glass scintillation vials and freeze dried prior to extraction. Dried samples were pooled together according to species, homogenized and three 100 mg replicates were subsequently weighted into glass vials for each animal. The next step involved the addition of 1.5 mL of 90% methanol that was added to each vial and then mixed on a shaker plate at 500 rpm for 30 minutes. Vials were then centrifuged at 2200 rpm for 20 minutes. The liquid layer was then pipetted off each pellet and into new glass scintillation vials, before being dried using a Speedvac vacuum concentrator. Samples were then resuspended in 1.5 mL of diluted assay buffer provided in each enzyme immunoassay (EIA) kit and frozen at -80°C until analysis. Testosterone parallelism testing was conducted with the Arbor Assays commercial progesterone EIA kit (testosterone kit #K032-H5; <https://www.arborassays.com>) by generating serial dilutions of each animal's extracted hormones. Seven dilutions were made of varying concentrations for testosterone: 1:2 -1:28. Diluted samples demonstrated parallelism with the standard curve, with dilutions falling within



the curve's range for each species, indicating that there is negligible impact by the matrix on measured response (Figure 10).

Sample dilutions were viable at above 50% binding in false killer whales which is the ideal dilution for accurate measurement of hormone concentrations. Pygmy sperm whale dilutions were viable up to 52% binding, which is still >50% and is therefore an accurate measurement of testosterone concentrations. Intra-assay CVs for both species met quality control requirements, with values below 15%, and false killer whales indicating high precision with CVs below 10%. Concentrations in the pygmy sperm whale pooled sample was generally higher in concentration than the false killer whale pooled samples, with is likely reflective of individual reproductive status that comprised the pool.

## ***SUMMARY***

Cetacean stranding events in the Pacific Islands during calendar year 2025 occurred across the central and Western Pacific, including locations in the main Hawaiian Islands, the remote Northwestern Hawaiian Islands, and the Commonwealth of the Northern Mariana Islands. Highlights from stranding investigations during this period include a *Brucella ceti* outbreak in striped dolphins on O'ahu, which resulted in confirmed cause-of-death determinations for four individuals. Three of these dolphins stranded in close proximity within an eight-day period, suggesting a much larger mortality event, particularly given that carcass recovery rates in the Hawaiian Islands average only 1% across the 20 cetacean species present. Other notable findings included evidence of blunt trauma in a Blainville's beaked whale at Midway Atoll, fatal pleuritis in a spinner dolphin stranded on O'ahu, and the first documented stranding of a Bryde's whale in the Hawaiian Islands. Importantly, stranding investigative findings indicated that no stranding events during calendar year 2025 were attributed to naval training or testing activities. Analyses of archival stranding samples during the reporting period provided critical contributions to our understanding of poorly known Pacific Islands cetacean populations, including life history, health status, and the identification of stressors that may impact these populations.

## ***ACKNOWLEDGEMENTS***

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