Comprehensive Stranding Investigations for High Priority Cetacean Species

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ABSTRACT

This project provides support for comprehensive stranding investigations in order to obtain increased baseline information about the health of marine mammals. Such support is essential when considering the Pacific Islands region (PIR) where unique geographical challenges exist. The PIR is comprised of isolated islands, spanning over 4 million square miles across the North, South and Western Pacific basins and includes the Hawai'i Range Complex and the Mariana Islands Range Complex. All cetacean stranding response and investigative efforts for the PIR are centralized at a dedicated stranding facility located at Marine Corps Base Hawai'i (MCBH). This specialized facility houses the University of Hawai'i (UH) Health and Stranding Lab, which plays a critical role as the only organization in the region to conduct cause of death investigations when dolphins and whales strand. This requires mounting an immediate response to each newly reported stranding event that occurs and conducting extensive necropsy examinations, including histopathology, disease surveillance, and tissue sampling in support of numerous research efforts aimed at better understanding Hawaiian cetaceans. In addition to this project facilitating advanced diagnostics in-house at the Health and Stranding Lab, we also report on progress towards a project option to measure fecal stress and reproductive hormones in Pacific Island cetaceans. An additional project option includes characterizing marine debris ingestion by abundance and mass in previously stranded short-finned pilot whales.

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SUMMARY OF STRANDING CASES DURING CALENDAR YEAR 2023

Stranding response, necropsy, and cause of death investigative summaries were provided for 15 stranding events in quarterly reports that occurred during the 2023 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 that reflects the calendar year of the initial report, a number of additional cetacean strandings were confirmed and responses mounted that are not reported as stranding case summaries because of limitations that precluded the necropsy and/or sample collection. Verified or unverified stranding reports may not result in the death of an animal and/or the collection of a biological sample. In these types of stranding response scenarios, a case number is not assigned, and the event is not documented with a unique specimen identification number that is tracked in the Health and Stranding Lab records. An example of one of these types of stranding events occurred on December 27, 2023 when a humpback whale calf was observed alone nearshore in Waikīkī, Oʻahu. Soon after the whale stranded on shore several times, ocean safety and the general public pushed the animal back out into the water. The Health and Stranding Lab was notified at that time and mounted an immediate on-site response. By the time Health and Stranding Lab personnel arrived on site, the animal was milling several hundred feet from shore, and slowly moving further out to sea. It was observed until sundown, when plans were developed among the Health and Stranding Lab and other stakeholders for a response led by the Health and Stranding Lab the following day but the whale calf was not sighted again. Other stranding response activities during the calendar year included mounting of responses by the Health and Stranding Lab to dead stranded sharks based on requests of other agencies combined with no organization in the Hawaiian Islands that recovers shark carcasses. The Health and Stranding Lab similarly does not include recovered shark specimens or samples with the unique specimen identified for tracking of samples but the Health and Stranding Lab responded to seven dead shark reports during calendar year 2023 where carcasses were recovered for three cases. Two of these were confirmed as a blacktip reef shark and sandbar shark (confirmed by genetic species identification). The third recovered shark is a suspected Galapagos shark where genetics will be conducted in the future to confirm.

The summary cases reported on in 2023 include cetacean strandings where full necropsies or sampling was conducted that are indicated by a unique specimen identification number used for the tracking of sample status. During the 2023 calendar year, the UH Health and Stranding Lab coordinated or conducted stranding responses, necropsy, and sample collection from six of the main Hawaiian Islands including Kaua'i (1), Oʻahu (5), Molokaʻi (1), Lānaʻi (2), Maui (2), and Hawaiʻi Island (3), as well as Kwajalein Atoll, Marshall Islands (1). These responses resulted in sample collections and specimen tracking at the UH Health and Stranding Lab from a confirmed total of 7 species including sperm whale, *Physeter macrocephalus* (3), pygmy sperm whale, *Kogia breviceps* (3), spinner dolphin, *Stenella longirostris* (5), bottlenose dolphin, *Tursiops truncatus* (1), short-finned pilot whale, *Globicephala macrorhynchus* (1), dwarf sperm whale, *Kogia sima* (1), and a probable Bryde's whale, *Balaenoptera spp.* (1). The Balaenoptera spp. is a suspected Bryde's whale stranding from Kwajalein Atoll that will be tested for specific identification confirmation by genetics. One of both the sperm whale and spinner dolphin cases

are suspected species identification with confirmation using in-house genetic analysis in progress. Specimens represented both male and female sexes, as well as various reproductive stages including newborn calves, juveniles and sexually mature adults.

Case highlights from quarterly reports submitted during the reporting period are included below. These include fatal marine debris ingestion in an adult sperm whale that stranded dead on the island of Kaua'i in 2023 that received national and international media attention, the first case of fatally disseminated toxoplasmosis in a bottlenose dolphin that stranded dead on the island of O'ahu in 2023 and a fatal lungworm diagnosis in a spinner dolphin calf that stranded on Maui in 2023.

Sperm Whale Stranding, Physeter macrocephalus, Kaua'i

A sperm whale was reported dead on 01/26/23 on a reef off of Lydgate Beach Park in Kaua'i. It looked relatively fresh from initial photographs and was estimated at approximately 40 feet in length. The response was coordinated by Jamie Thomton, NOAA's Kaua'i Marine Wildlife Response Coordinator, and a team from DLNR, including Dr. Mimi Olry, who monitored the animal to determine whether it would strand closer to shore the following morning.

At approximately 7:00 am on 01/27/23, the animal had drifted to shore and efforts were initiated to coordinate a necropsy. Cultural practioners were contacted and excavators coordinated by NOAA and DLNR partners in attempt to move the animal further up the beach for necropsy and burial (Figure 1). Nicholas Hofmann, from the UH Health and Stranding Lab, traveled to Kaua'i early in the morning on 1/27/23 to aid with heavy equipment efforts. The rest of the UH Health and Stranding Lab necropsy team traveled to Kaua'i late morning on 01/27/23 to prepare for an on-site necropsy.

Figure 1. Stranded adult sperm whale being moved by heavy equipment on the island of Kaua'i in 2023 that died from a gastrointestinal blockage caused by marine debris.

Once the animal had moved closer to shore, it was measured at 56 feet in length and estimated at 54431 kg (120,000 lbs). The animal was too large to move intact any significant distance even with coordinated heavy equipment usage. From approximately 10:30 am to 5:30 pm, excavators worked collaboratively to move the sperm whale onto sand from within the wave wash and into a position where it would be possible for the necropsy team to safely access the animal for sampling. There was a slight depression noted behind the head, but due to the mass of the animal and the position it was in onshore, it is unknown if this observed depression was related to poor body condition. Blubber samples were collected for blubber histology that may provide further quantitative insight into the overall body condition of this individual at the time of its death.

The necropsy began around 6:30 pm on 01/27/23. High winds, rain, and waves crashing over the top of the animal resulted in pausing of the necropsy at approximately 3:30 am on 01/28/23. The necropsy and sampling continued the following morning with additional organs sampled with the assistance of the excavators. Limited sampling was conducted due to the size and weight of the animal and requests from cultural practioners. The whale had scarring around the mouth and on the head, but it was difficult to distinguish scarring between damage done by excavators, travel over the reef and onshore and natural predation from scavengers. The mouth was examined and a few teeth were noted as having wear from what was likely a result of fishing line or rope. There were 22 teeth on the bottom left with one large tooth missing and 23 teeth on the bottom right. The blubber was noted to have phyllobothrium, a tapeworm-like parasite commonly found in Hawaiian cetaceans. Heart, testes, lungs, bladder, brain, esophagus, trachea, liver and tongue were collected. While examining the reproductive organs, the animal was confirmed as a mature male. Blood was collected and frozen. Infectious disease screening for the pathogens responsible for cetacean brucellosis and circovirus were negative from a suite of organ samples collected during necropsy.

Some of the stomach contents were collected, which included hundreds of squid beaks, an intact squid tentacle as well as numerous fish vertebrae. Additionally, a large amount of marine debris was removed from the stomach by the necropsy team. At least eight different types of netting were removed, as well as a fly-fishing line with plastic baits still attached. Two plastic bags were found within the stomachs of the animals and at least six hagfish traps (Figure 2). One plastic lightbulb protector was found and a plastic floater from a net. Not all the stomach chambers were fully examined because of the positioning of the whale and the inability to reach all areas of the stomach, so it is highly likely that more debris was ingested by this whale than what was recovered at the time of necropsy. However, it was possible to examine almost the full intestinal tract during the necropsy and no formation of feces was observed from the start of the small intestine to the caudal end of the large intestine. The intestinal tract had only water throughout that is believed to be a direct result of a gastrointestinal blockage from the marine debris recovered from this individual. The inability to form feces is consistent with an intestinal blockage in an actively feeding animal. The stomach contents collected demonstrated recent and active feeding of this individual with all stages of digestion observed among the recovered prey remains.

Blood tests carried out on frozen blood resulted in high levels of liver enzymes, consistent with hepatic damage. While most values obtained from frozen blood of dead animals may not be significant, protein values (enzymes) are likely close to the real value at the time of death. A tenfold elevation of ALT (Alanine transaminase) was consistent with liver injury; a threefold elevation of BUN (Blood Urea Nitrogen) indicated metabolic stress and multiorgan disease. A

CPK (Creatine phosphokinase) of 9,000 was likely related to muscle trauma of stranding and perhaps gastric inflammation. Histopathology findings showed significant skeletal muscle degeneration and peritonitis in connective tissue was also evident, inflammation that is also consistent with gastric obstruction.

Following the necropsy, the team returned to Oahu and started to clean and process the marine debris that was recovered. The debris was photographed and video obtained for release as part of a joint press release between DLNR and UH on findings from this whale. This resulted in widespread media attention, including interviews with local stations and live broadcasts from the UH Health and Stranding Lab at MCBH. International coverage included Canada, Australia and the United Kingdom among other countries. This event and the associated publicity significantly raised awareness on the global problem of marine debris and the threat it poses to whales and other marine wildlife.

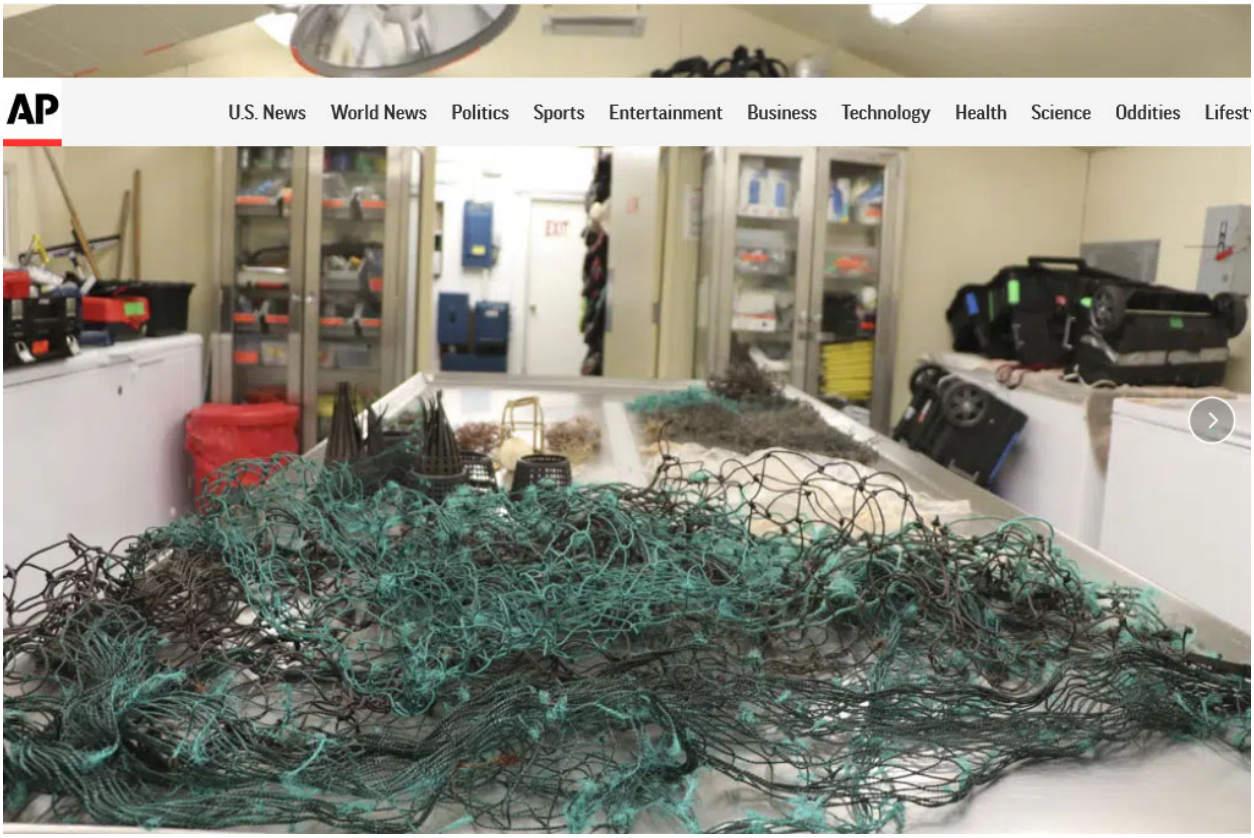

Figure 2. Fishing nets, hagfish traps, plastic bags, light protector plastic and other marine debris recovered from the stomach of the stranded sperm whale in 2023 and reported on by the Associated Press.

Bottlenose Dolphin Stranding, Tursiops truncatus, Lāi'e, O'ahu

Around 6:15 am on 05/27/2023, a fresh dead dolphin was reported at Laniloa beach in Lā'ie, O'ahu. The UH Health and Stranding Lab team responded to the call, arriving at the beach around 8:00 am. The animal was moved out of the water and onto the beach where it was transferred into a fish bag for transport by the Health and Stranding Lab for necropsy at MCBH. The Marine

Mammal Research Program (MMRP) conducted a 3D scan of the animal at MCBH, external morphometrics were taken and the animal was weighed prior to necropsy. The body condition was fresh (Code 2) with a straight length of 234 cm, and weight of 268 lbs. The animal, a male sub-adult based on size and weight, was scored as thin in the external examination of body condition.

The necropsy began around noon on 05/27/2023. The dorsal fin had several abnormal notches possibly secondary to fishery interactions. The carcass had many rake marks noted and a significant scar on the left lateral side in the peduncle region was likely due to a fishery interaction. The size of the animal, as well as obvious whisker pores located along the rostrum, was consistent with a sub-adult animal and was confirmed by examination of internal reproductive organs.

The internal examination of the mouth cavity indicated discoloration and ulcers, especially under the tongue, measuring 2 mm to 8 mm in size. In the chest cavity, significant changes were observed in the respiratory system. The margins of both lung lobes were rounded indicating expanded volume and a localized area of hemorrhage on the surface of the left main lobe. Small hemorrhages were noted on the thoracic surface of the diaphragm. Bronchial surfaces were lined by yellow-brown material that could easily be removed and once peeled, a bright red color was exposed. The cut surface of the lung showed soft yellow-brown discoloration around bronchi that felt firm upon palpation that is likely indicative of consolidation. Lymph nodes in the thoracic cavity were enlarged, dark brown and mottled in appearance and bulged on cut surface. The esophagus had multiple darker, depressed areas consistent with ulcerative lesions. A single ulcer, 3 mm in diameter, was found in the main chamber of the stomach (glandular stomach). There were minimal gut contents and liquid feces in the rectum.

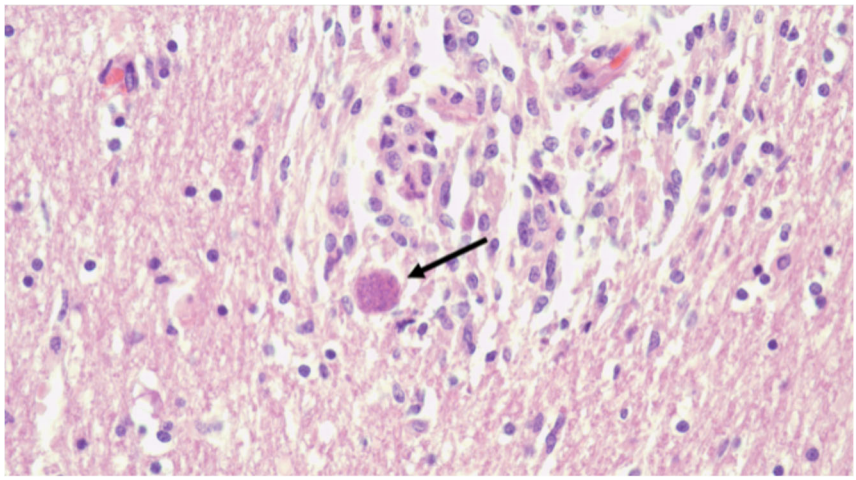

Figure 3. Toxoplasma cyst (arrow) evident upon histopathology in the brain of the stranded bottlenose dolphin.

A hemorrhage was noted in the pancreatic lymph node. The urinary bladder, which held around 20 mls of brown colored urine had punctuate hemorrhages on the internal surface. The cerebrum and cerebellum had superficial hemorrhages along both lobes, and meningeal blood vessels were prominent (congested). The brain stem, which was left attached to the cerebellum, had significant superficial hemorrhaging. Histological evaluation of tissues revealed evident inflammation in the brain, lung, lymph nodes, adrenal gland, liver and heart with intralesional *Toxoplasma* organisms. The lining of bronchi and adjacent areas of the lung were necrotic due to fungal infection, and fungal hyphae are seen in blood vessels in the brain, heart and other organs. Efforts to conduct fungal identification by PCR are on-going.

Infectious disease screening for pathogens known to impact the health of Hawaiian cetaceans was performed. A suite of tissues screened for cetacean morbillivirus and *Brucella* were negative. *Toxoplasma* was positive in all tissues screened which included cerebrum, cerebellum, meninges, left and right lung, left kidney, liver, spleen, lymph nodes (mesenteric, mediastinal, left hilar, left prescapular, right marginal) and feces. Sequencing confirmed *Toxoplasma gondii*, making this the first documented case of fatal disseminated toxoplasmosis in a cetacean species other than spinner dolphins (*Stenella longirostris*) in Hawaiian waters. *Toxoplasma* strain determination indicated genotype #24, the same genotype previously described in two Hawaiian spinner dolphins that died of toxoplasmosis and from feral pigs in the Hawaiian Islands. Genotype determination from this case allows for comparison with prior spinner dolphin fatalities as a result of this parasite, as well as for comparison to the genotype responsible for monk seal deaths and that of terrestrial mammals in the Hawaiian Islands.

Spinner Dolphin, S. longirostris, Maui

At approximately 9:00 am on 08/04/2023, a recently deceased dolphin approximately 90 cm in length was reported on Maui. Nicole Davis (NOAA Island Response Coordinator) confirmed the report and coordinated response efforts to retrieve and transport the carcass to the MCBH facility for necropsy. Timely cargo transport was not available and given the small size of the carcass, it was prepared for shipment in a cooler with ice packs and brought to O'ahu by Maura Schonwald (NOAA contractor) via commercial airline. Maura and the animal were escorted to the UH Health and Stranding Lab facility on MCBH along with MMRP team members. External examination began upon arrival at 5:00 pm. 3D scanning was performed by MMRP, while water displacement and external morphometrics were measured by the Health and Stranding Lab. The animal was a female spinner dolphin calf in robust body condition, with a straight length of 86.1 cm and weight of 5.9 kg (13.1 lbs).

The necropsy began at 6:30 pm on 08/04/2023. Several skin lesions were identified during the external examination. The first was an open wound measuring 2 x 0.5 cm on the right ventral-lateral surface, posterior to the insertion of the pectoral fin. The edges were irregular and raised suggesting active healing. An oval-shaped wound measuring 4 x 1.5 cm was observed on the dorsal surface of the rostrum. A 1 x 0.8cm skin irregularity was seen on the head, caudal to the blowhole. The lesion was superficial, but the texture was irregular. These three lesions were sampled for histological examination. Other external abnormalities included a series of scratches near the umbilicus and right eye (which were likely result of the stranding), and discoloration of the skin above the left eye (0.5 cm) and right lateral surface below the dorsal fin (6 x 1.5 cm and 2.3 x 0.5 cm). The teeth were unerupted, and whiskers, fetal folds, and frills along the tongue were present

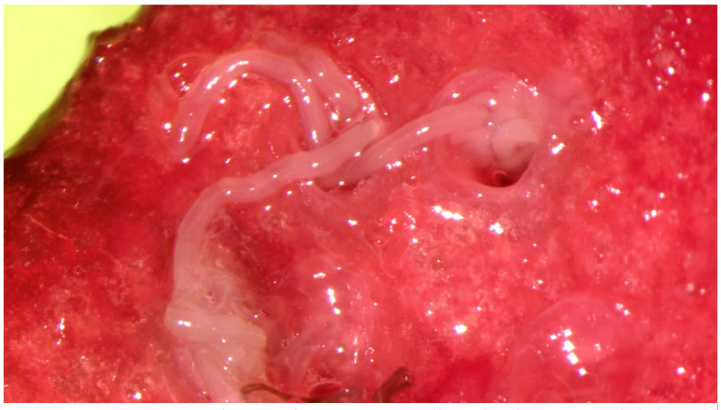

Figure 4. Lungworms observed microscopically in both spinner dolphin calf lungs.

however the umbilicus was healed, suggesting that the animal was not a neonate. We estimate that this calf was weeks old at the time of death. The internal examination included discolored right and left lungs with mottled surfaces and hemorrhaging along the margins. Cut surfaces of the lungs revealed lungworms throughout the parenchyma and bronchi (Figure 4). The right lung appeared to have a higher concentration of lungworms than the left lung. Parasites were collected both in 70% ethanol and frozen at -80ºC for later analysis. The lymphatic duct to the right marginal lymph node contained a yellow-tinged fluid. The left hilar and aortic lymph nodes were enlarged and normal in color. The stomach had minimal amounts of liquid content, and less than 0.5 ml of fecal material was collected from the colon. The liver had hemorrhaging throughout, but notably in the right and left lobes. Urine was not observed in the bladder, but urine may have been lost during examination. Hemorrhaging was observed in the occipital and parietal bones. Moderate hemorrhage was also observed in both lobes of the cerebellum. The female reproductive tract was sampled for later histological examination.

The following twelve tissues were tested for *Brucella spp.*, *Toxoplasma* and beaked whale circovirus by polymerase chain reaction (PCR): left and right lung, liver, brain (cerebrum and cerebellum), right kidney, spleen, feces, and lymph tissue (right prescapular, left hilar, right marginal, and mesenteric). *Brucella spp.* and *Toxoplasma* were not detected in any tissues. The mesenteric lymph node was confirmed positive for circovirus by DNA sequencing. PCR was also used to identify the parasites found in the lungs with the closest DNA match to the lungworm species *Halocerus delphini*.

FECAL HORMONES OPTION

During the last quarter of calendar year 2023, initial experimental trials were conducted to begin validation of commercial enzyme immunoassays for the detection of steroid hormones in the feces of Hawaiian odontocetes. Fecal samples from two previously stranded animals were selected for initial trials: an adult male pilot whale (*Globicephala macrorhynchus,* KW2017005) and an adult female pygmy sperm whale (*Kogia breviceps*, KW2018005). Fecal samples were extracted and analyzed for progesterone concentrations using methods adapted from Lemos et al. (2020). Fecal samples were directly collected from the intestines of both animals, in the case of the stranded individuals that were the focus of our initial trials no filtration or centrifugation steps were necessary to remove salt water contaminants. Approximately 10 g of feces from each animal was collected in glass scintillation vials and freeze dried prior to extraction. Dried samples were homogenized and three 100 mg replicates were weighed 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 HPLC grade water and frozen at -80C until analysis.

Extracted samples from each animal were analyzed using commercially available enzyme immunoassay kits for progesterone (Enzo Life Sciences, ADI-901-011). 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 were performed for each of the pilot whale and pygmy killer whale samples to assess fecal matrix

effects by generating serial dilutions of each animal's extracted hormones. Seven dilutions were made of varying concentrations for progesterone: 1:5 – 1:800. Diluted samples demonstrated parallelism with the standard curve, with three dilutions falling within the curve's range for each animal, indicating that there is negligible impact by the matrix on measured response. Sample dilutions were viable at approximately 50% binding in both animals, which is the ideal dilution for accurate measurement of hormone concentrations. Intra-assay CVs met quality control requirements, with values below 10% for all measured dilutions except for KW2018005 1:200 (CV: 21.7%), which was the likely due to it being the lowest concentration dilution investigated, however it still exhibited the desired pattern of parallel response to the standard curve.

The hormone progesterone was successfully extracted and concentrations measured within the standard curves from the feces of both whale species (one male and one female) and these findings suggest that feces is a viable sample matrix for investigating biochemical processes in multiple species of Hawaiian cetaceans. Progesterone concentrations were found to be higher in the female pygmy sperm whale extracts (KW2018005: 86.0 ng/mL) than the male pilot whale (KW2017005: 5.1 ng/mL).

The results of our initial progesterone trials indicate that the methods described above are anticipated to be successful in the assessment of fecal samples from multiple cetacean species. Next steps will include accuracy validation trials by conducting progesterone hormone spike recoveries to ensure the extraction procedure is removing all hormone present in the sample. We will also expand the demographics of individual animals to test for biological relevance of the progesterone hormone according to reproductive status. Future testing of individual animals will include different age classes (calf, sub adult, adult) and reproductive stages (not pregnant, pregnant, nursing) to determine the concentration of progesterone hormone present in the feces of individuals and to establish a range of expected values per species. The steps described above will also be conducted to conduct validations for additional fecal hormones (other reproductive and stress hormones).

MARINE DEBRIS OPTION

The UH Health and Stranding Lab has removed significant masses of marine debris from three previously necropsied pilot whales. Sorting, counting, and categorizing by type and size class was completed for two of the three animals (pilot whales previously stranded in Kauai and Oahu) in calendar year 2022 and some progress was made towards the third animal. In quarter four of calendar year 2023 we continued this project and focused on increasing the data collected from the third animal, a pilot whale that stranded in 2013 on the island of Lānaʻi that had significant marine debris ingestion. The total counts of marine debris items from the 2013 pilot whale stranded on Lānaʻi increased from 12,924 pieces of marine debris that had been sorted and categorized prior to calendar year 2023 to a total 19,135 pieces of marine debris. The data for the third animal is only partially complete.

Of the 19,315 marine debris pieces sorted and categorized to date for the 2013 Lānaʻi animal, the majority of the marine debris by count was monofilament line (53.9%). The second most abundant category of marine debris represented by count data was sheet plastic (37.9%) and that was followed by multifilament line (7%). We compared the count data by category between the two

previously completed pilot whales with marine debris ingestion and the partially completed third animal that stranded on Lānaʻi in 2013. All individuals had marine debris that was dominated by monofilament line that ranged between 53% and 61% of the total counts by category in all three of the individual whales that stranded separately temporally and spatially time (Figure 5). Multifilament line was the second most abundant marine debris represented in the stomachs of the Kauaʻi 2017 and Oʻahu 2014 individuals but sheet plastic was the second most abundant category by count in the Lānaʻi 2013 individual (Figure 5). However, differences apparent between the Lānaʻi 2013 animal and the other two individuals may change as we continue data collection for the third animal and final comparisons are made when all three pilot whales are complete.

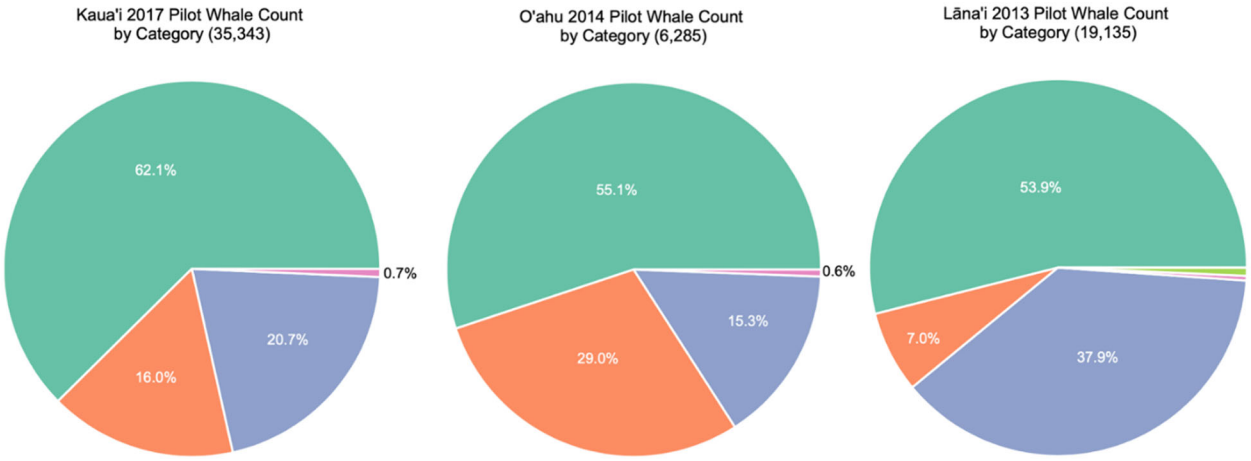

Figure 5. Marine debris categorization by count in three stranded pilot whales. The 2013 Lānaʻi stranded individual data collection is only partially complete. Monofilament is represented by teal green color, multifilament by blue, sheet plastic by orange and hard fragments by pink and miscellaneous by light green.

Weight data of the categorized marine debris was also obtained for the individuals where data collection was previously completed as well as partial weight data for the third animal that stranded on Lānaʻi in 2013. The partially completed third animal marine debris weight totaled 944 grams with the majority of the weight represented by the multifilament debris category at 72%. The second most abundant marine debris category by weight in the partially complete animal was sheet plastic at 21% and then monofilament line at 3%. The partial weight data for the Lānaʻi 2013 third animal was similarly compared to the completed Kauaʻi 2017 and Oʻahu 2014 animals. In all cases, marine debris categorization was dominated by multifilament line in all animals and ranged between 72% and 80% of the total marine debris in each of the three whales (Figure 6). The second most abundant category by weight varied among the three individuals, with sheet plastic most abundant in two of the animals and hard fragments the most abundant by weight in the other individual (Figure 6). We anticipate that the weight data contribution and comparisons among the individuals may change once the final data is complete for the third animal.

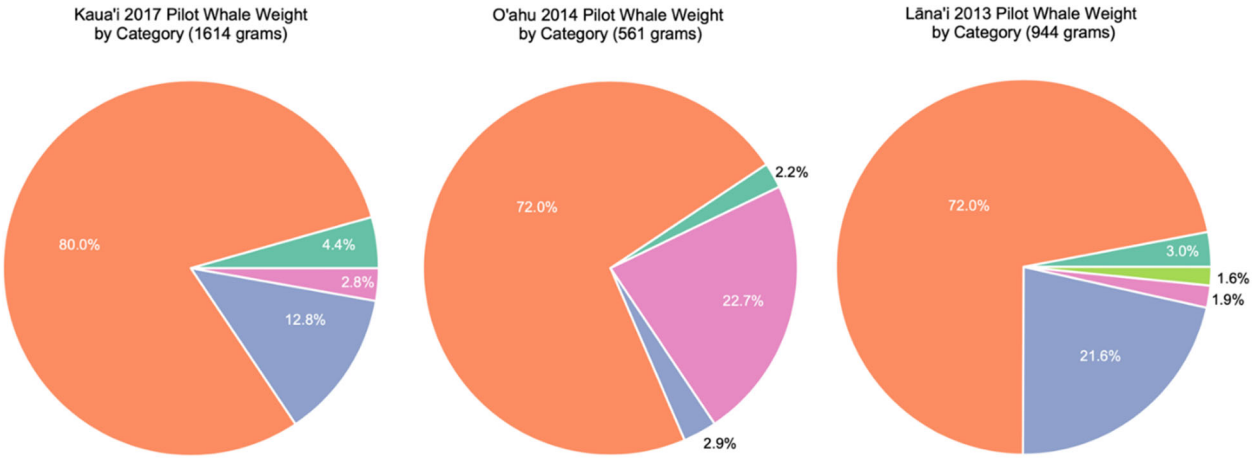

Figure 6. Marine debris categorization by count in the three stranded pilot whales. The 2013 Lāna'i stranded individual is only partially complete. Monofilament is represented by teal green, multifilament by blue, sheet plastic by orange, hard plastic by pink and miscellaneous by light green.

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