

APPENDIX D

CETACEAN MONITORING IN THE MARIANA ISLANDS RANGE COMPLEX, 2014

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14. ABSTRACT

The Pacific Islands Fisheries Science Center's (PIFSC) Cetacean Research Program (CRP) has been conducting visual surveys for cetaceans in the waters surrounding Guam and the Commonwealth of the Northern Mariana Islands (CNMI) and collecting long-term passive acoustic monitoring data at two sites in CNMI as part of an ongoing effort to develop a record of cetacean occurrence in the region. Visual surveys, conducted aboard small boats (7.6 – 12.2 m), have been ongoing since 2010 off the southern Mariana Islands of Guam, Rota, Saipan, Tinian, and Aguijan. These surveys include the collection of photographs for individual identification, tissue samples for genetic analysis of population structure, and the deployment of satellite tags for assessment of individual movements through the broader region. These surveys have been carried out in partnership with the Commander, U.S. Pacific Fleet Environmental Readiness Division. PIFSC has also maintained long-term acoustic monitoring sites in CNMI since 2010. The various datasets from these efforts are collectively being used to evaluate the seasonal occurrence and distribution, stock structure, and movements of cetaceans within the study area. This report includes a summary of the most recent survey (summer 2014), updates on the status of existing photo-identification catalogs and the creation of new photo-identification catalogs, and summaries of genetic analyses of bottlenose dolphin and short-finned pilot whale samples collected in the region, preliminary interpretation of satellite telemetry datasets, and the year-round occurrence of cetacean sounds recorded. The Appendices contain the more detailed reports on the mitochondrial and nuclear DNA analyses of bottlenose dolphins and short-finned pilot whales sampled in the Mariana Islands.

The PIFSC CRP conducted small boat visual surveys within the waters surrounding Guam, Saipan, Tinian, Aguijan, and Rota between 15 May and 20 June 2014 and surveyed 2,958 km of trackline. The survey team encountered 37 groups of cetaceans that were identified to species. In order of encounter frequency from highest to lowest, those species included spinner dolphin, pantropical spotted dolphin, short-finned pilot whale, bottlenose dolphin, false killer whale, Blainville's beaked whale, and Cuvier's beaked whale. Additional encounters included 2 groups of unidentified Mesoplodont whales, a group of unidentified beaked whales, and an unidentified small whale. The overall encounter rate was 1.39 encounters/100km of survey effort. Over 22,000 photos were taken during the 37 encounters and 36 biopsy samples were collected from false killer whales, pilot whales, and bottlenose dolphins. Thirty-one green sea turtles and 34 sea turtles of unknown species were observed during the surveys. A total of 13 Wildlife Computers satellite tags were deployed on 3 cetacean species (short-finned pilot whale, false killer whale, and bottlenose dolphin). To date, individual photo-identification catalogs have been created for 6 species (short-finned pilot whales, bottlenose dolphins, spinner dolphins, false killer whales, pygmy killer whales, and rough-toothed dolphins). Genetic analyses confirmed hybridization between Fraser's and bottlenose dolphins now evident in bottlenose dolphins sampled in the Marianas. The data suggest that the Fraser's dolphin ancestry in the Mariana Islands bottlenose dolphin population is the result of a single hybridization event in the past. The samples of Mariana Islands bottlenose dolphins exhibited low genetic diversity compared to other bottlenose dolphin populations, suggesting that they represent a small, genetically isolated population. Complete mitochondrial genomes from 99 short-finned pilot whales were sequenced to infer evolutionary relationships and patterns in the Pacific. Results indicate that there are three major groups in the pilot whale phylogeny, corresponding to the two known morphotypes (called Naisa and Shiho based on original descriptions in Japan), and a third, widely distributed group that spans the range of the other two groups in the Pacific. These results suggest evolutionary divergence of multiple types of pilot whales.

PIFSC maintains long-term passive acoustic datasets collected by High-frequency Acoustic Recording Packages (HARPs) at 2 sites in the Marianas; one west of Saipan since 2010 and another east of Tinian since 2011. Analyses reported here were conducted on passive acoustic data collected from 21 July 2013 to 13 June 2014 at the Tinian site. Several odontocete species were detected within the 2013-14 Tinian HARP dataset including sperm whales, Kogia spp, Blainville's beaked whales, unidentified beaked whale classified as BWC, killer whales, short-finned pilot whales, false killer whales, and Risso's dolphins

15. SUBJECT TERMS

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Cetacean Monitoring in the Mariana Islands Range Complex, 2014

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Mission

The Pacific Islands Fisheries Science Center's (PIFSC) Cetacean Research Program (CRP) has been conducting visual surveys for cetaceans in the waters surrounding Guam and the Commonwealth of the Northern Mariana Islands (CNMI) and collecting long-term passive acoustic monitoring data at two sites in CNMI as part of an ongoing effort to develop a record of cetacean occurrence in the region. Visual surveys, conducted aboard small boats (7.6 – 12.2 m), have been ongoing since 2010 off the southern Mariana Islands of Guam, Rota, Saipan, Tinian, and Aguijan (Figure 1). These surveys include the collection of photographs for individual identification, tissue samples for genetic analysis of population structure, and the deployment of satellite tags for assessment of individual movements through the broader region. These surveys have been carried out in partnership with the Commander, U.S. Pacific Fleet Environmental Readiness Division. PIFSC has also maintained long-term acoustic monitoring sites in CNMI since 2010. The various datasets from these efforts are collectively being used to evaluate the seasonal occurrence and distribution, stock structure, and movements of cetaceans within the study area. This report includes a summary of the most recent survey, updates on the status of existing photo-identification catalogs and the creation of new photo-identification catalogs, and summaries of genetic analyses of bottlenose dolphin (*Tursiops truncatus*) and short-finned pilot whale (*Globicephala macrorhynchus*) samples collected in the region, preliminary interpretation of satellite telemetry datasets, and the year-round occurrence of cetacean sounds recorded. The Appendices contain the more detailed reports on the mitochondrial and nuclear DNA analyses of bottlenose dolphins and short-finned pilot whales sampled in the Mariana Islands.

Methods

Visual Surveys

Visual surveys were conducted in spring and summer 2014. Summary results from the spring survey are included in Hill et al. (2014) and will not be detailed further here. Summer surveys were conducted aboard chartered vessels between 15 May and 20 June 2014 (Table 1). Off of Guam surveys were conducted aboard two different vessels, the *Lucky Strike* and *Mieko*. Surveys off of Saipan, Tinian, and Aguijan were conducted aboard two different vessels, the *Sea Hunter* and *Regulator*. Surveys were conducted off of Rota aboard a single vessel, *Asakaze*.

Field Methods

Visual survey effort was designed to cover representative habitat within the study area and did not conform to systematic (e.g. line-transect) design. Vessel tracks were spread out from day to day to ensure broad survey coverage over a wide range of depths and were also dictated by weather and sea conditions. The survey vessels traveled at a speed of 15-26 km/h, depending on the size of the vessel and sea conditions. Five vessels were chartered for these surveys ranging from 5.8 to 12.2 m length. *Lucky Strike*, *Mieko*, and *Sea Hunter* had flying bridges. The vessels were operated by locally experienced captains, with knowledge of cetacean sighting locations. Captains allowed the research team to operate the vessel when approaching cetaceans for photo-identification, biopsy, and satellite tagging. Between four and

six observers scanned for marine mammals with unaided eye or occasional use of 7x and 10x binoculars, collectively searching 360-degrees around the vessel.

All cetacean groups encountered were approached for species confirmation, group size estimates, and photo-identification. During encounters with certain species, biopsy sampling and satellite tagging operations were conducted. Photo-identification and biopsy protocols were identical to those described by Hill et al. 2014.

Satellite tagging was conducted using a Dan Inject air rifle and deployment arrows designed by Wildlife Computers. Two types of tags were deployed. One type was a location-only Wildlife Computers SPOT5 tag. The other tag type was the Wildlife Computers SPLASH10, which provided location as well as depth, temperature, and light level. Both tag types were deployed in the LIMPET configuration. The tags were attached to the dorsal fin with two sterilized, titanium darts with backward facing petals. Two dart lengths were used depending on the species (4.5 cm for small to medium odontocetes or 6.5 cm for large odontocetes). The programming of the tag configurations varied depending on the species and followed the specifications used by Cascadia Research Collective (CRC) based on the average number of respirations per hour, speed of surfacing, and the likelihood that a tag would remain attached for longer than a month, which were determined in previous tagging studies by CRC (Baird et al. 2013). SPLASH10 location-dive tags were programmed to collect time-series dive data every 1.25 minutes for false killer whales and 2.5 minutes for short-finned pilot whales. Dive statistics (number of dives, dive depths, and dive durations) were also collected for dives equal to 30m depth or greater and durations of 2 minutes or greater. To conserve battery life, the tag sensors were duty cycled to collect dive data for the first 2 days of deployment then alternately 3 days off and 1 day on for false killer whales, and 1 day off and 1 day on for pilot whales.

The occurrences and locations of turtles were recorded but neither photos nor biological samples were collected.

Passive Acoustic Data Collection

PIFSC maintains long-term passive acoustic datasets collected at 2 sites in the Marianas; 1 west of Saipan since 2010 and another east of Tinian since 2011. High-frequency Acoustic Recording Packages (HARPs; Wiggins & Hildebrand 2007) were used to record underwater sounds from 10 Hz to 100 kHz with 16-bit quantization. The HARP sensor and mooring package are described in Wiggins and Hildebrand (2007). For the Marianas deployments, the HARP was configured as a mooring, anchored on the seafloor with the hydrophone suspended 30 m above.

Analyses reported here were conducted on passive acoustic data collected from 21 July 2013 to 13 June 2014 at the Tinian site (15° 2.40' N, 145° 45.38' E, 695 m depth). Data were collected with a duty-cycle, such that data were collected for 5 minutes and then the recorder was off for 2 minutes. This duty-cycle was chosen to allow for year-round recording at 200 kHz sample rate, and was facilitated through use of high energy-density lithium batteries housed within two pressure cases.

Data Processing and Analyses

Visual Surveys and Encounters

The methods and bathymetry data used in the processing and analysis of the visual survey and encounter data are identical to those described in Hill et al. 2014.

Satellite Telemetry

The methods used to process and analyze the satellite tag location data are identical to those described in Hill et al. 2014. The SPLASH10 tag dive data were extracted as .csv files using Wildlife Computer's DAP Processor 3.0 and were analyzed for median and maximum dive depths and durations in Microsoft Excel.

Photo-Identification

Photo analysis was continued to add to existing individual photo-identification catalogs for short-finned pilot whales, bottlenose dolphins, and spinner dolphins (*Stenella longirostris*) (Hill et al. 2014) and to create new catalogs for false killer whales (*Pseudorca crassidens*), rough-toothed dolphins (*Steno bredanensis*), and pygmy killer whales (*Feresa attenuata*). The details of how the photos were processed and analyzed are described in detail in Hill et al. (2014). Photos used in the creation of and comparison to current catalogs include those taken of all species by PIFSC in 2010-2014, photos taken of spinner dolphins, bottlenose dolphins, and short-finned pilot whales by HDR in 2011-2012 (HDR 2011, 2012)¹, and photos taken of bottlenose dolphins, short-finned pilot whales, and false killer whales by a Navy contractor in 2007 during the MISTCS (Mariana Islands Sea Turtle and Cetacean Survey) (U.S. Navy 2007, Fulling et al. 2011)².

Tissue Sample Analysis

Two genetics projects were conducted in 2014 on existing biopsy samples collected from Marianas animals. Previously, Martien et al. (2014) conducted mitochondrial DNA analyses and found that 5 of the 15 bottlenose dolphins sampled in the Marianas shared a haplotype with Fraser's dolphins (*Lagenodelphis hosei*) leading to the conclusion that introgressive hybridization may have occurred within this bottlenose dolphin population. Here, we further investigated the extent and origin of hybrid ancestry in Mariana Islands bottlenose dolphins by analyzing mitochondrial DNA sequence data and nuclear microsatellite genotype data. Bottlenose dolphin biopsy samples collected in the Marianas were compared to biopsy and stranding samples from Hawai'i and other North Pacific areas, as well as to Fraser's dolphin samples from the Philippines and elsewhere in the Pacific and Indian Oceans. Analysis methods are detailed in the report by Martien et al. (Appendix I).

Martien et al. (2014) also detailed analyses of Marianas short-finned pilot whale samples and revealed that significant differences were found in mitochondrial DNA haplotype

¹ HDR conducted small boat surveys in the waters surrounding Guam and Saipan during 17 February – 3 March, 2011 and 15-29 March, 2012. All photos were contributed by the Navy to PIFSC for photo-identification analysis.

² A Navy contractor conducted shipboard surveys within the CNMI EEZ during 1 January – 14 April, 2007. All photos were contributed by the Navy to PIFSC for photo-identification analysis.

frequencies between short-finned pilot whales samples near Guam and those sampled in CNMI. Here, more detailed analyses of the entire mitogenome are evaluated to determine the relationship between short-finned pilot whales in the Marianas and two sub-types identified near Japan, as well as those elsewhere in the Pacific. Short-finned pilot whale samples collected in the Marianas were reanalyzed along with samples of short-finned pilot whales from throughout the Pacific. Analysis methods are detailed in Appendix II authored by Morin et al.

Passive Acoustics

The 2013-2014 Tinian datasets was analyzed for hourly occurrence of all cetacean sounds. The original HARP data were decimated into 2 lower frequency datasets to allow more efficient viewing at the appropriate frequency and time resolution for a subset of sound types. A low-frequency (LF) dataset was created by decimating the HARP data to 2 kHz sample rate, and a mid-frequency (MF) dataset was created by decimating the full-bandwidth data to 10 kHz sample rate.

Either manual or automated scanning of the datasets was carried out depending on the species of interest. Table 2 lists the species, call types, and primary literature source of each of the sound types searched for as part of this analysis. Low-frequency data were manually scanned for the occurrence of blue (*Balaenoptera musculus*), fin (*B. physalus*), sei (*B. borealis*), and Bryde's (*B. edeni*) whales, as well as other low-frequency sounds likely to be produced by baleen whales, but whose source is not currently known. The MF datasets were manually scanned for minke (*B. acutorostrata*) and humpback (*Megaptera novaeangliae*) whales. All baleen whale detections were noted in hourly bins; that is, if at least one call was detected in an hour, the analyst did not search for further calls from the same species in the same hour, as encounters may consist of individual calls or long calling bouts by a single individual. Use of hourly bins reduces bias associated with oversampling an individual caller. The full-bandwidth dataset was used for detection of all odontocete species. Sperm whales (*Physeter macrocephalus*) were manually detected within the full-bandwidth data, but with the analysis viewing window extending only up to 40 kHz. Delphinid species were manually marked within the full 100 kHz viewing area and beaked whales were automatically detected and manually classified following the methods of Baumann-Pickering et al. (2013) and as described in Oleson et al. (2015). All odontocete detections were noted as the start and end of the calling bout and presented as cumulative detection time, such that it is possible that overall detection of individual species is much less than one hour.

Each data set was visually and aurally analyzed using the program Triton, a Matlab-based software package for acoustic data display and analysis (Wiggins 2003). A long-term spectral average (LTSA) was computed for each data set by averaging power spectral density (Welch 1967) in 5-s time bins and 1-Hz frequency bins for LF data, 10-Hz bins for MF data, and 100-Hz bins for full-bandwidth data. The analyst visually inspected the LTSA spectrogram display to search for potential calls of each species, and calls were verified by visual examination of spectrograms and, in some cases, audio playback. For those species marked manually, the presence of calls and other sounds was logged on a per hour basis. Calls were assigned to species based on resemblance to known calls published in the literature.

Results

Visual Surveys and Encounters

The PIFSC CRP conducted small boat visual surveys within the waters surrounding Guam, Saipan, Tinian, Aguijan, and Rota between 15 May and 20 June 2014 and surveyed 2,958 km of trackline (Table 1, Figures 2-4). Less than half (43%, 1268 km) of the on-effort trackline was surveyed in Beaufort sea state conditions of 0-3, while a nearly equal amount was surveyed in Beaufort sea state conditions of 4 (42%, 1253 km)(Figure 5). Most (96%, 2839 km) of the on-effort trackline was surveyed in swell heights of 0-4 ft (Figure 6). Approximately 1/5 (21%, 40 hours) of the total time on-effort was surveyed inside of the 100 m depth contour (Figure 4). Effort was distributed fairly evenly over 101 – 1100 m depth bins and was reduced gradually over depths of 1200 – 2800 m (Figure 7).

The survey team encountered 37 groups of cetaceans that were identified to species (Tables 3-4, Figures 2-4). In order of encounter frequency from highest to lowest, those species included spinner dolphin, pantropical spotted dolphin (*Stenella attenuata*), short-finned pilot whale, bottlenose dolphin, false killer whale, Blainville's beaked whale (*Mesoplodon densirostris*), and Cuvier's beaked whale (*Ziphius cavirostris*) (Table 4). Additional encounters included 2 groups of unidentified Mesoplodont whales, a group of unidentified beaked whales, and an unidentified small whale (Tables 3-4, Figures 2-4). The overall encounter rate was 1.39 encounters/100km of survey effort (Table 4). Over 22,000 photos were taken during the 37 encounters and 36 biopsy samples were collected from false killer whales, pilot whales, and bottlenose dolphins (Table 3).

Thirty-one green sea turtles (*Chelonia mydas*) and 34 sea turtles of unknown species were observed during the surveys (Table 5, Figure 8).

Satellite Telemetry

A total of 13 Wildlife Computers satellite tags were deployed on 3 cetacean species (short-finned pilot whale, false killer whale, and bottlenose dolphin) (Table 6). Eight satellite tags were deployed on short-finned pilot whales during 4 encounters; 2 off Guam and 2 off Rota. Two of the individuals tagged off Guam on 25 May (tag IDs 128910 and 128914) had been previously photographed together off Guam in July 2013. The other two individuals tagged off Guam on 19 May (tag IDs 128889 and 128920) were photographed for the first time and were subsequently resighted off of Rota a month later on 17 June. On 16 June three satellite tags were deployed on short-finned pilot whales off Rota. Two of the individuals (tag IDs 128899 and 137726) were photographed for the first time and were resighted during 2 additional encounters off Rota on 17 and 18 June. The third individual tagged on 16 June (tag ID 137727) had been previously photographed off Rota in September 2011 and off Guam in June 2013. On 17 June the last satellite tag for these surveys was deployed on a short-finned pilot whale (tag ID 137728) off Rota. This individual was photographed for the first time off Guam on 19 May and was accompanied by the previously tagged individuals (tag IDs 128889 and 128920). The median distance of the Douglas Argos filtered tag locations from shore for all 8 individuals was

17.1 km and the median depth was 1188 m (Table 6, Figure 9). Tag 128889 was a SPLASH10 location-dive tag. It provided 443.9 hrs of dive and surfacing data and 1321 distinct dives with median and maximum depths of 167.5 m and 1167.5 m, and median and maximum dive durations of 9.93 min and 27.27 min (Table 7, Figure 10).

Four satellite tags were deployed on false killer whales during 2 separate encounters; the first off Guam on 21 May (tag IDs 128887 and 128902) and the other off Tinian on 12 June (tag IDs 128888 and 128901). None of the four individuals had been previously photographed. The median distance of the Douglas Argos filtered tag locations from shore for the 4 individuals was 48.0 km and the median depth was 3180 m (Table 6, Figure 11). Two of the satellite tags that were deployed on false killer whales were SPLASH10 location-dive tags (tag IDs 128887 and 128888). They provided 868.1 hrs of dive and surfacing data (658.9 hr for tag 128887 and 209.2 hrs for tag 128888) (Table 7). Tag 128887 recorded 167 dives with median and maximum dive depths of 240.5 m and 1359.5 m, and median and maximum dive durations of 5.37 min and 17.57 min (Table 7, Figure 12). Tag 128888 registered 332 dives with a median depth of 95.5 m and maximum dive depth of 847.5 m. Median and maximum dive durations were 4.20 min and 13.13 min (Table 7, Figure 13).

A single satellite tag was deployed on a bottlenose dolphin during an encounter off Saipan/Tinian on 12 June (tag ID 128912). The dorsal fin of the individual was not well marked; therefore its sighting history is unknown. The median distance of the Douglas Argos filtered tag locations from shore was 4.6 km and the median depth was 503 m (Table 6, Figure 14).

Photo-Identification

To date, individual photo-identification catalogs have been created for 6 species (short-finned pilot whales, bottlenose dolphins, spinner dolphins, false killer whales, pygmy killer whales, and rough-toothed dolphins). Tables 8-13 list, by species, details of the photo data from each encounter used in the analyses and creation of the individual photo-identification catalogs. Hill et al. (2014) provided a summary of the data through 2013 for short-finned pilot whales and spinner dolphins and through April 2014 for bottlenose dolphins.

During the May-June 2014 PIFSC surveys, 5 encounters with short-finned pilot whales provided resights of individuals within the catalog, as well as the addition of 32 new individuals to the catalog bringing the total to 178 individuals (Table 8). In addition, 4 short-finned pilot whale encounters during the 2007 Navy-contracted MISTCS (Mariana Islands Sea Turtle and Cetacean Survey) were analyzed. Four individuals from an encounter in March 2007 off the northeast side of Guam were matched to the existing catalog. These individuals were photographed together off the west side of Tinian in September 2011 and off the west side of Guam in March 2012. Although there were other distinctive short-finned pilot whale individuals photographed during the MISTCS encounters, no other matches or additions to the catalog were made because the photographic quality did not meet the threshold for new additions.

There were 4 bottlenose dolphin encounters during the May - June 2014 PIFSC surveys. Thirteen individuals were matched to the existing catalog and 5 individuals were added to the catalog bringing the total to 52 individuals (Table 9). Two encounters during the 2007 MISTCS surveys were analyzed; both were outside of the EEZ boundary. No matches or additions were made to the catalog from those encounters.

During the 2014 April and May-June PIFSC surveys there were 27 encounters with spinner dolphins (Table 10). The initial processing of photos and within-encounter matching has been completed for 8 encounters from the 2014 May-June surveys. Individuals noted within encounters have not yet been compared to the catalog, such that no new matches or additions to the catalog have been made. There are currently 307 individuals in the catalog. Spinner dolphins were photographed during a single sighting during the 2007 MISTCS surveys. There was 1 distinctive individual that did not match to the existing photo-identification catalog and the quality rating of the photograph did not meet the threshold for entry into the catalog.

New individual photo-identification catalogs were created for 3 species (false killer whales, pygmy killer whales, and rough-toothed dolphins). Five false killer whale encounters during the June - July 2013 and May - June 2014 PIFSC surveys, and 7 encounters during the January - April 2007 MISTCS surveys were analyzed (Table 11). The resulting catalog contains 40 individuals. Nine of those individuals were photographed twice. Two individuals were photographed off Guam on 22 June 2013 and again off Guam on 21 May 2014. Two individuals were photographed off Rota on 6 July 2013 and then off Tinian on 12 June 2014. Five individuals were photographed off Rota on 7 July 2013 and then off Guam on 21 May 2014. A single individual, photographed within the offshore waters of the southern part of the EEZ during a February 2007 MISTCS survey, was added to the catalog but was not photographed during any subsequent surveys (Table 11). There were 6 additional distinctive individuals from MISTCS encounters on 16 February and 17 March 2007, but the quality ratings of the photographs did not meet the threshold for entry into the catalog.

The individual photo-identification catalog of pygmy killer whales resulted from 2 encounters off the west side of Guam by PIFSC (Table 12). The first encounter occurred in June 2013 just north of Orote Pt. Eight individuals were present during the encounter and 6 of those individuals had sufficiently distinctive marks to be entered into the catalog. The second encounter occurred in April 2014 northwest of Cocos Island. The same 8 individuals were present, as well as a calf. One of indistinct individuals from 2013 had a changed fin that made it distinctive enough for the catalog but the quality ratings of the photographs did not meet the threshold for entry into the catalog. Pygmy killer whales were not photographed during the 2007 MISTCS surveys.

The rough-toothed dolphin photo-identification catalog includes 6 individuals that were originally photographed by PIFSC off Aguijan on 15 July 2013 (Table 13). Four of the 6 individuals were subsequently photographed off Saipan on 20 July 2013. The same 4 individuals were photographed off Aguijan on 16 April 2014. Rough-toothed dolphins were not photographed during the 2007 MISTCS surveys.

Tissue Sample Analysis

Appendix I describes the detailed results of genetic analyses to examine introgression of Fraser's dolphin DNA into bottlenose dolphins in the Marianas. Previous analyses (Martien et al. 2014) revealed that 5 of 15 individual bottlenose dolphins sampled from the Mariana Islands had a Fraser's dolphin haplotype. The analyses described in Appendix I are based on a dramatically expanded set of samples, including greater geographic coverage of bottlenose dolphins and the addition of Fraser's dolphin samples, and include nuclear microsatellite loci in addition to mitochondrial sequence data. Assessment of nuclear loci confirmed hybridization between Fraser's and bottlenose dolphins now evident in bottlenose dolphins sampled in the Marianas. On average, approximately 14% of the Marianas bottlenose dolphin nuclear DNA ancestry was derived from Fraser's dolphins. This was in contrast to findings that the Fraser's dolphin samples and bottlenose dolphin samples from other locations received, on average, over 99% of their nuclear DNA ancestry from Fraser's dolphins and bottlenose dolphins respectively. The data suggest that the Fraser's dolphin ancestry in the Mariana Islands bottlenose dolphin population is the result of a single hybridization event in the past, though the possibility of ongoing hybridization cannot be rejected. In addition, the bottlenose dolphin samples from the Mariana Islands exhibited low genetic diversity compared to other bottlenose dolphin populations.

Appendix II describes the detailed results of analysis of short-finned pilot whale mitogenome structure across the central and western Pacific. Previous studies have identified two genetically distinct groups of short-finned pilot whales in the Pacific, which correlate with two morphologically distinct forms identified off of Japan (Oremus et al. 2009, Van Cise et al. submitted). Van Cise et al. (submitted) have shown that the two groups have non-overlapping ranges, with the Shiho-like group restricted to northern Japan and the eastern Pacific and the more broadly distributed Naisa-like group occurring in Hawai'i, and the western and southern Pacific Ocean, and Indian and Atlantic Oceans. A hypothesized third group (hereafter 'stock 3') appears to be restricted to the western and southern Pacific Ocean. The three groups are sufficiently distinct that it has been suggested that they represent separate subspecies (Kasuya et al. 1998, Oremus et al. 2009). However, previous genetic studies have been limited to only sequence from the control region of the mitochondrial genome and have therefore lacked the necessary resolution to evaluate the taxonomic status of the groups. The study described in Appendix II used full mitochondrial genome sequences from 100 samples taken from throughout the Pacific Ocean to examine the evolutionary relationships between the Naisa, Shiho, and stock 3 groups of SFPWs. This expanded data set revealed the three groups fall into three strongly supported clades on a phylogenetic tree, which is consistent with possible subspecific status. However, additional data from the Indian and Atlantic Oceans and data from nuclear markers will be necessary to date the divergence of the clades and provide a definitive answer regarding their taxonomic status. The samples from the Mariana Islands included sequences associated with both the Naisa-like group and stock 3, indicating that it is an area of unusually high diversity and overlap between divergent types.

Passive Acoustics

Four species of mysticetes were recorded within the 2013-14 Tinian dataset: blue, fin, sei, and humpback whales (Figure 15). No known Bryde's whale sounds were detected, though 2 unidentified whale sounds were commonly heard, and these may have been produced by Bryde's whales based on their similarity to Bryde's whale sounds recorded in other regions. Fin whale 20 Hz downsweeps were the most commonly detected baleen whale call identified to species; heard on 20 days from January to April 2014, with a peak in occurrence in mid- to late-March. Humpback song was detected on 15 days from January to March 2014 with periods of occasional singing lasting 2-4 days, followed by several days to weeks with no humpback song detected. Central Pacific blue whale calls were detected on 4 days in May and June 2014 and downswept D calls were heard on 2 consecutive days in December 2013. Sei whale call detections were rare, heard on only 3 days over the monitoring year in February and March 2014. Minke calls were not detected within the dataset.

Two unidentified whale sounds were detected within the Tinian dataset. The more commonly detected call was a slight downsweep from an average start frequency of 42 Hz and end frequency of 32 Hz over an average duration of 2.5 s (Figure 16). These calls occurred throughout the year, but with a marked peak in detection from September through November. These calls were only sporadically detected from January through May (Figure 17). The second unidentified call type was a pulsed call that often occurred in a series of 2-3 pulses. These calls had an average start frequency of 116 Hz and end frequency of 102 Hz with 0.7 s duration. Pulsed calls were heard sporadically from the start of the deployment in July 2014 to March 2014, but with 2 periods of intense calling in November and December (Figure 17).

Several odontocete species were detected within the 2013-14 Tinian HARP dataset including sperm whales, *Kogia* spp, Blainville's beaked whales, unidentified beaked whale classified as BWC (see Baumann-Pickering et al. 2013), killer whales (*Orcinus orca*), short-finned pilot whales, false killer whales, and Risso's dolphins (*Grampus griseus*). A variety of additional delphinid sounds were detected that could not be identified to species. Sperm whales, *Kogia* spp, and both species of beaked whales were detected year-round (Figure 18) with no distinct seasonal cycle. Sperm whales were the most commonly detected within this group, with detections of Blainville's beaked whales occurring regularly, but with short overall encounter durations. Detections of BWC were relatively rare. Short-finned pilot whales and false killer whales were heard year-round, but were heard for only a few hours each week on average (Figure 19). Risso's dolphins were heard on only a few occasions, primarily in December and January, but also during January and February. Killer whales were heard on 3 days in October and November 2013 and April 2014, with very short encounter durations, suggesting they are uncommon in the region and not highly vocal when they occur. Unidentified dolphins were commonly detected year-round, with only one week during the monitoring effort with no detections (Figure 19). This group may comprise several species of small and medium odontocete, as well as undescribed whistles produced by pilot whales, false killer whales, killer whales, or Risso's dolphins that did not occur with distinctive echolocation clicks. Unidentified odontocetes are the only group that was detected with a distinct diel pattern, occurring

primarily at night (Figure 20). Individual species differences among this collective group are likely, but cannot be separated until additional species-specific reference signals are available for species classification.

Discussion

The May – June 2014 visual surveys and assessment of 2013-14 year-round passive acoustic data collected near Tinian represent a continuation of the collaborative effort between the PIFSC's CRP and the U.S. Navy towards a better understanding of the occurrence and distribution of cetaceans in waters off of Guam and the southernmost islands of CNMI (Saipan, Tinian, Aguijan, and Rota) (Hill et al. 2014, Oleson et al. 2015).

The NMFS (PIFSC) is responsible for the assessment of marine mammal stocks in the Exclusive Economic Zone (EEZ) waters of Guam and CNMI. The U.S. Navy is mandated by permits and Biological Opinions issued under the Marine Mammal Protection Act (MMPA) and the Endangered Species Act (ESA) to monitor cetacean presence within the Mariana Island Range Complex (MIRC). Although addressed in greater detail by Hill et al. (2014), additional preliminary results for questions presented within the U.S. Navy's monitoring plan are discussed below.

1. What species of beaked whales and other odontocetes occur around Guam and Saipan?

During the 2014 May-June PIFSC visual surveys 7 cetacean species were encountered in the waters surrounding Guam, Saipan, Tinian, Aguijan, and Rota. Five of these species (bottlenose dolphin, spinner dolphin, pantropical spotted dolphin, short-finned pilot whale, and false killer whale) had been encountered in previous years during other PIFSC surveys (see Hill et al. 2014). Although beaked whales had been encountered during previous PIFSC surveys, they had not been identified to species. The 2014 May-June encounters were the first confirmed sightings of Cuvier's and Blainville's beaked whales. The Cuvier's beaked whale encounter occurred 19 km off the west side of Saipan in 1700m-deep water (Table 4, Figure 3). The Blainville's beaked whale encounter occurred 11 km west-southwest of Rota in 1200m-deep water (Table 4, Figure 4).

Analysis of the passive acoustic dataset collected near Tinian in 2013-14 reveals the occurrence of 11 species, including 6 not previously seen during prior PIFSC surveys in the region. Blue, fin, humpback, and sei whales, and two types of unidentified whale calls were detected, though fin and humpback whales were the most common within this group. The unidentified whale calls were similar in structure to calls previously reported from Bryde's whales in other parts of the Pacific (Oleson et al. 2003), though there are currently no visually-verified reference signals from the Marianas or elsewhere in the western Pacific to determine species-ID of these signals. None of these species have been observed during PIFSC visual surveys, though all except sei whales were present within passive acoustic datasets collected near Tinian and Saipan from 2010 through mid-2013 (Oleson et al. 2015). Fin whales were

significantly more common in the 2013-14 dataset than in previous years at either monitoring location, though detection of blue whales remained rare. Minke whales were detected in prior years at this site (Oleson et al. 2015), though were not common. Although there were several detections of minke and sei whales during the MISTCS survey (Norris et al. 2012), most of the MISTCS minke whale detections were offshore and none occurred near the Tinian HARP and the reported sei whale signals were not detected in our dataset. Sperm whales, *Kogia* spp, Blainville's beaked whales, unidentified beaked whale BWC, killer whales, false killer whales, short-finned pilot whales, and Risso's dolphins were also detected within the passive acoustic dataset. All of these species except killer whales and BWC have been seen during prior PIFSC surveys in the region. The occurrence of beaked whales has been evaluated in earlier Tinian and Saipan datasets (Oleson et al. 2015), with the only notable difference being the absence of Cuvier's beaked whale within the 2013-14 Tinian dataset.

2. Are there locations of greater relative cetacean abundance around Guam and Saipan?

Patterns of habitat use (depth and distance from shore) evident from the 2014 May-June visual surveys were similar to those described by Hill et al. 2014. Spinner dolphins remained the most frequently encountered species and were seen at Marpi Reef and at all islands except for Tinian (Figures 2-4). Most of the encounters were within 1 km of shore and in water depths less than 300 m (Tables 3-4).

Pantropical spotted dolphins remained the second most frequently encountered species (Table 4) as was reported by Hill et al. 2014. Except for a single encounter off Guam, all encounters occurred around Rota during the 2014 May-June visual surveys (Figures 2, 4). All of the encounters occurred within 8 km from shore and were in locations where the water depth was 500 – 1600 m (Table 4). Hill et al. 2014 reported a median distance from shore of 6.4 km and a median depth of 784 m.

Short-finned pilot whales were encountered off Guam and Rota during the 2014 May-June visual surveys with an encounter rate of 0.17 encounters/100 km surveyed (Table 4, Figures 2, 4). Hill et al. 2014 reported a rate of 0.09 encounters/100 km surveyed. The increase observed during the 2014 May-June visual surveys is related to the repeated encounters with the same group (or part of the same group) over a 3-day period off Rota. The encounter locations and filtered satellite tag locations demonstrate the continued use of areas close to shore by short-finned pilot whales as was reported by Hill et al. 2014 (Figures 2, 4, 6). None of the satellite-tagged short-finned pilot whales traveled long distances offshore as the individual with tag 128885 had done in 2013 (Hill et al. 2014). Median distances from shore for encounter locations and filtered satellite tag locations were 3.8 km and 17.1 km respectively (Table 4). The median depth of encounter locations was 794 m and that of satellite tag locations was 1188 m (Table 4) compared to 720 m and 1086 m reported by Hill et al. 2014. Preliminary dive data from a single SPLASH10 tag revealed that short-finned pilot whales in the Marianas will dive to a maximum depth of 1168 m and for maximum periods of 24.4 min (Table 7, Figure 10). In addition, the tag recorded deep dives (> 800 m) during the day and night. Baird et al. 2003 reported that short-finned pilot whales in Hawai'i dove to maximum depths of 800 m for

maximum periods of 27 min during the nighttime. The photo-identification data continue to show that individual short-finned pilot whales associate with the southern islands of the Mariana Archipelago and do so over many years.

False killer whales were encountered off Guam and Tinian and continued to exhibit a broad range of habitat use based on encounter locations and filtered satellite tag locations from the 2014 May-June visual surveys (Tables 4, 6, Figures 2, 3, 7, 11). Most of the filtered satellite tag locations were to the west of the islands with some as far out as the West Mariana Ridge (Figure 11). Individuals with tag IDs 128888 and 128902 traveled up the island chain as far north as Pagan (Figure 11). Distances from shore ranged 5.9 km – 8.4 km for encounter locations and 0.3 km – 216 km for filtered satellite tag locations (Tables 4, 6). Depths of encounter locations were 673 m – 1003 m and those of filtered satellite tag locations were 52 m – 4959 m (Tables 4, 6). Preliminary dive data from 2 SPLASH10 tags revealed that false killer whales in the Marianas will dive to depths of 1360 m and for periods as long as 17.6 min (Table 7, Figures 12, 13). Baird et al. 2013 recorded a maximum dive depth of 1272 m with a 14.7 min duration for a false killer whale tagged off the island of O‘ahu in Hawai‘i and recorded deep dives during the day and night. The photo-identification data suggest that some individuals repeatedly associate with the southernmost islands of the Marianas but that there is likely a larger population that travels throughout the EEZ waters and beyond.

Bottlenose dolphins were encountered at locations with higher median values for both distance from shore (6.0 km) and water depth (800 m) during the 2014 May-June visual surveys than previously observed (Table 4). Hill et al. 2014 reported that the median distance from shore for bottlenose dolphin locations was 0.9 km and the median water depth was 88 m. The filtered satellite tag locations from the single bottlenose dolphin tagged on 12 June 2014 reveal the individual’s use of a wide range of depths (12 m – 1407 m) over the 3.7 days of the satellite tag’s deployment (Table 6, Figure 14). The photo identification data demonstrate that most of the cataloged individuals move between all of the southernmost islands of the Marianas and associate with the islands over periods of years. Analyses of the mitochondrial and nuclear DNA suggest that the Mariana Islands bottlenose dolphin population is a small, genetically isolated population with a history of hybridization with Fraser’s dolphins (Appendix I, Martien et al. 2014).

The Blainville’s beaked whale encounter off Rota was closer to shore (10.9 km) than the two unidentified Mesoplodont whale encounters off Guam (at Tracey Seamount) and Saipan (30.6 km and 20.3 km respectively) during the 2014 May-June visual surveys (Table 4, Figures 2-4). The depth of the Blainville’s beaked whale encounter location of 1200 m fell within the range of the unidentified Mesoplodont encounter location depths of 1074 m and 1614 m off Guam and Saipan respectively (Table 4).

3. What is the baseline abundance and population structure of odontocetes which may be exposed to sonar and/or explosives in the nearshore areas of Guam, Saipan, Tinian, and Rota?

As previously addressed by Hill et al. 2014, baseline abundance and population structure is not straightforward and requires further research to determine which cetaceans may be exposed to sonar and explosives.

Based on filtered satellite tag locations from pilot whales and false killer whales, as well as the observed habitat use of pilot whales, false killer whales, pantropical spotted dolphins and beaked whales during the 2014 May-June surveys it is possible that these species could be exposed to underwater detonations at the Piti Floating Mine Neutralization Area and the Agat Bay UNDET Area sites off Guam (Tables 4, 6, Figures 7, 21).

4. What is the seasonal occurrence of baleen whales around Guam, Saipan, Tinian, and Rota?

Baleen whales were not observed during May-June 2014 visual surveys, nor have they been observed on any previous PIFSC visual survey. The passive acoustic data collected in 2013-14 from near Tinian reveal that blue, fin, sei, and humpback whales were in the region during this period. In contrast to analyses of passive acoustic data collected near Saipan and Tinian from 2010 through mid-2013 (Oleson et al. 2015), fin whales were detected more frequently at Tinian in 2013-14 than in prior years. Two sounds that were likely produced by Bryde's whales, but whose species-identity cannot be confirmed at this time, occurred year-round and were more prevalent in 2013-14 than in previous years. All baleen whale calls were detected in the winter and spring, with very few acoustic detections outside of that period, with the exception of the unidentified tonal and pulsed calls.

Ongoing and Future Work

The analysis of photos and the creation of new photo-identification catalogs will be ongoing. Work has begun on the creation of a catalog for melon-headed whales.

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References

- Au, W.W.L., J.K.B. Ford, J.K. Horne, Newman Allman KA. 2004. Echolocation signals of free-ranging killer whales (*Orcinus orca*) and modeling of foraging for chinook salmon (*Oncorhynchus tshawytscha*). *Journal of the Acoustical Society of America* 115: 901-909.
- Baird, R.W., D.J. McSweeney, M.R. Heithaus and G.J. Marshall. 2003. Short-finned pilot whale diving behavior: deep feeders and day-time socialites. In *Abstracts of the 15th Biennial Conference on the Biology of Marine Mammals*, Greensboro, NC, December 2003.
- Baird, R.W., D.L. Webster, S.D. Mahaffy, G.S. Schorr, J.M. Aschettino, and A.M. Gorgone. 2013. *Movements and Spatial Use of Odontocetes in the western Main Hawaiian Islands: Results of a Three year Study off O'ahu and Kaua'i*. Final report under Grant No. N00244-10-1-0048 from the Naval Postgraduate School. 30 pp.
- Baird, R.W., S.M. Jarvis, D.L. Webster, B.K. Rone, J.A. Shaffer, S.D. Mahaffy, A.M. Gorgone, and D.J. Moretti. 2014. *Odontocete studies on the Pacific Missile Range Facility in July/August 2013: satellite-tagging, photo-identification, and passive acoustic monitoring*. Prepared for U.S. Pacific Fleet, submitted to NAVFAC PAC by HDR Environmental, Operations and Construction, Inc.
- Baumann-Pickering, S., M. A. McDonald, A. E. Simonis, A. Solsona Berga, K. P. B. Merckens, E. M. Oleson, M. A. Roch, S. M. Wiggins, S. Rankin, T. M. Yack and J. A. Hildebrand. 2013. Species-specific beaked whale echolocation signals. *Journal of the Acoustical Society of America* 134:2293-2301.
- Baumann-Pickering, S. A.E. Simonis, E.M. Oleson, R.W. Baird, M.A. Roch, and S.M. Wiggins. *In review*. False killer whale and short-finned pilot whale acoustic identification. *Endangered Species Research*.

Fulling, G.L., P.H. Thorson, and J. Rivers. 2011. Distribution and abundance estimates for Cetaceans in the waters off Guam and the Commonwealth of the Northern Mariana Islands. *Pacific Science* 65(3):321-343.

HDR. 2011. Guam marine species monitoring survey: Vessel-based monitoring surveys winter 2011. Final Report submitted by HDR to U.S. Navy NAVFAC Pacific under Contract No. N62470-10-D-3011 Task Order KB08. 15 pp.

HDR. 2012. Guam-Saipan marine species monitoring winter-spring survey March 2012. Final Report submitted by HDR to U.S. Navy NAVFAC Pacific under Contract No. N62470-10-D-3011 Task Order KB17. 15 pp.

Hill, M.C., A.D. Ligon, M.H. Deakos, A.C. Ü, A. Milette-Winfree, A.R. Bendlin, and E.M. Oleson. 2014. Cetacean surveys in the waters of the southern Mariana Archipelago (February 2010 – April 2014). Prepared for the U.S. Pacific Fleet Environmental Readiness Office. PIFSC Data Report DR-14-013. 49 pp. + Appendix.

Kasuya, T., T. Miyashita, and F. Kasamatsu. 1988. Segregation of two forms of short-finned pilot whales off the Pacific coast of Japan. *Scientific Reports of the Whales Research Institute* 39:77-90.

Madsen, P.T., R. Payne, N.U. Kristiansen, M. Wahlberg, I. Kerr and B. Mohl. 2002. Sperm whale sound production studied with ultrasound time/depth-recording tags. *Journal of Experimental Biology* 205: 1899-1906.

Madsen, P.T., D.A. Carder, K. Bedholm, and S.H. Ridgway. 2005. Porpoise clicks from a sperm whale nose—Convergent evolution of 130 kHz pulses in toothed whale sonars?. *Bioacoustics*, 15(2), 195-206.

Martien, K. K., M. C. Hill, A. M. Van Cise, K. M. Robertson, S. M. Woodman, L. L. Dolar, V. L. Pease, and E. M. Oleson. 2014. Genetic diversity and population structure in four species of cetaceans around the Mariana Islands. NOAA technical Memorandum NOAA-TM-NMFS-SWFS-536.

McDonald, M.A., S.L. Mesnick, J.A. Hildebrand. 2006. Biogeographic characterisation of blue whale song worldwide: using song to identify populations. *Journal of Cetacean Research and Management* 8: 55-65.

Norris, T.F., J. Oswald, T. Yack, E. Ferguson, C. Hom-Weaver, K. Dunleavy, S. Coates, and T. Dominello. 2012. An Analysis of Acoustic Data from the Mariana Islands Sea Turtle and Cetacean Survey (MISTCS). Prepared for Commander, Pacific Fleet, Pearl Harbor, HI. Submitted to Naval Facilities Engineering Command Pacific (NAVFAC), EV2 Environmental Planning, Pearl Harbor, HI, 96860-3134, under Contract No. N62470-10D-3011 CTO KB08, Task Order #002 issued to HDR, Inc. Submitted by Bio-Waves Inc., Encinitas, CA 92024.

Oleson, E.M., J. Barlow, J. Gordon, S. Rankin, and J.A. Hildebrand. 2003. Low frequency calls of Bryde's whales. *Marine Mammal Science* 19(2): 160-172.

- Oleson, E.M., S. Baumann-Pickering, A. Sirovic, K.P. Merckens, L.M. Munger, J.S. Trickey, and P. Fisher-Pool. 2015. Analysis of long-term acoustic datasets for baleen and beaked whales within the Mariana Islands Range Complex (MIRC) for 2010-2013. PIFSC Data Report DR-15-002, 19p.
- Oremus, M., R. Gales, M. L. Dalebout, N. Funahashi, T. Endo, T. Kage, D. Steel, and C. S. Baker. 2009. Worldwide mitochondrial DNA diversity and phylogeography of pilot whales (*Globicephala* spp.). *Biological Journal of the Linnean Society* 98:729-744.
- Oswald, J.N., J. Barlow, and T.F. Norris. 2003. Acoustic identification of nine delphinid species in the eastern tropical Pacific Ocean. *Marine Mammal Science* 19: 20-37.
- Payne, R.S. and S. McVay. 1971. Songs of humpback whales. *Science* 173: 585-597.
- Rankin, S. and J. Barlow. 2005. Source of the North Pacific “boing” sound attributed to minke whales. *Journal of the Acoustical Society of America* 118: 3346-3351.
- Rankin, S. and J. Barlow. 2007. Vocalizations of the sei whale *Balaenoptera borealis* off the Hawaiian Islands. *The International Journal of Animal Sound and its Recording* 16: 137-145.
- Soldevilla, M.S., E.E. Henderson, G.S. Campbell, S.M. Wiggins, J.A. Hildebrand, and M.A. Roch. 2008. Classification of Risso's and Pacific white-sided dolphins using spectral properties of echolocation clicks. *Journal of the Acoustical Society of America* 124: 609-624.
- Thomsen, F., D. Franck, and J.K.B. Ford. 2002. On the communicative significance of whistles in wild killer whales (*Orcinus orca*). *Naturwissenschaften* 89: 404-407.
- Thompson, P.O., L.T. Findley, and O. Vidal. 1992. 20-Hz pulses and other vocalizations of fin whales, *Balaenoptera physalus*, in the Gulf of California, Mexico. *Journal of the Acoustical Society of America* 92: 3051-3057.
- Thompson, P.O., L.T. Findley, O. Vidal, and W.C. Cummings. 1996. Underwater sounds of blue whales, *Balaenoptera musculus*, in the Gulf of California, Mexico. *Marine Mammal Science* 12: 288-293.
- U.S. Navy. 2007. Marine Mammal and Sea Turtle Survey and Density Estimates for Guam and the Commonwealth of the Northern Mariana Islands Final Report. Prepared by SRS-Parsons Joint Venture, Geo-Marine, Inc., and Bio-Waves, Inc. Prepared for Naval Facilities Engineering Command Pacific Commander, U.S. Pacific Fleet.
- Van Cise, A. M., P. A. Morin, R. W. Baird, A. M. Lang, K. M. Robertson, S. J. Chivers, R. L. Brownell Jr, and K. K. Martien. In review. Redrawing the map: mtDNA provides new insights into the distribution and diversity of short-finned pilot whales in the Pacific Ocean. *Marine Mammal Science*.

Watkins, WA. 1981. Activities and underwater sounds of fin whales. Scientific Reports of the Whales Research Institute, Tokyo 33: 83-117.

Wiggins, S. M. 2003. Autonomous Acoustic Recording Packages (ARPs) for long-term monitoring of whale sounds. Marine Technology Society Journal 37(2): 13-22.

Wiggins, S. M. and J. A. Hildebrand. 2007. High-frequency Acoustic Recording Package (HARP) for broad-band, long-term marine mammal monitoring. Pages 551-557 International Symposium on Underwater Technology 2007 and International Workshop on Scientific Use of Submarine Cables & Related Technologies 2007. Institute of Electrical and Electronics Engineers, Tokyo, Japan.

Zimmer, W.M.X., M.P. Johnson, P.T. Madsen, P.L. Tyack. 2005. Echolocation clicks of free-ranging Cuvier's beaked whales (*Ziphius cavirostris*). Journal of the Acoustical Society of America 117: 3919-3927.

Tables

Table 1.- Summary of cetacean visual surveys in the waters surrounding Guam, Rota, Saipan, Tinian, and Aguijan (May-June 2014).

Date (2014)	Location	Vessel	Survey Description	Time On Effort (h:mm)	On Effort Distance (km)
15-May	Guam	<i>Lucky Strike</i>	Hagåtña north to Rota Bank	8:47	121.4
16-May	Guam	<i>Lucky Strike</i>	Hagåtña west to Tracey Seamount	6:36	97.8
17-May	Guam	<i>Mieko</i>	Agat north - offshore loop down to Facpi Pt.	5:12	92.3
19-May	Guam	<i>Mieko</i>	Agat - SW zig zag	6:48	118.1
21-May	Guam	<i>Mieko</i>	Hagåtña - northwest	5:55	55.1
22-May	Guam	<i>Mieko</i>	Agat - SW loop to then north to Piti	5:29	92.2
23-May	Guam	<i>Mieko</i>	Cabras - NW loop nearshore-offshore	5:39	93.5
24-May	Guam	<i>Mieko</i>	Agat - SW spiral	5:39	120.9
25-May	Guam	<i>Mieko</i>	Agat - Agat Bay west loop	4:33	25.7
26-May	Guam	<i>Mieko</i>	Agat- SW loop	3:53	72.0
27-May	Guam	<i>Mieko</i>	Hagåtña - NW zig zag	5:58	117.0
30-May	CNMI-Saipan	<i>Sea Hunter</i>	Saipan - west circuit	5:31	83.5
31-May	CNMI-Saipan/Tinian	<i>Sea Hunter</i>	Saipan-Tinian west loop offshore to inshore	6:19	104.6
1-Jun	CNMI-Saipan	<i>Sea Hunter</i>	Saipan-NW loop	6:57	98.3
2-Jun	CNMI-Saipan/Tinian	<i>Sea Hunter</i>	Tinian circumnavigation	5:35	91.5
4-Jun	CNMI-Saipan	<i>Sea Hunter</i>	Saipan-NW loop	5:52	75.3
5-Jun	CNMI-Saipan/Tinian	<i>Sea Hunter</i>	Saipan-Tinian west offshore loop	6:35	104.3
6-Jun	CNMI-Saipan/Marpi Reef	<i>Regulator</i>	Saipan-west to Marpi Reef then Saipan-east offshore	6:18	115.3
7-Jun	CNMI-Saipan/Tinian/Aguijan	<i>Regulator</i>	Tinian east to Aguijan and south to "Marie's Reef" return on west side	7:55	147.2
8-Jun	CNMI-Saipan/Tinian/Esmeralda Bank	<i>Regulator</i>	Saipan-Tinian west out to Esmeralda Bank and Coke Reef	6:33	133.7
10-Jun	CNMI-Saipan	<i>Regulator</i>	Saipan west offshore triangle	5:16	97.4
11-Jun	CNMI-Saipan	<i>Regulator</i>	Saipan nearshore circumnavigation	5:50	84.3
12-Jun	CNMI-Saipan/Tinian	<i>Regulator</i>	Saipan-Tinian west offshore	7:35	99.9
13-Jun	CNMI-Saipan	<i>Regulator</i>	Saipan west spiral	5:41	115.1

Date (2014)	Location	Vessel	Survey Description	Time On Effort (h:mm)	On Effort Distance (km)
14-Jun	CNMI-Saipan/Tinian/Aguijan	<i>Regulator</i>	Saipan-Tinian west and partial Aguijan circumnavigation	6:04	118.1
16-Jun	CNMI-Rota	<i>Asakaze</i>	Rota NW loop offshore	7:46	88.8
17-Jun	CNMI-Rota	<i>Asakaze</i>	Rota SSE loop	6:30	89.7
18-Jun	CNMI-Rota	<i>Asakaze</i>	Rota circumnavigation offshore	8:20	127.3
19-Jun	CNMI-Rota	<i>Asakaze</i>	Rota circumnavigation at 2-4km distance	3:33	70.0
20-Jun	CNMI-Rota	<i>Asakaze</i>	Rota circumnavigation along shore then loop of north side	7:40	107.5
Total				186:35	2957.7

Table 2. Species and associated call types searched for as part of the analysis of the 2013-14 Tinian HARP dataset. Publications generally refer to the earliest description of a given call type. In most cases there are no published reference signals recorded in the Marianas Archipelago or western Pacific, so other Pacific call types are referenced.

Species	Signal type	Reference
Mysticetes		
Blue whale	central Pacific song	McDonald et al 2006
	D call	Thompson et al 1996
Fin whale	20Hz downsweep	Thompson et al 1992
	40Hz downsweep	
Sei whale	Low-frequency downsweep	Rankin & Barlow 2007
Bryde's whale	All Pacific types	Oleson et al 2003
Humpback whale	general song structure	Payne & McVay 1971
Minke whale	Boing	Rankin & Barlow 2005
Unidentified whale	Call-tonal, Call-pulsed	
Odontocetes		
Sperm whales	clicks, creaks, codas, slow clicks	Madsen et al 2002, Watkins & Schevill 1977
Kogia spp.	clicks	based on very high frequency (extending above 1000kHz), see Madsen et al 2005
Blainville's beaked whale	clicks	Johnson et al 2006
Cuvier's beaked whale	clicks	Zimmer et al 2005
Unidentified beaked whale- "BWC"	clicks	Baumann-Pickering et al 2013
Unidentified beaked whale- other	Upswept unclassified click types	see Baumann-Pickering et al 2013

Short-finned pilot whale	clicks, whistles	Baumann-Pickering et al <i>in review</i> , Oswald et al 2003
False killer whale	clicks, whistles	Baumann-Pickering et al <i>in review</i> , Oswald et al 2003
Risso's dolphin	clicks	based on similarity in structure to Soldevilla et al 2008
Killer whale	clicks, pulsed calls, whistles	based on similarity to Au et al 2004, Thomsen et al 2002
Unidentified dolphin	Clicks<20kHz, Clicks >20kHz, Whistles<10khz, Whistles >10khz	

Table 3.-- Details of encounters with cetacean groups during small vessel visual surveys off Guam, Saipan, Tinian, Aguijan, and Rota (15 May - 20 June 2014) including within-day resights. The number of calves includes the best estimate of the young of the year and neonates combined.

Date (2014)	Sight	Common Name	Time (GMT +10)	Location	Latitude	Longitude	Total Best	Calves Best	Behaviors	Bft.	Swell Height (ft)	Depth Bin (m)	Shore Distance (km)	No. Biopsy Samples	No. Tags	No. Photos
15-May	1	Spinner dolphin	5:51	Guam	13.4864	144.7446	65	1	slow travel, mill, boat approach, bow ride, synch dive/surface, head slap	1	2 to 4	0-100	0.59	0	0	530
15-May	2	Unid. small whale	7:38	Guam	13.6346	144.8002	1	0	log, dive	1	2 to 4	501-600	4.12	0	0	0
16-May	3	Unid. Mesoplodont	9:01	Guam	13.6252	144.4015	1	0	dive	4	2 to 4	1001-1100	30.59	0	0	0
19-May	4	Short-finned pilot whale	12:49	Guam	13.4360	144.6175	23	0	slow travel, spy hop, boat approach, bow ride, tail slap	3	0 to 2	301-400	0.64	2	2	719
21-May	5a	False killer whale	7:41	Guam	13.5533	144.7059	13	0	mod travel	4	2 to 4	1001-1100	8.36	9	2	1227
21-May	5b	Bottlenose dolphin	9:57	Guam	13.6326	144.7348	4	0	boat approach, bow ride, porpoise, wave ride	5	2 to 4	801-900	11.06	0	0	83
22-May	6	Spinner dolphin	9:48	Guam	13.4697	144.6937	15	0	mill, synch dive/surface, rest	4	2 to 4	0-100	0.42	0	0	293
23-May	7	Spinner dolphin	7:45	Guam	13.4862	144.7585	16	0	slow travel, boat approach, bow ride, synch dive/surface	4	2 to 4	0-100	0.73	0	0	475

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Date (2014)	Sight	Common Name	Time (GMT +10)	Location	Latitude	Longitude	Total Best	Calves Best	Behaviors	Bft.	Swell Height (ft)	Depth Bin (m)	Shore Distance (km)	No. Biopsy Samples	No. Tags	No. Photos
23-May	8	Spinner dolphin	8:21	Guam	13.5110	144.7885	31	0	slow travel, synch dive/surface, leap, boat approach, bow ride	4	2 to 4	0-100	0.54	0	0	530
23-May	9	Pantropical spotted dolphin	10:12	Guam	13.5842	144.7462	105	4	leap, mod trav, porpoise, boat approach, bow ride, tail slap	5	2 to 4	801-900	7.73	0	0	871
25-May	10	Short-finned pilot whale	6:29	Guam	13.3788	144.6274	19	0	slow travel, log, boat approach, spy hop, low swim	2	0 to 2	501-600	2.46	5	2	1047
26-May	11	Spinner dolphin	10:32	Guam	13.3665	144.6406	5	0	synch dive/surface, social, boat approach, bow ride, spy hop	4	2 to 4	101-200	0.92	0	0	122
30-May	12	Spinner dolphin	6:52	CNMI-Saipan	15.2638	145.7760	47	2	slow travel, boat approach, bow ride, spin, leap	4	2 to 4	0-100	0.74	0	0	389
1-Jun	13	Unid. Mesoplodont	8:58	CNMI-Saipan	15.3528	145.5871	1	0	slow travel	4	2 to 4	1601-1700	20.28	0	0	21
4-Jun	14	Cuvier's beaked whale	8:45	CNMI-Saipan	15.3098	145.5683	4	0	slow travel	4	2 to 4	1701-1800	18.82	0	0	230

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Date (2014)	Sight	Common Name	Time (GMT +10)	Location	Latitude	Longitude	Total Best	Calves Best	Behaviors	Bft.	Swell Height (ft)	Depth Bin (m)	Shore Distance (km)	No. Biopsy Samples	No. Tags	No. Photos
5-Jun	15	Spinner dolphin	6:03	CNMI-Saipan	15.2283	145.6909	29	1	slow travel, mill, synch dive/surface, boat approach, bow ride	1	0 to 2	0-100	2.93	0	0	695
6-Jun	16	Spinner dolphin	7:35	CNMI-Marpi Reef	15.4251	145.8682	98	3	slow travel, synch dive/surface, boat approach, spin, leap, head slap, bow ride, social, porpoise	3	2 to 4	0-100	16.14	0	0	1168
7-Jun	17	Spinner dolphin	6:14	CNMI-Saipan	15.2307	145.6832	28	1	leap, mill, boat approach, bow ride, synch dive/surface, social	0	0 to 2	101-200	3.8	0	0	640
7-Jun	18	Spinner dolphin	8:58	CNMI-Aguijan	14.8563	145.5815	135	1	boat approach, bow ride, leap, spin, mill, synch dive/surface, social, head slap, porpoise	2	2 to 4	201-300	0.37	0	0	935
8-Jun	19	Spinner dolphin	12:06	CNMI-Saipan	15.2231	145.6990	21	1	mill, synch dive/surface, leap, boat approach, bow ride, head slap, social	4	0 to 2	0-100	1.93	0	0	776

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Date (2014)	Sight	Common Name	Time (GMT +10)	Location	Latitude	Longitude	Total Best	Calves Best	Behaviors	Bft.	Swell Height (ft)	Depth Bin (m)	Shore Distance (km)	No. Biopsy Samples	No. Tags	No. Photos
11-Jun	20	Spinner dolphin	6:41	CNMI-Saipan	15.2578	145.7480	19	0	slow travel, boat approach, bow ride, leap, spin, porpoise, tail slap, synch dive/surface	2	0 to 2	0-100	1.77	0	0	778
11-Jun	21	Spinner dolphin	8:06	CNMI-Saipan	15.2746	145.8312	50	0	mill, surf waves, boat approach, bow ride, leap, porpoise	5	2 to 4	0-100	0.19	0	0	688
11-Jun	22	Spinner dolphin	9:14	CNMI-Saipan	15.2381	145.8107	19	0	boat approach, bow ride, slow travel, synch dive/surface	3	2 to 4	0-100	0.26	0	0	190
12-Jun	23a	False killer whale	8:13	CNMI-Tinian	14.9908	145.5301	2	0	mod travel, boat approach, bow ride	5	2 to 4	601-700	5.89	1	1	220
12-Jun	23b	Bottlenose dolphin	8:14	CNMI-Tinian	14.9908	145.5301	1	0	boat approach	5	2 to 4	601-700	5.89	0	0	0
12-Jun	23b-resight	Bottlenose dolphin	10:14	CNMI-Tinian	15.0361	145.5312	1	0	boat approach, bow ride, evasive	5	2 to 4	701-800	5.83	1	0	46
12-Jun	23a-resight	False killer whale	10:18	CNMI-Tinian	15.0344	145.5081	9	0	mod travel, boat approach, bow ride	5	2 to 4	801-900	8.19	6	1	735
12-Jun	23c	Bottlenose dolphin	12:28	CNMI-Tinian	15.1237	145.5593	16	0	mod travel, boat approach, bow ride	5	2 to 4	901-1000	8.2	0	1	524

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Date (2014)	Sight	Common Name	Time (GMT +10)	Location	Latitude	Longitude	Total Best	Calves Best	Behaviors	Bft.	Swell Height (ft)	Depth Bin (m)	Shore Distance (km)	No. Biopsy Samples	No. Tags	No. Photos
14-Jun	24	Spinner dolphin	9:18	CNMI-Aguijan	14.8625	145.5797	67	0	bow ride, mill, synch dive/surface, leap, spin	4	2 to 4	0-100	0.13	0	0	571
16-Jun	25	Pantropical spotted dolphin	8:01	CNMI-Rota	14.2445	145.2745	36	0	boat approach, bow ride, mill	2	2 to 4	901-1000	5.2	0	0	739
16-Jun	26	Short-finned pilot whale	9:17	CNMI-Rota	14.2159	145.3139	48	1	slow travel	1	2 to 4	701-800	3.8	9	3	2102
16-Jun	27	Spinner dolphin	14:03	CNMI-Rota	14.1649	145.1487	5	0	slow travel	1	2 to 4	0-100	0.77	0	0	99
17-Jun	28	Pantropical spotted dolphin	6:32	CNMI-Rota	14.1750	145.0994	19	0	mill, slow travel	2	2 to 4	901-1000	5.5	0	0	214
17-Jun	29	Short-finned pilot whale	9:00	CNMI-Rota	14.0544	145.2383	36	0	mod travel, slow travel	0	2 to 4	1401-1500	7.29	2	1	1558
17-Jun	30	Pantropical spotted dolphin	10:20	CNMI-Rota	14.1018	145.2719	16	0	boat approach, bow ride, porpoise	0	2 to 4	1501-1600	5.48	0	0	100
18-Jun	31	Spinner dolphin	6:19	CNMI-Rota	14.1391	145.1311	5	1	slow travel, boat approach, bow ride, synch dive/surface	0	0 to 2	0-100	0.28	0	0	149
18-Jun	32	Pantropical spotted dolphin	7:24	CNMI-Rota	14.2640	145.2206	40	1	mill, feed, boat approach, bow ride, leap, porpoise	1	2 to 4	801-900	7.38	0	0	470
18-Jun	33	Unid. Ziphiid whale	8:54	CNMI-Rota	14.2227	145.3457	2	0	slow travel	2	2 to 4	901-1000	7.02	0	0	0
18-Jun	34	Blainville's beaked whale	12:25	CNMI-Rota	14.0692	145.0340	1	0	slow roll, evasive	3	2 to 4	1101-1200	10.9	0	0	107

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Date (2014)	Sight	Common Name	Time (GMT +10)	Location	Latitude	Longitude	Total Best	Calves Best	Behaviors	Bft.	Swell Height (ft)	Depth Bin (m)	Shore Distance (km)	No. Biopsy Samples	No. Tags	No. Photos
18-Jun	35	Short-finned pilot whale	13:22	CNMI-Rota	14.1674	145.0890	15	0	slow travel, dive	2	2 to 4	901-1000	5.7	0	0	162
20-Jun	36a	Bottlenose dolphin	7:05	CNMI-Rota	14.1922	145.2926	2	0	boat approach, bow ride, mill, leap	1	2 to 4	201-300	0.37	1	0	77
20-Jun	36b	Spinner dolphin	7:08	CNMI-Rota	14.1905	145.2947		0	spin, mill, boat approach, bow ride	1	2 to 4	101-200	0.49	0	0	0
20-Jun	36b-resight	Spinner dolphin	8:30	CNMI-Rota	14.1621	145.2853	64	0	spin, mill, boat approach, bow ride, synch dive/surface, social	1	2 to 4	101-200	0.16	0	0	721
20-Jun	37	Pantropical spotted dolphin	12:41	CNMI-Rota	14.1937	145.1388	145	0	boat approach, bow ride, leap, porpoise, social, slow travel	1	2 to 4	501-600	3.62	0	0	1212
Total:														36	13	22213

Table 4.—Summary of cetacean encounters during the 2014 May-June visual surveys including encounter rates, distances from shore, and water depth. The total distance surveyed was 2,958 km. The number of encounters and the encounter rate calculation excludes within-day resights. Summaries of shore distance and depth for those species denoted with * include 1 within-day resight encounter location.

Species	No. Encounters	Encounters/100km effort	Shore Distance (km) - median (min-max)	Water Depth (m) - median (min-max)
Spinner dolphin*	18	0.61	0.6 (0.1-16.1)	56 (2-260)
Pantropical spotted dolphin	6	0.20	5.5 (3.6-7.7)	907 (528-1543)
Short-finned pilot whale	5	0.17	3.8 (0.6-7.3)	794 (341-1443)
Bottlenose dolphin*	4	0.14	6.0 (0.4-11.1)	800 (243-934)
False killer whale*	2	0.07	8.2 (5.9-8.4)	872 (673-1003)
Unid. Mesoplodont	2	0.07	25.4 (20.3-30.6)	1344 (1074-1614)
Blainville's beaked whale	1	0.03	10.9	1200
Cuvier's beaked whale	1	0.03	18.8	1706
Unid. small whale	1	0.03	4.1	568
Unid. Ziphiid whale	1	0.03	7.0	972
Total:	41	1.39		

Table 5.-- Turtle sightings during cetacean visual surveys in the waters off Guam, Saipan, Tinian, Aguijan, and Rota (May - June 2014).

Date (2014)	Time	Island	Latitude	Longitude	Description
15-May	6:17	Guam	13.4851	144.7500	Green Turtle-large (>2.5 ft)
15-May	6:20	Guam	13.4850	144.7513	Turtle-med (1.5-2.5 ft)
19-May	7:01	Guam	13.3663	144.6464	Turtle-small (<1.5 ft)
22-May	9:52	Guam	13.4724	144.6927	Turtle-med (1.5-2.5 ft)
22-May	10:24	Guam	13.4709	144.6934	Green Turtle-med (1.5-2.5 ft)
22-May	10:26	Guam	13.4727	144.6916	Turtle-large (>2.5 ft)
23-May	6:51	Guam	13.4571	144.6573	Green Turtle-med (1.5-2.5 ft)
23-May	7:35	Guam	13.4833	144.7309	Turtle-med (1.5-2.5 ft)
23-May	8:50	Guam	13.5228	144.7992	Green Turtle-large (>2.5 ft)
23-May	8:59	Guam	13.5430	144.8039	Turtle-large (>2.5 ft)
23-May	9:01	Guam	13.5503	144.8068	Turtle-large (>2.5 ft)
23-May	9:03	Guam	13.5555	144.8086	Turtle-med (1.5-2.5 ft) x 2
24-May	8:47	Guam	13.4069	144.6560	Turtle-med (1.5-2.5 ft)
24-May	9:52	Guam	13.2693	144.6584	Green Turtle-med (1.5-2.5 ft)
26-May	8:36	Guam	13.3992	144.6571	Turtle-small (<1.5 ft)
26-May	8:44	Guam	13.4125	144.6458	Green Turtle-med (1.5-2.5 ft)
27-May	11:01	Guam	13.5127	144.7918	Turtle-med (1.5-2.5 ft)
30-May	11:44	Saipan	15.2274	145.7207	Green Turtle-med (1.5-2.5 ft)
31-May	10:09	Tinian	14.9302	145.6282	Green Turtle-med (1.5-2.5 ft)
31-May	11:11	Saipan	15.0467	145.5926	Green Turtle-med (1.5-2.5 ft) x2
31-May	12:29	Saipan	15.2085	145.6947	Green Turtle-large (>2.5 ft)
31-May	12:31	Saipan	15.2128	145.6958	Turtle-med (1.5-2.5 ft)
31-May	12:34	Saipan	15.2195	145.6979	Green Turtle-med (1.5-2.5 ft)
31-May	12:43	Saipan	15.2278	145.7156	Turtle-med (1.5-2.5 ft)
1-Jun	13:05	Saipan	15.2256	145.6906	Turtle-med (1.5-2.5 ft)
2-Jun	6:11	Saipan	15.2084	145.6950	Green Turtle-large (>2.5 ft)
2-Jun	11:46	Saipan	15.2277	145.7170	Green Turtle-med (1.5-2.5 ft)
4-Jun	12:02	Saipan	15.2283	145.7100	Green Turtle-med (1.5-2.5 ft)
5-Jun	12:35	Saipan	15.2272	145.7033	Turtle-small (<1.5 ft) x2
5-Jun	12:40	Saipan	15.2279	145.7157	Turtle-small (<1.5 ft)
5-Jun	12:42	Saipan	15.2270	145.7198	Turtle-med (1.5-2.5 ft)
5-Jun	12:43	Saipan	15.2259	145.7211	Turtle-med (1.5-2.5 ft)
6-Jun	6:10	Saipan	15.2288	145.6952	Green Turtle-small (<1.5 ft) x2
6-Jun	6:10	Saipan	15.2288	145.6942	Green Turtle-small (<1.5 ft)
6-Jun	6:11	Saipan	15.2287	145.6907	Green Turtle-small (<1.5 ft)
6-Jun	6:12	Saipan	15.2292	145.6878	Turtle-med (1.5-2.5 ft)
6-Jun	12:05	Saipan	15.1661	145.6800	Green Turtle-large (>2.5 ft)

Date (2014)	Time	Island	Latitude	Longitude	Description
6-Jun	12:25	Saipan	15.2207	145.7006	Green Turtle-large (>2.5 ft)
6-Jun	12:27	Saipan	15.2251	145.7029	Turtle-med (1.5-2.5 ft)
6-Jun	12:32	Saipan	15.2256	145.7208	Turtle-med (1.5-2.5 ft)
7-Jun	14:09	Saipan	15.2259	145.7200	Turtle-med (1.5-2.5 ft)
8-Jun	6:15	Saipan	15.2284	145.6973	Turtle-small (<1.5 ft)
8-Jun	6:16	Saipan	15.2286	145.6942	Turtle-small (<1.5 ft)
8-Jun	6:16	Saipan	15.2286	145.6917	Turtle-small (<1.5 ft)
10-Jun	11:41	Saipan	15.2267	145.7164	Turtle-small (<1.5 ft)
10-Jun	11:42	Saipan	15.2253	145.7199	Turtle-large (>2.5 ft)
11-Jun	9:07	Saipan	15.2531	145.8141	Green Turtle-large (>2.5 ft)
11-Jun	10:24	Saipan	15.1585	145.7942	Turtle-large (>2.5 ft)
11-Jun	10:27	Saipan	15.1489	145.7936	Turtle-large (>2.5 ft)
11-Jun	10:46	Saipan	15.1377	145.7449	Green Turtle-large (>2.5 ft)
11-Jun	10:53	Saipan	15.1224	145.7575	Green Turtle-large (>2.5 ft)
11-Jun	11:54	Saipan	15.2008	145.6952	Green Turtle-med (1.5-2.5 ft)
11-Jun	12:01	Saipan	15.2195	145.6971	Turtle-med (1.5-2.5 ft) x2
11-Jun	12:07	Saipan	15.2276	145.7152	Green Turtle-small (<1.5 ft)
11-Jun	12:08	Saipan	15.2273	145.7162	Green Turtle-small (<1.5 ft)x2
12-Jun	13:50	Saipan	15.2253	145.7210	Green Turtle-med (1.5-2.5 ft)
12-Jun	13:50	Saipan	15.2243	145.7220	Green Turtle-med (1.5-2.5 ft)
13-Jun	12:02	Saipan	15.2256	145.7195	Green Turtle-med (1.5-2.5 ft)
13-Jun	12:02	Saipan	15.2249	145.7208	Turtle-med (1.5-2.5 ft)
14-Jun	9:54	Aguijan	14.8615	145.5852	Green Turtle-large (>2.5 ft)
14-Jun	12:06	Saipan	15.2027	145.6916	Turtle-large (>2.5 ft)
14-Jun	12:08	Saipan	15.2082	145.6952	Turtle-med (1.5-2.5 ft)
14-Jun	12:18	Saipan	15.2270	145.7159	Green Turtle-small (<1.5 ft)
20-Jun	6:32	Rota	14.1891	145.2040	Green Turtle-med (1.5-2.5 ft)
20-Jun	8:59	Rota	14.1729	145.2868	Turtle-med (1.5-2.5 ft)

Table 6.-- Summary of satellite tags deployed during cetacean visual surveys in the waters off Guam, Saipan, Tinian, Aguijan, and Rota (May - June 2014) including depths and distances from shore of the Douglas Argos filtered locations.

Species and Tag IDs	Tag Type	Deployment Location	Deployment Date-Time (GMT +10)	Duration (Days)	Shore Distance (km) - median (min-max)	Water Depth (m) - median (min-max)
Short-finned pilot whale					17.1 (0.03-110.1)	1188 (15-4615)
128889	SPLASH10	Guam	05/19/2014 13:26	34.7	17.3 (1.4-91)	887 (24-4176)
128920	SPOT5	Guam	05/19/2014 13:54	39.7	20.7 (0.5-96.1)	912 (26-4249)
128914	SPOT5	Guam	05/25/2014 8:10	35.3	11.1 (0.03-109.8)	1085 (17-4191)
128910	SPOT5	Guam	05/25/2014 9:50	62.4	13.5 (0.1-110.1)	1300 (15-4277)
128899	SPOT5	CNMI-Rota	06/16/2014 9:36	83.5	19.1 (1.3-88.1)	1386 (17-4615)
137726	SPOT5	CNMI-Rota	06/16/2014 11:27	50.9	18.1 (0.3-88.4)	1386 (52-4571)
137727	SPOT5	CNMI-Rota	06/16/2014 13:12	94.6	17.5 (0.4-85.3)	1212 (29-4498)
137728	SPOT5	CNMI-Rota	06/17/2014 10:07	10.5	11.2 (0.6-39.9)	1104 (36-2317)
False killer whale					48 (0.3-216.4)	3180 (52-4959)
128887	SPLASH10	Guam	05/21/2014 7:50	31.4	16.9 (0.8-154.7)	3286 (482-4792)
128902	SPOT5	Guam	05/21/2014 8:44	39.0	95.7 (1.5-203.1)	3437 (351-4416)
128888	SPLASH10	CNMI-Tinian	06/12/2014 8:29	22.3	35.9 (0.6-108.3)	2756 (53-4308)
128901	SPOT5	CNMI-Tinian	06/12/2014 11:05	30.7	53.9 (0.3-216.4)	3000 (52-4959)
Bottlenose dolphin					4.6 (0.2-13.9)	503 (12-1407)
128912	SPOT5	CNMI-Saipan/ Tinian	06/12/2014 12:44	3.7	4.6 (0.2-13.9)	503 (12-1407)

Table 7.-- Summary of dive data (depths and durations) from SPLASH10 satellite tags deployed on 2 false killer whales and a short-finned pilot whale during cetacean visual surveys in the waters off Guam, Saipan, Tinian, Aguijan, and Rota (May - June 2014).

Species and Tag IDs	Total Dive/Surface Data (hrs)	No. of Dives \geq 30m	Median Dive Depth (m)	Maximum Dive Depth (m)	Median Dive Duration (min)	Maximum Dive Duration (min)
Short-finned pilot whale						
128889	443.9	1321	167.5	1167.5	9.9	24.4
False killer whale	868.1	499				
128887	658.9	167	240.5	1359.5	5.4	17.6
128888	209.2	332	95.5	847.5	4.2	13.1

Table 8.-- Details of short-finned pilot whale encounters analyzed for individual photo-identification including the number of cataloged individuals identified during each encounter (including new individuals) and the number of new individuals added to the catalog after each encounter. The total number of cataloged individuals identified represents all encounters with cataloged individuals including resights. The total number of new cataloged individuals represents the current catalog size.

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
2/11/2007	41	MISTCS	West Mariana Ridge	17.1000	142.8500	23	0	0
3/16/2007	111a	MISTCS	High Seas- south of CNMI EEZ	10.1833	144.1167	108	0	0
3/20/2007	127	MISTCS	Guam	13.6167	145.0667	23	4	0
3/28/2007	133	MISTCS	Mariana Trough-central	17.7833	143.7167	42	0	0
2/22/2011	5a	HDR	Guam	13.5785	144.7613	649	13	13
8/27/2011	2	PIFSC	Guam	13.5791	144.7501	389	10	10
9/8/2011	2	PIFSC	Saipan	15.3039	145.7113	445	19	10
9/15/2011	1	PIFSC	Rota	14.1136	145.1259	996	32	32
9/29/2011	3	PIFSC	Tinian	15.0219	145.5413	792	30	30

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
3/21/2012	6	HDR	Guam	13.3889	144.5954	583	20	0
5/26/2012	3	PIFSC	Guam	13.7076	144.8246	676	19	0
6/8/2012	14a	PIFSC	Aguijan	14.7827	145.4912	533	20	20
6/8/2012	14c	PIFSC	Aguijan	14.7960	145.5292	200	5	5
6/10/2012	17	PIFSC	Esmeralda Bank	14.9935	145.2356	373	9	9
6/30/2013	6b	PIFSC	Guam	13.4847	144.6589	1004	20	2
6/30/2013	6c	PIFSC	Guam	13.5526	144.7137	379	4	0
7/1/2013	11	PIFSC	Guam	13.4023	144.6097	1179	15	15
5/19/2014	4	PIFSC	Guam	13.4360	144.6175	719	21	12
5/25/2014	10	PIFSC	Guam	13.3789	144.6274	1047	20	1
6/16/2014	26	PIFSC	Rota	14.2159	145.3139	2102	41	13
6/17/2014	29	PIFSC	Rota	14.0544	145.2383	1558	35	6
6/18/2014	35	PIFSC	Rota	14.1674	145.0890	162	13	0
Total:						13982	350	178

Table 9.-- Details of bottlenose dolphin encounters analyzed for individual photo-identification including the number of cataloged individuals identified during each encounter (including new individuals) and the number of new individuals added to the catalog after each encounter. The total number of cataloged individuals identified represents all encounters with cataloged individuals including resights. The total number of new cataloged individuals represents the current catalog size.

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
3/16/2007	111b	MISTCS	High Seas -south of CNMI EEZ	10.1833	144.1167	3	0	0
3/18/2007	126b	MISTCS	High Seas -south of CNMI EEZ	10.4667	142.0833	21	0	0
2/22/2011	5b	HDR	Guam	13.5785	144.7613	90	3	3

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
8/29/2011	2	PIFSC	Rota Bank	13.7996	144.9539	158	9	9
9/9/2011	2	PIFSC	Saipan	15.1351	145.7456	307	8	5
9/10/2011	3	PIFSC	Tinian	15.0990	145.6365	222	7	0
3/24/2012	8	HDR	Saipan	15.2619	145.7347	134	3	3
5/29/2012	5	PIFSC	Rota	14.1621	145.1491	340	12	6
6/8/2012	14b	PIFSC	Aguijan	14.7785	145.5184	116	4	4
6/26/2012	27	PIFSC	Rota Bank	13.7958	144.9563	141	5	1
6/29/2012	30	PIFSC	Guam	13.4410	144.6093	285	4	3
6/30/2013	6a	PIFSC	Guam	13.4823	144.6507	67	3	1
7/6/2013	14b	PIFSC	Rota	14.1405	145.1260	3	0	0
7/9/2013	19	PIFSC	Rota	14.1470	145.1375	805	12	2
7/10/2013	21	PIFSC	Rota	14.1976	145.2267	337	11	0
7/15/2013	29a	PIFSC	Aguijan	14.8576	145.5831	123	5	2
7/17/2013	30	PIFSC	Saipan	15.2505	145.7060	132	3	1
7/17/2013	31	PIFSC	Saipan	15.2041	145.6968	447	6	5
7/23/2013	39	PIFSC	Saipan	15.2989	145.7068	200	5	0
4/16/2014	7a	PIFSC	Aguijan	14.8378	145.5424	703	9	2
5/21/2014	5b	PIFSC	Guam	13.63255	144.7348	83	2	2
6/12/2014	23b	PIFSC	Tinian	14.99084	145.5301	46	1	1
6/12/2014	23c	PIFSC	Tinian	15.12367	145.5593	524	13	2
6/20/2014	36a	PIFSC	Rota	14.19217	145.2926	77	2	0
Total:						5364	127	52

Table 10.-- Details of spinner dolphin encounters analyzed for individual photo-identification including the number of cataloged individuals identified during each encounter (including new individuals) and the number of new individuals added to the catalog

after each encounter. The total number of cataloged individuals identified represents all encounters with cataloged individuals including resights. The total number of new cataloged individuals represents the current catalog size. TBD = To Be Determined.

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
2/17/2007	56	MISTCS	Saipan	15.3167	145.8333	22	0	0
2/9/2010	1	PIFSC	Guam	13.4080	144.6580	274	15	15
2/9/2010	2	PIFSC	Guam	13.4070	144.6580	167	12	0
2/10/2010	1	PIFSC	Guam	13.3970	144.6580	250	8	1
2/10/2010	2	PIFSC	Guam	13.3350	144.6430	353	7	7
2/11/2010	1	PIFSC	Guam	13.4050	144.6570	491	20	11
2/12/2010	1	PIFSC	Guam	13.3960	144.6580	505	28	9
2/13/2010	1	PIFSC	Guam	13.4080	144.6560	240	19	0
2/14/2010	1	PIFSC	Guam	13.4070	144.6570	78	7	4
2/22/2010	1	PIFSC	Saipan	15.2487	145.7023	72	2	2
2/22/2010	2	PIFSC	Marpi Reef	15.4392	145.8839	187	7	7
2/23/2010	1	PIFSC	Saipan	15.2655	145.8346	34	2	2
2/23/2010	2	PIFSC	Saipan	15.1791	145.7890	232	4	4
2/23/2010	3	PIFSC	Saipan	15.1063	145.7575	186	3	3
2/18/2011	1	HDR	Guam	13.3898	144.6422	565	20	10
2/20/2011	2	HDR	Guam	13.4142	144.6449	102	3	3
2/21/2011	4	HDR	Guam	13.4883	144.7618	336	8	2
2/22/2011	6	HDR	Guam	13.5155	144.7940	101	9	1
3/1/2011	8	HDR	Guam	13.4032	144.6564	136	3	0
3/1/2011	9	HDR	Guam	13.3932	144.6521	252	6	1
08/28/2011	1	PIFSC	Guam	13.5159	144.7951	266	9	1
08/29/2011	1	PIFSC	Rota Bank	13.7955	144.9532	428	15	15
08/30/2011	1	PIFSC	Guam	13.2720	144.7571	320	11	8

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
09/01/2011	1	PIFSC	Guam	13.5630	144.9430	439	18	13
9/7/2011	2	PIFSC	Aguijan	14.8557	145.5823	615	22	19
9/8/2011	4	PIFSC	Marpi Reef	15.4110	145.8704	343	13	9
9/9/2011	1	PIFSC	Saipan	15.2680	145.7790	696	14	10
9/10/2011	1	PIFSC	Tinian	14.9790	145.6681	480	3	3
9/10/2011	2	PIFSC	Tinian	14.9202	145.6415	27	1	0
9/14/2011	2	PIFSC	Rota	14.1095	145.1775	315	14	14
9/15/2011	2	PIFSC	Rota	14.1156	145.1243	142	5	5
9/17/2011	2	PIFSC	Rota	14.1953	145.2935	462	9	1
9/18/2011	1	PIFSC	Rota	14.1839	145.2938	338	5	2
9/18/2011	2	PIFSC	Rota	14.1279	145.2310	212	9	0
9/19/2011	1	PIFSC	Rota	14.1306	145.1409	262	7	1
9/19/2011	2	PIFSC	Rota	14.1832	145.2947	205	7	0
9/24/2011	1	PIFSC	Marpi Reef	15.4328	145.8862	391	11	3
9/25/2011	1	PIFSC	Saipan	15.1926	145.7849	373	6	2
9/25/2011	2	PIFSC	Saipan	15.0922	145.7532	69	1	1
9/25/2011	3	PIFSC	Saipan	15.1200	145.6864	26	1	0
3/21/2012	5	HDR	Guam	13.4034	144.6572	319	7	0
5/25/2012	1	PIFSC	Guam	13.6085	144.9086	374	8	2
6/4/2012	9	PIFSC	Rota	14.1831	145.2920	27	2	1
6/8/2012	12	PIFSC	Saipan	15.1765	145.6872	399	6	5
6/8/2012	13	PIFSC	Aguijan	14.8525	145.5788	119	1	0
6/9/2012	15	PIFSC	Marpi Reef	15.4218	145.8792	706	22	5
6/11/2012	18	PIFSC	Saipan	15.2292	145.6915	326	4	3
6/11/2012	19	PIFSC	Saipan	15.2896	145.8181	177	4	1
6/16/2012	21	PIFSC	Saipan	15.2730	145.8341	673	8	0
6/16/2012	22	PIFSC	Saipan	15.1631	145.7994	22	1	0

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
6/24/2012	24	PIFSC	Marpi Reef	15.4210	145.8763	104	1	0
6/26/2012	28	PIFSC	Rota Bank	13.7950	144.9584	189	12	3
6/29/2012	31	PIFSC	Guam	13.5140	144.7942	215	15	2
6/30/2012	32	PIFSC	Guam	13.3473	144.6346	346	3	0
7/2/2012	33	PIFSC	Guam	13.3277	144.6481	440	4	1
7/2/2012	34	PIFSC	Guam	13.2775	144.6607	987	29	14
7/2/2012	35	PIFSC	Guam	13.4779	144.7144	360	13	0
7/3/2012	37	PIFSC	Guam	13.4862	144.7475	796	11	0
06/23/2013	2	PIFSC	Guam	13.4007	144.6595	520	19	5
06/28/2013	5	PIFSC	Guam	13.4845	144.7543	381	12	0
06/30/2013	7	PIFSC	Guam	13.6514	144.8784	95	1	0
06/30/2013	8	PIFSC	Guam	13.6140	144.9079	421	12	3
06/30/2013	9	PIFSC	Guam	13.5673	144.9506	432	14	10
07/07/2013	16	PIFSC	Rota	14.1673	145.2878	392	10	6
07/09/2013	18	PIFSC	Rota	14.1389	145.1287	34	1	0
07/12/2013	22	PIFSC	Saipan	15.2661	145.7792	538	10	5
07/12/2013	23	PIFSC	Saipan	15.2725	145.8340	406	14	12
07/12/2013	24	PIFSC	Saipan	15.1164	145.7585	313	8	6
07/13/2013	26	PIFSC	Saipan	15.2283	145.7057	565	13	3
07/14/2013	27	PIFSC	Saipan	15.2054	145.6808	45	2	0
07/15/2013	28	PIFSC	Aguijan	14.8625	145.5803	463	11	7
07/18/2013	33	PIFSC	Marpi Reef	15.4135	145.8752	616	18	6
07/19/2013	34	PIFSC	Saipan	15.2179	145.6702	239	2	1
07/21/2013	36	PIFSC	Saipan	15.2104	145.6957	511	7	2
07/21/2013	37	PIFSC	Saipan	15.1734	145.6914	44	2	0
07/24/2013	40	PIFSC	Saipan	15.1923	145.6830	404	10	0
07/24/2013	41	PIFSC	Tinian	14.9912	145.6727	664	18	10

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
07/27/2013	42	PIFSC	Aguijan	14.8593	145.5817	449	13	5
04/11/2014	1	PIFSC	Saipan	15.2347	145.6905	118	TBD	TBD
04/12/2014	2	PIFSC	Tinian	14.9912	145.6733	357	TBD	TBD
04/14/2014	3	PIFSC	Saipan	15.2279	145.6929	303	TBD	TBD
04/14/2014	4	PIFSC	Saipan	15.2282	145.7126	388	TBD	TBD
04/15/2014	5	PIFSC	Marpi Reef	15.4321	145.8854	746	TBD	TBD
04/16/2014	6	PIFSC	Aguijan	14.8640	145.5801	405	TBD	TBD
04/25/2014	11	PIFSC	Guam	13.4851	144.7331	188	TBD	TBD
04/26/2014	13	PIFSC	Guam	13.4082	144.6571	318	TBD	TBD
04/27/2014	14	PIFSC	Guam	13.4859	144.7595	188	TBD	TBD
5/15/2014	1	PIFSC	Guam	13.4864	144.7446	530	Initial sorting completed	TBD
5/22/2014	6	PIFSC	Guam	13.4697	144.6937	293	Initial sorting completed	TBD
5/23/2014	7	PIFSC	Guam	13.4862	144.7585	475	Initial sorting completed	TBD
5/23/2014	8	PIFSC	Guam	13.5110	144.7885	530	Initial sorting completed	TBD
5/26/2014	11	PIFSC	Guam	13.3665	144.6406	122	TBD	TBD
5/30/2014	12	PIFSC	Saipan	15.2638	145.7760	389	Initial sorting completed	TBD
6/5/2014	15	PIFSC	Saipan	15.2283	145.6909	695	Initial sorting in completed	TBD

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
6/6/2014	16	PIFSC	Marpi Reef	15.4251	145.8682	1168	Initial sorting in completed	TBD
6/7/2014	17	PIFSC	Saipan	15.2307	145.6832	640	TBD	TBD
6/7/2014	18	PIFSC	Aguijan	14.8563	145.5815	935	TBD	TBD
6/8/2014	19	PIFSC	Saipan	15.2231	145.6990	776	TBD	TBD
6/11/2014	20	PIFSC	Saipan	15.2578	145.7480	778	TBD	TBD
6/11/2014	21	PIFSC	Saipan	15.2746	145.8312	688	TBD	TBD
6/11/2014	22	PIFSC	Saipan	15.2381	145.8107	190	TBD	TBD
6/14/2014	24	PIFSC	Aguijan	14.8625	145.5797	571	TBD	TBD
6/16/2014	27	PIFSC	Rota	14.1649	145.1487	99	TBD	TBD
6/18/2014	31	PIFSC	Rota	14.1391	145.1311	149	TBD	TBD
6/20/2014	36b	PIFSC	Rota	14.1905	145.2947	721	Initial sorting completed	TBD
Total:						37841	712	307

Table 11.-- Details of false killer whale encounters analyzed for individual photo-identification including the number of cataloged individuals identified during each encounter (including new individuals) and the number of new individuals added to the catalog after each encounter. The total number of cataloged individuals identified represents all encounters with cataloged individuals including resights. The total number of new cataloged individuals represents the current catalog size.

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
2/16/2007	52	MISTCS	West Mariana Ridge	16.1167	142.3833	56	0	0
2/19/2007	66	MISTCS	CNMI EEZ - southeast	14.5500	147.4667	3	0	0
2/20/2007	68b	MISTCS	CNMI EEZ - southeast	13.7500	146.2833	12	0	0
2/25/2007	90	MISTCS	CNMI EEZ - south central	13.4833	144.4333	6	1	1
3/13/2007	99	MISTCS	High Seas- south of CNMI EEZ	11.5500	147.5333	3	0	0
3/13/2007	101	MISTCS	High Seas- south of CNMI EEZ	11.2833	147.3000	24	0	0
3/17/2007	112	MISTCS	CNMI EEZ - south	11.7667	143.7500	2	0	0
6/22/2013	1	PIFSC	Guam	13.5310	144.6114	28	2	2
7/6/2013	14a	PIFSC	Rota	14.1405	145.1260	2161	13	13
7/7/2013	17	PIFSC	Rota	14.1143	145.0680	1162	14	14
5/21/2014	5a	PIFSC	Guam	13.5533	144.7059	1227	10	3
6/12/2014	23a	PIFSC	Tinian	14.9908	145.5301	955	9	7
Total:						5639	49	40

Table 12.-- Details of pygmy killer whale encounters analyzed for individual photo-identification including the number of cataloged individuals identified during each encounter (including new individuals) and the number of new individuals added to the catalog after each encounter. The total number of cataloged individuals identified represents all encounters with cataloged individuals including resights. The total number of new cataloged individuals represents the current catalog size.

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
6/25/2013	4	PIFSC	Guam	13.4744	144.6402	578	6	6
4/26/2014	12	PIFSC	Guam	13.2370	144.6299	212	6	0
Total:						790	12	6

Table 13.-- Details of rough-toothed dolphin encounters analyzed for individual photo-identification including the number of cataloged individuals identified during each encounter (including new individuals) and the number of new individuals added to the catalog after each encounter. The total number of cataloged individuals identified represents all encounters with cataloged individuals including resights. The total number of new cataloged individuals represents the current catalog size.

Date	Sighting	Research Group	Location	Latitude	Longitude	No. Photos	No. Cataloged Individuals ID'd	No. New Cataloged Individuals
7/15/2013	29b	PIFSC	Aguijan	14.8567	145.5820	297	6	6
7/20/2013	35	PIFSC	Saipan	15.2340	145.6200	299	4	0
4/16/2014	7b	PIFSC	Aguijan	14.8359	145.5420	703	4	0
Total:						1299	14	6

Figures

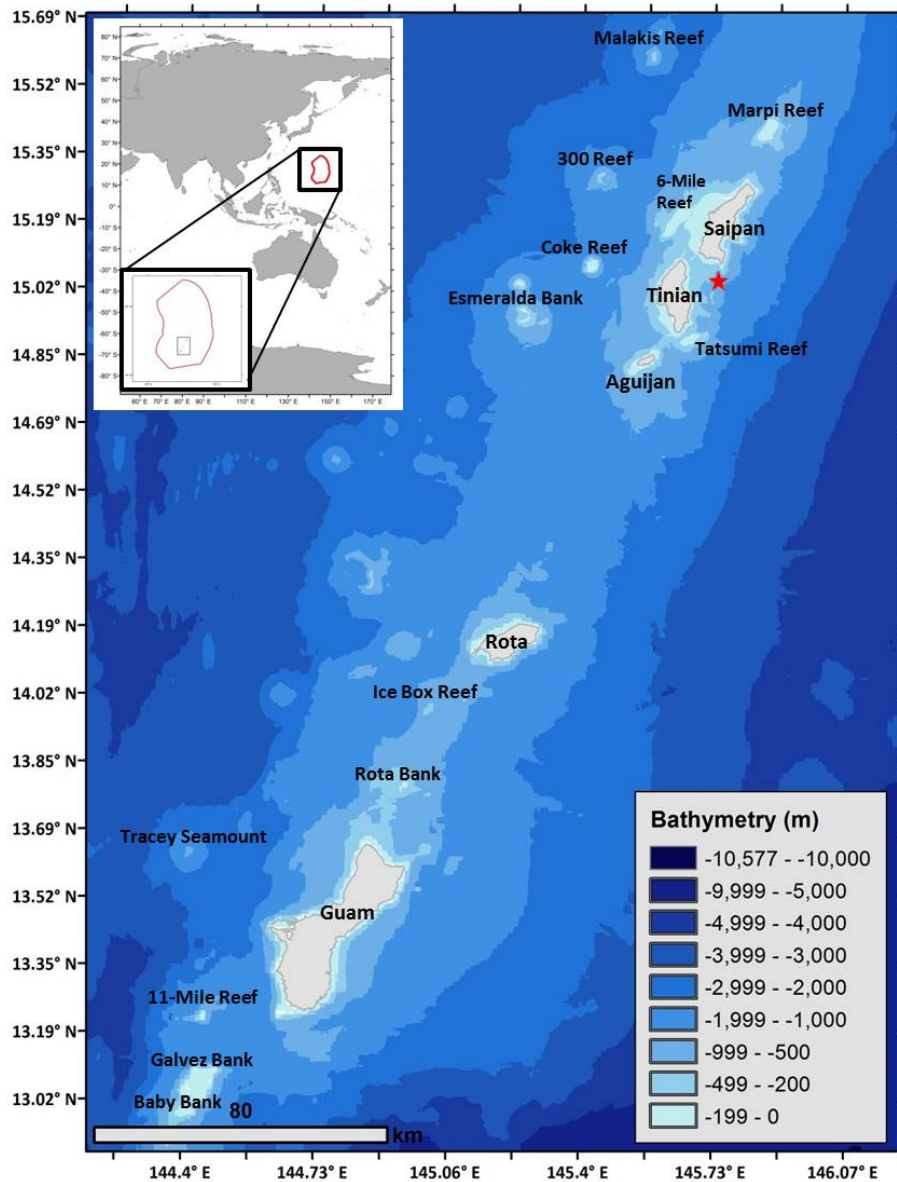


Figure 1.-- Survey area within the southern portion of the Mariana Archipelago. The red star indicates the location of the 2013-14 Tinian HARP.

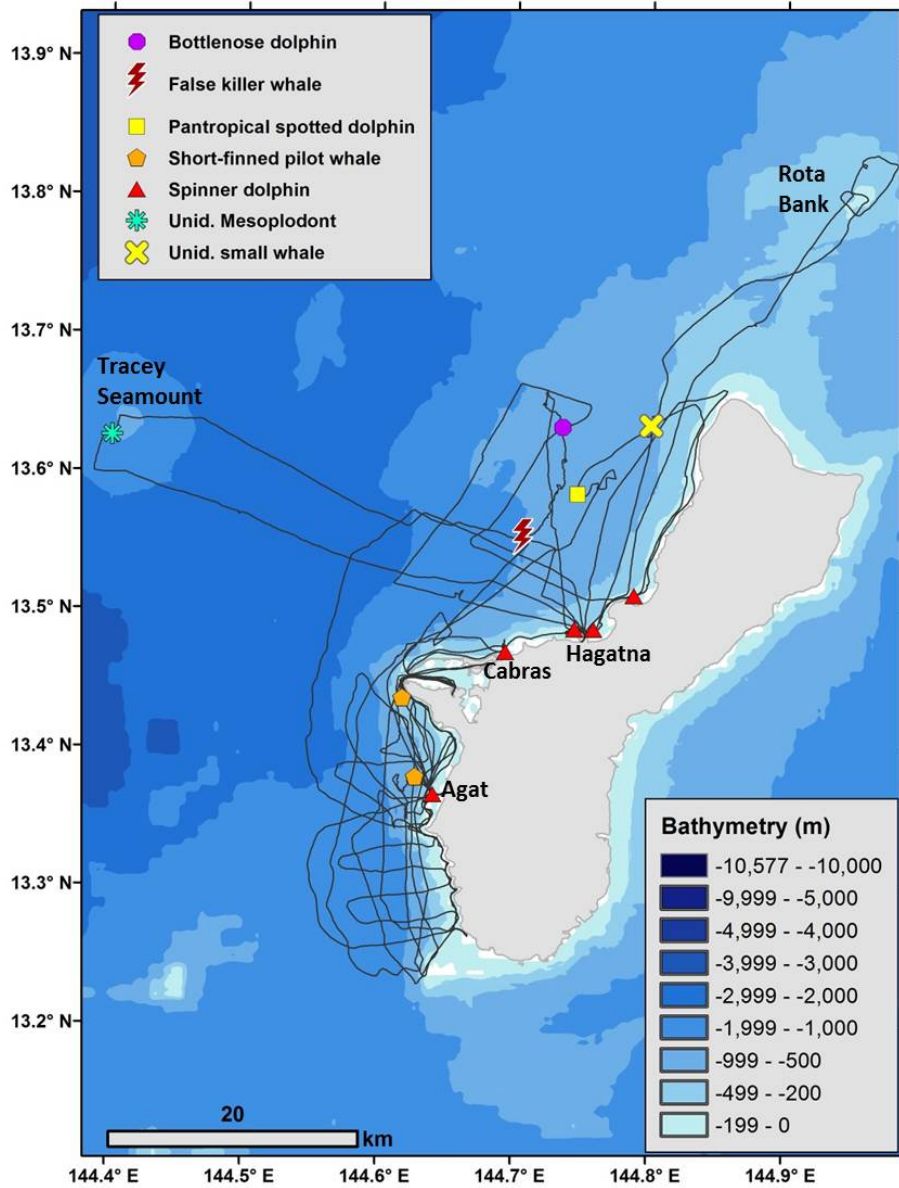


Figure 2.-- Visual survey tracklines and cetacean encounters off Guam (15-27 May 2014).

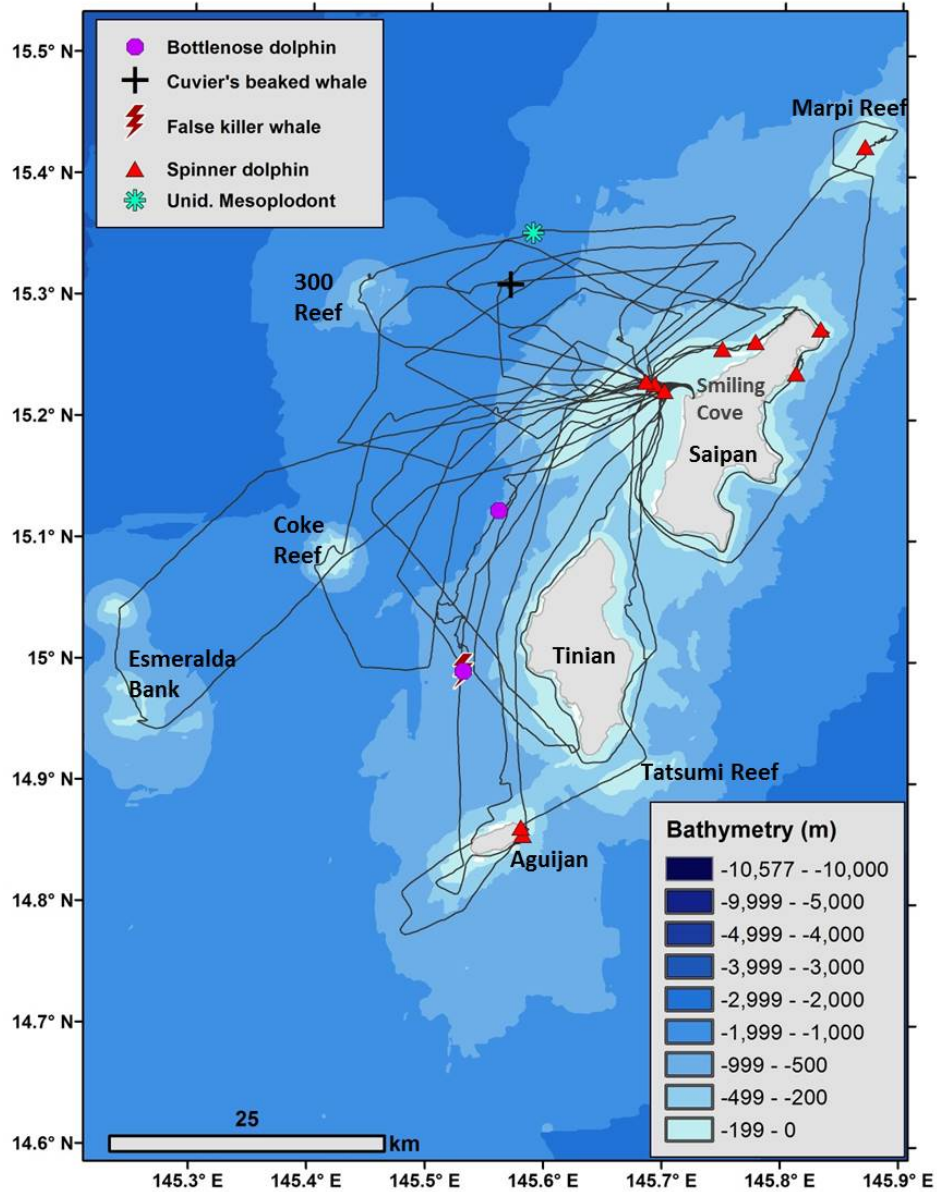


Figure 3.-- Visual survey tracklines and cetacean encounters off Saipan, Tinian, and Aguijan (30 May - 14 June 2014).

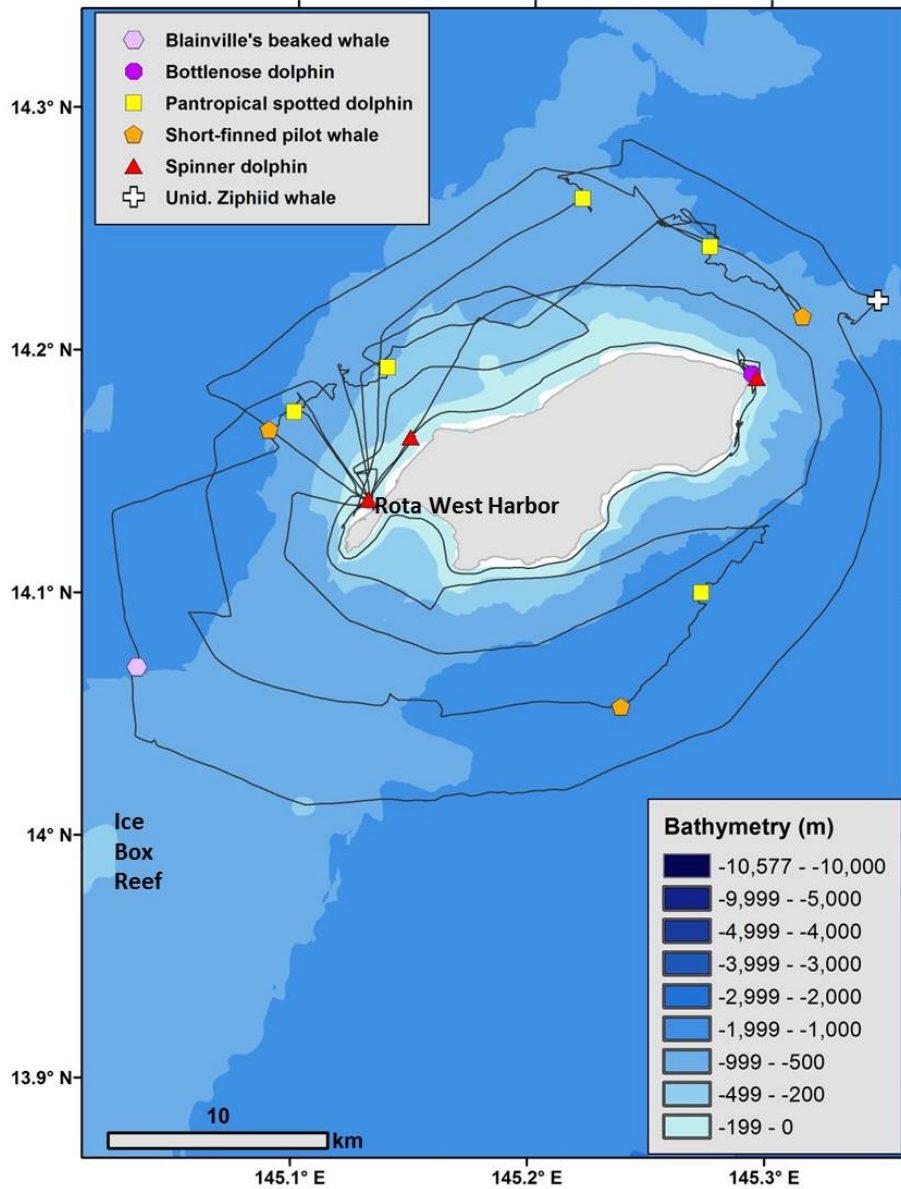


Figure 4.-- Visual survey tracklines and cetacean encounters off Rota (16 - 20 June 2014).

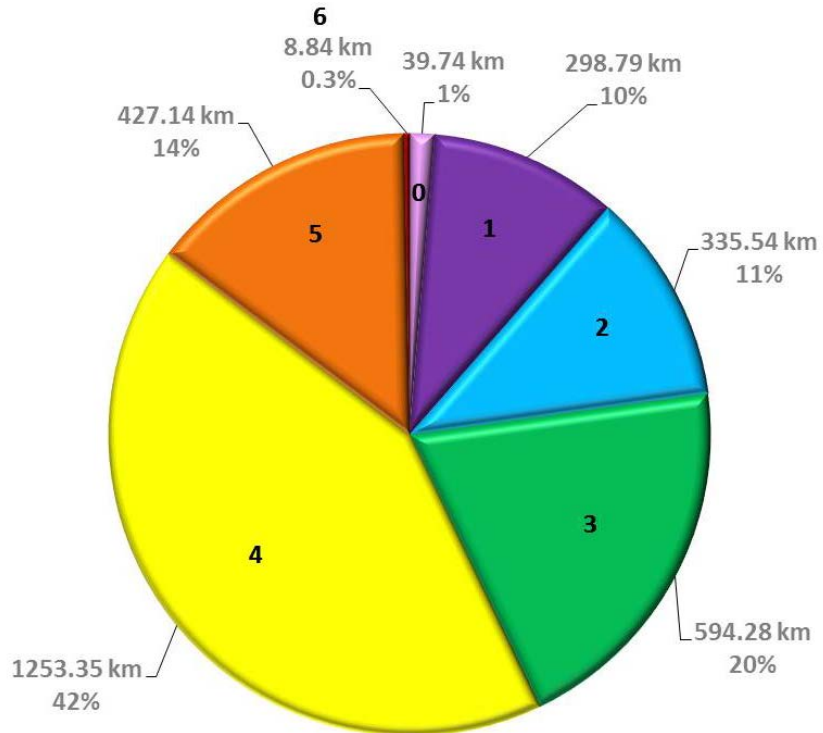


Figure 5.-- Survey effort by Beaufort Sea State for May-June 2014 visual survey.

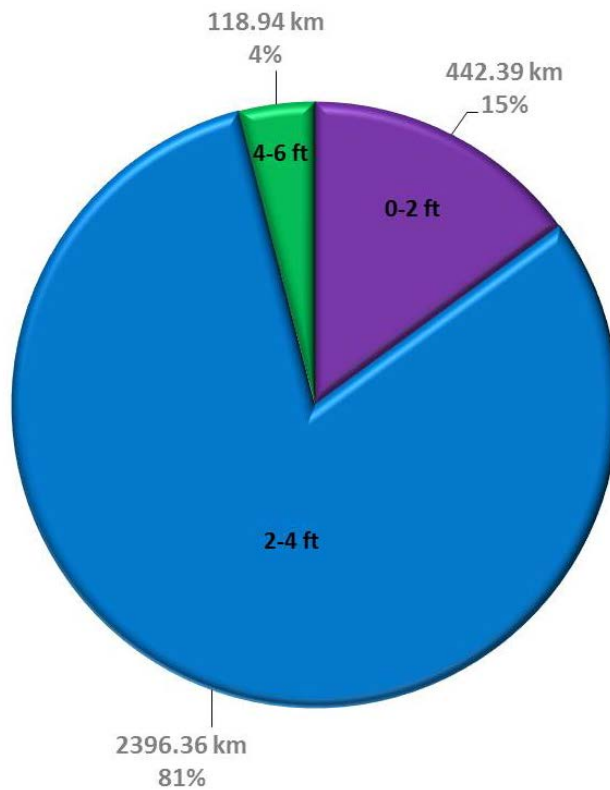


Figure 6.-- Survey effort by swell height (ft) for May-June 2014 visual survey.

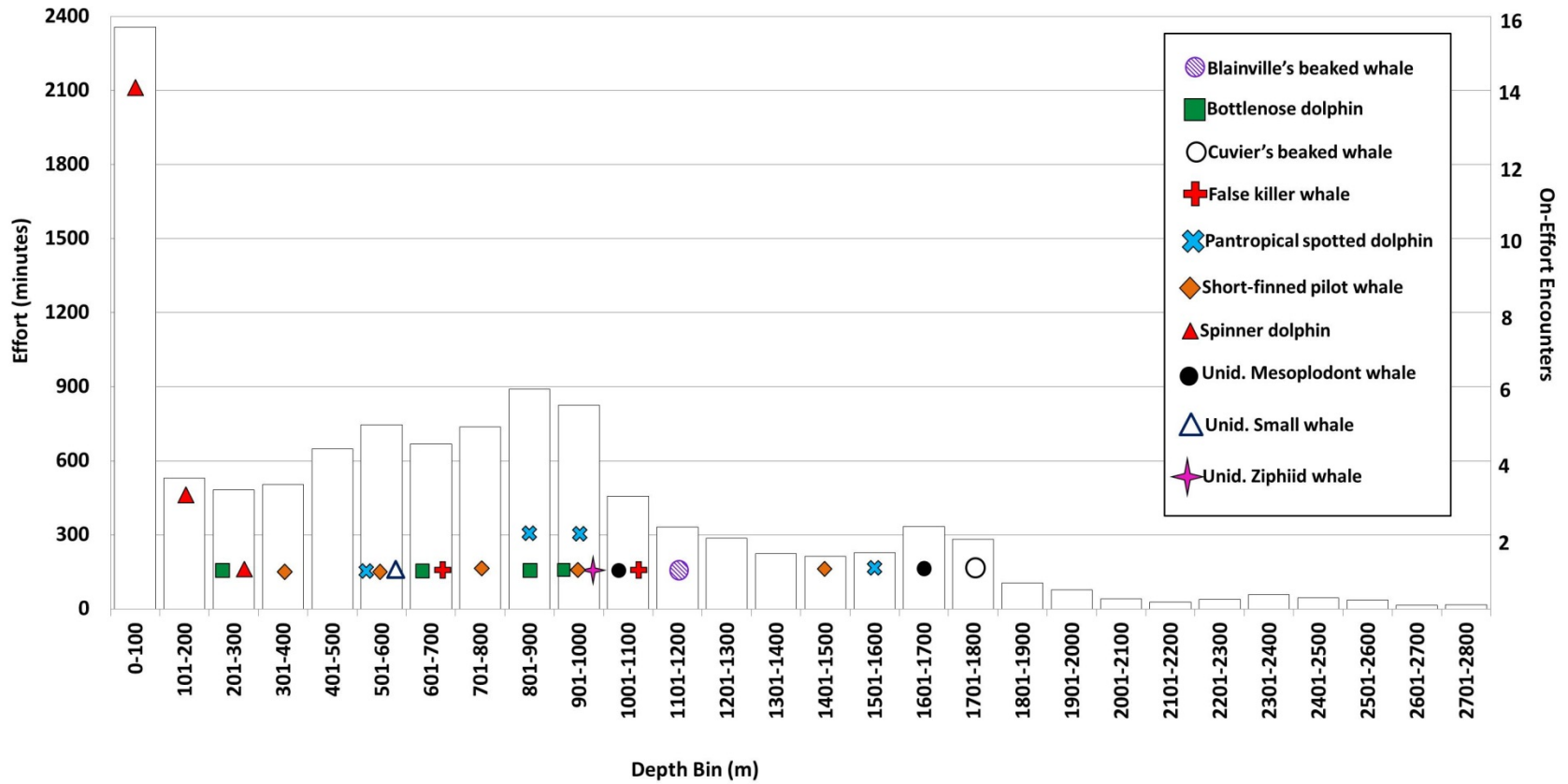


Figure 7.-- Survey effort and cetacean encounters by depth during the May-June 2014 visual survey. Total on-effort survey time was 186.6 hours (11,196 minutes).

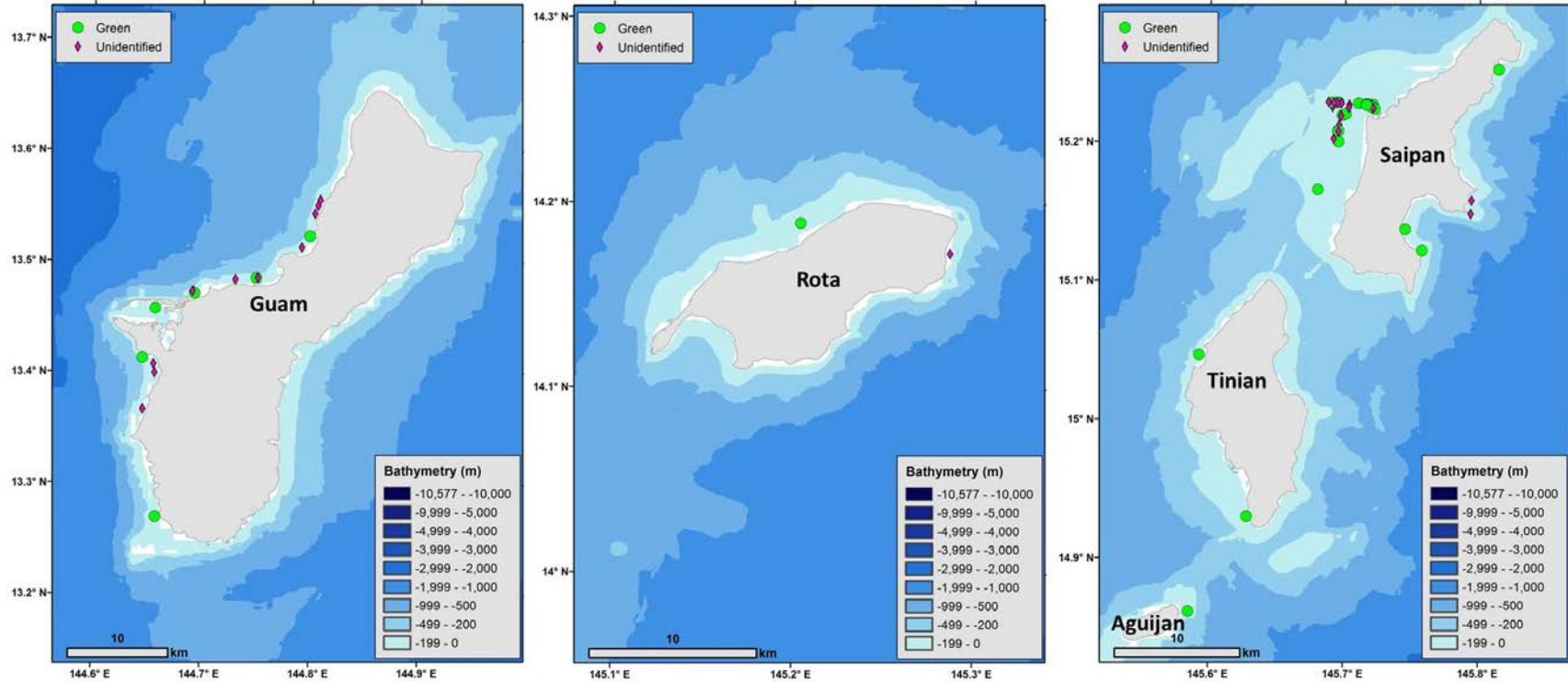


Figure 8.-- Sea turtles observed during the cetacean visual surveys off the islands within the southern Mariana Archipelago (15 May - 20 June 2014).

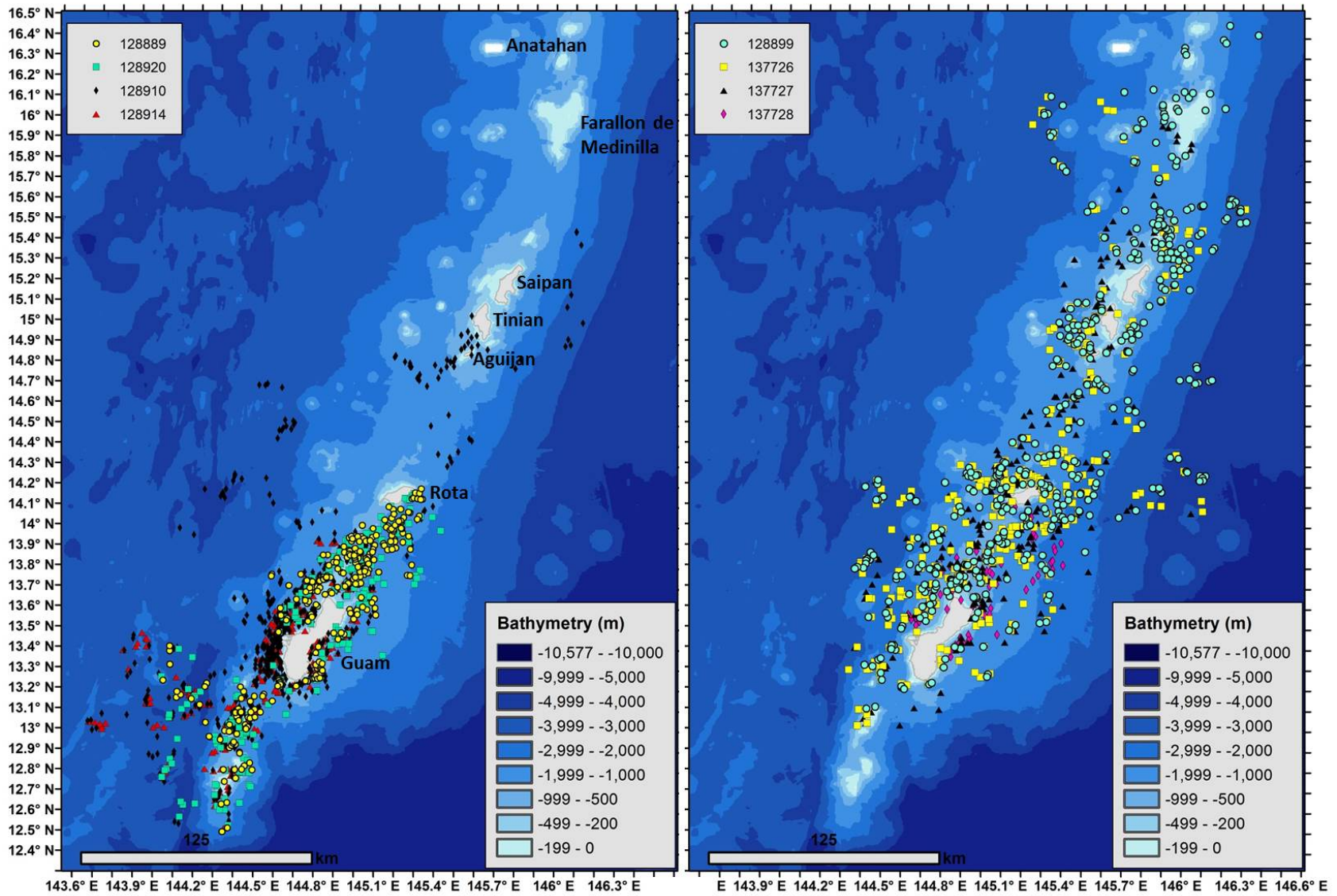


Figure 9.-- Douglas Argos filtered satellite locations for tags deployed on short-finned pilot whales. Left panel: tags 128889, 128920, 128910, 128914 deployed on individuals encountered off Guam (19 and 25 May 2014) with island locations labeled. Deployment durations were 34.7 d, 39.7 d, 35.3 d, 62.4 d respectively. Right panel: tags 128899, 137726, 137727, 137728 deployed on individuals encountered off Rota (16 and 17 June 2014). Deployment durations were 83.5 d, 50.9 d, 94.6 d, 10.5 d respectively.

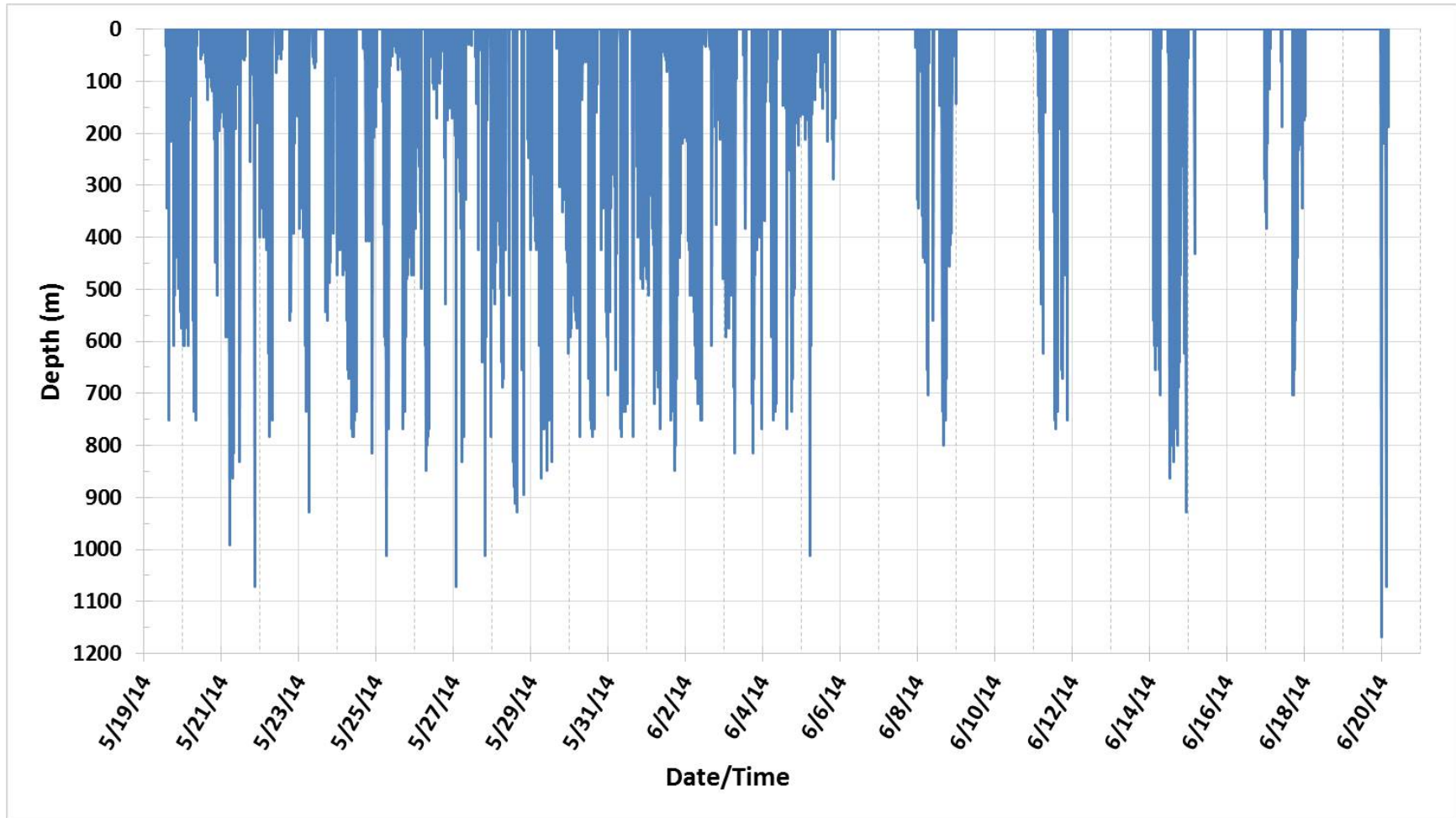


Figure 10: Maximum dive depths for each of the 1321 dives recorded from the short-finned pilot whale with SPLASH10 satellite tag ID 128889. Apparent gaps in dive data are due to tag duty-cycling (see Methods for details).

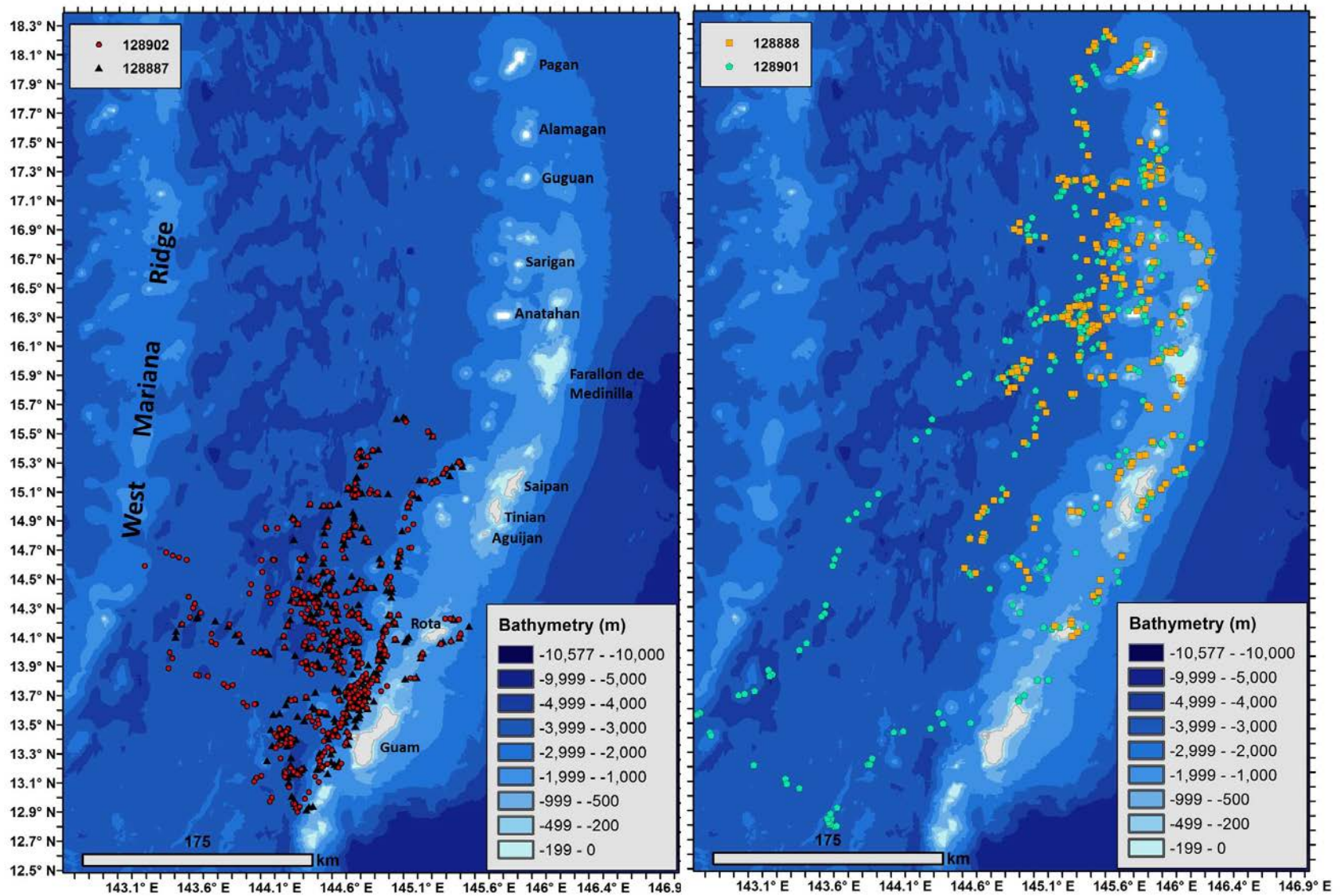


Figure 11.-- Douglas Argos filtered satellite locations from tags deployed on false killer whales. Left panel: tags 128887 and 128902 deployed on individuals encountered off Guam (21 May 2014) with island locations labeled. Deployment durations were 31.4 d and 39.0 d respectively. Right panel: tags 128888 and 128901 deployed on individuals off Tinian (12 June 2014). Deployment durations were 22.3 d and 30.7 d respectively.

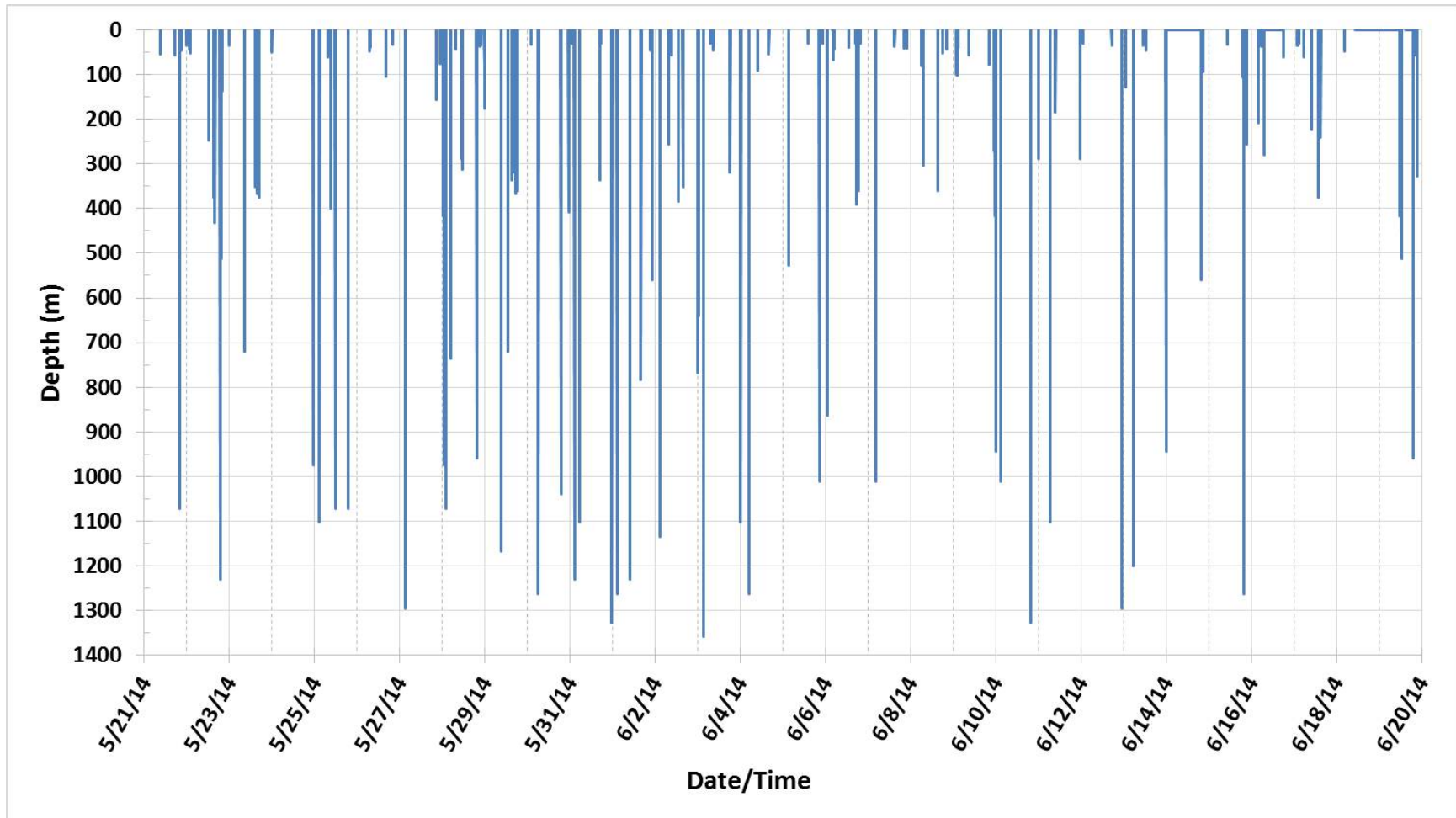


Figure 12: Maximum dive depths for each of the 167 dives recorded from the false killer whale with SPLASH10 satellite tag ID 128887, deployed off Guam. Apparent gaps in dive data are due to tag duty-cycling (see Methods for details).

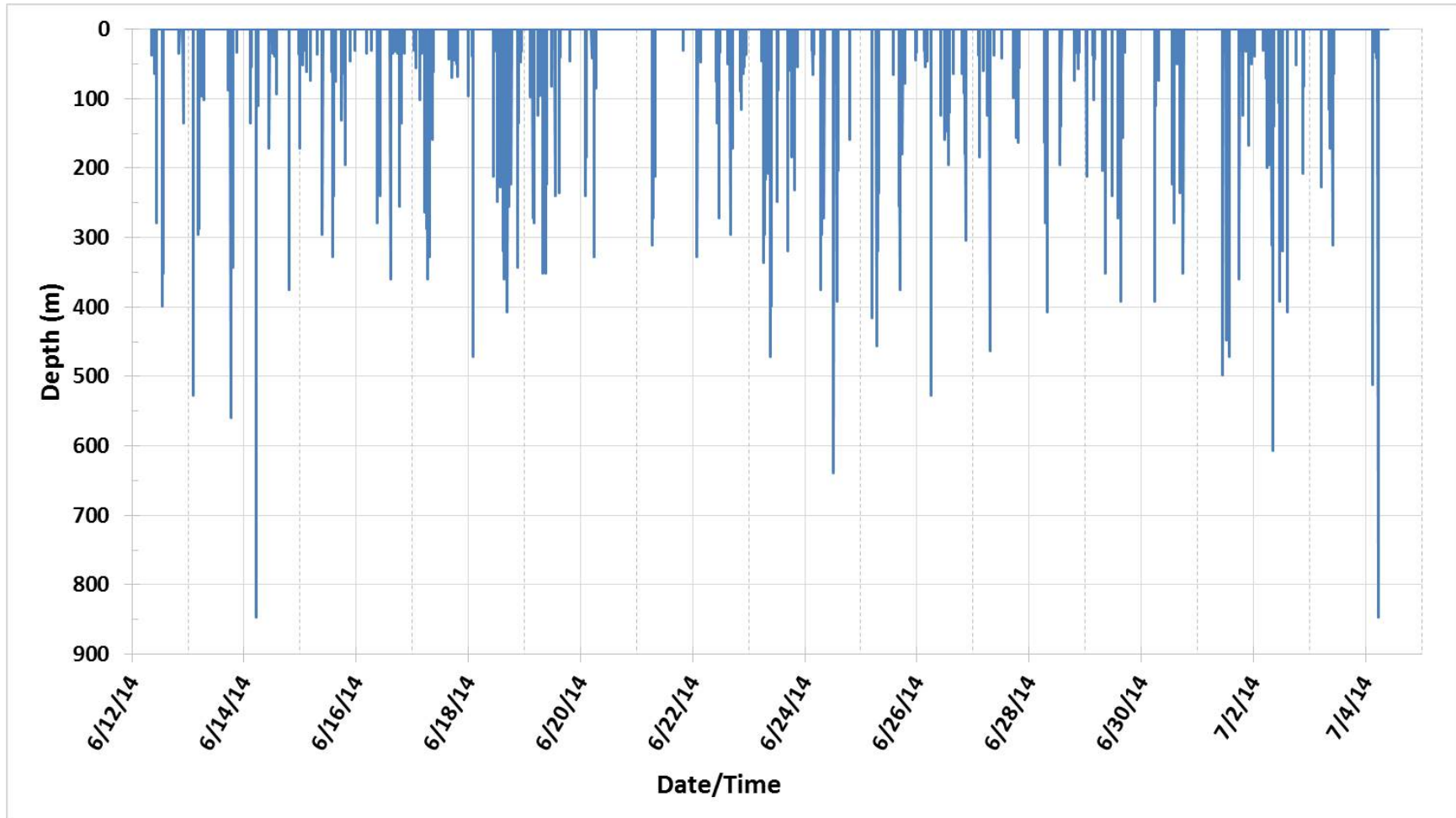


Figure 13: Maximum dive depths for each of the 332 dives recorded from the false killer whale with SPLASH10 satellite tag ID 128888, deployed off Tinian. Apparent gaps in dive data are due to tag duty-cycling (see Methods for details).

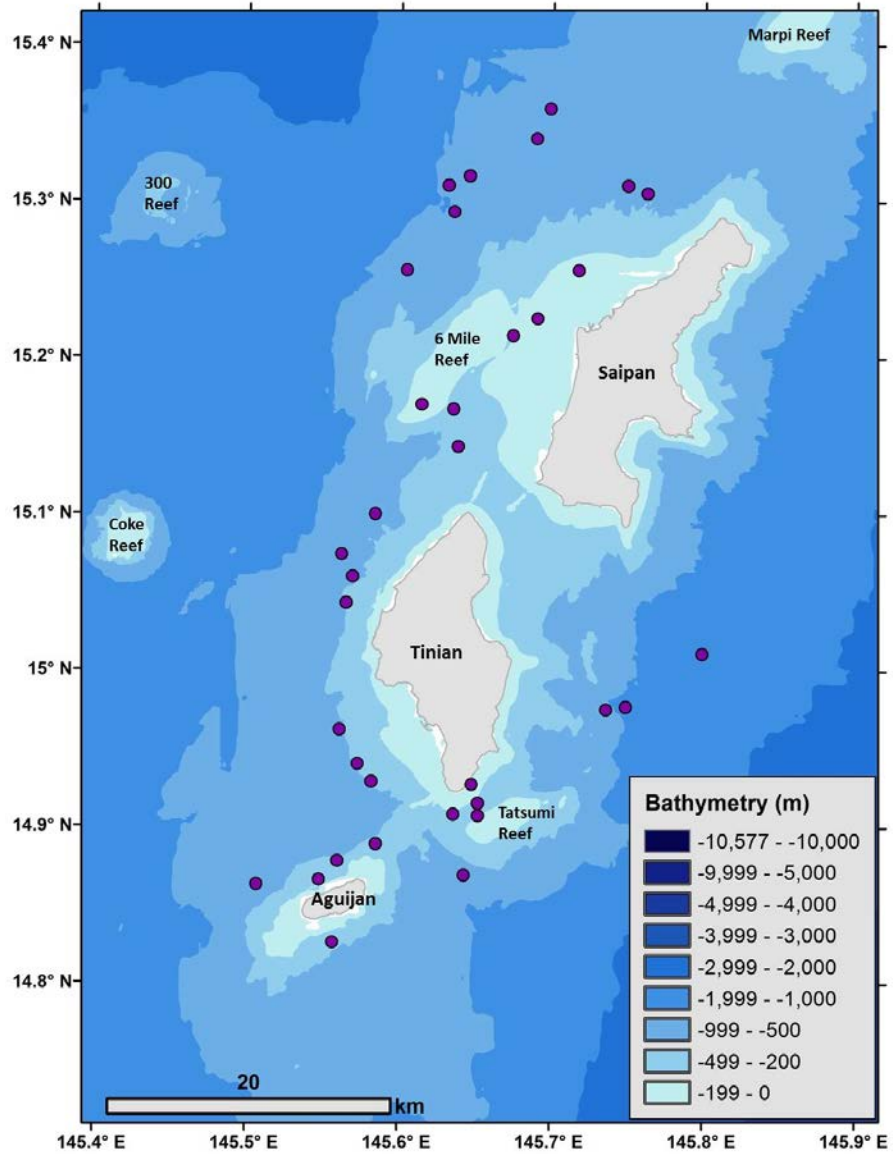


Figure 14.-- Douglas Argos filtered satellite locations from tag 128912 deployed on a bottlenose dolphin off the west side of Saipan and Tinian (12 June 2014). Deployment duration was 3.7 d.

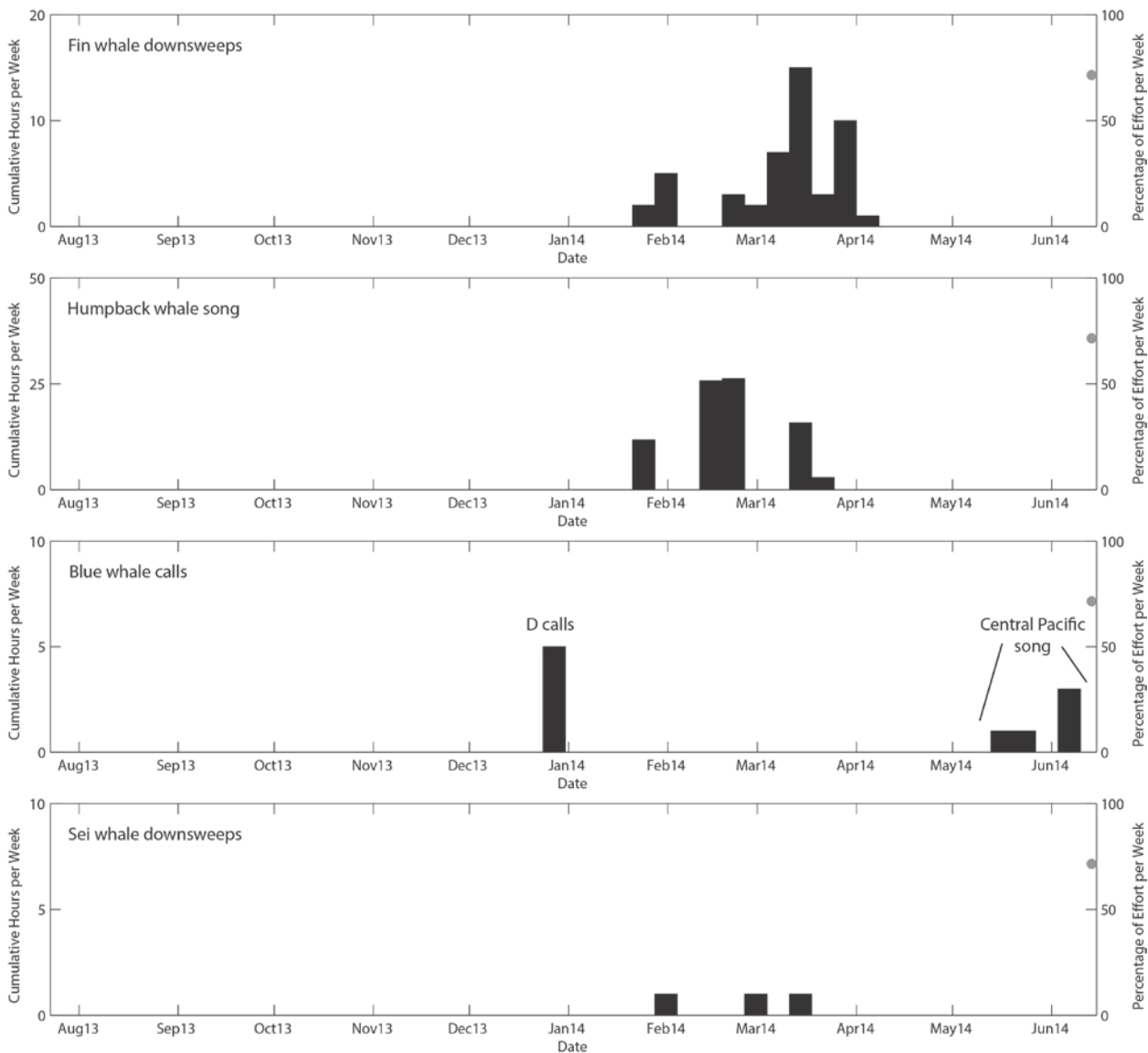


Figure 15.—Acoustic detections of baleen whale calls within the 2013-14 Tinian HARP dataset. Baleen whale calls are marked in hourly bins, not as individual calls or calling bouts, such that detection of a single or multiple calls within an hour is counted as one hour of detection. Y-axis scaling varies among plots. The light gray dot represents recording effort during the final monitoring week. Recording effort was 71.4% (5 minutes every 7 minutes).

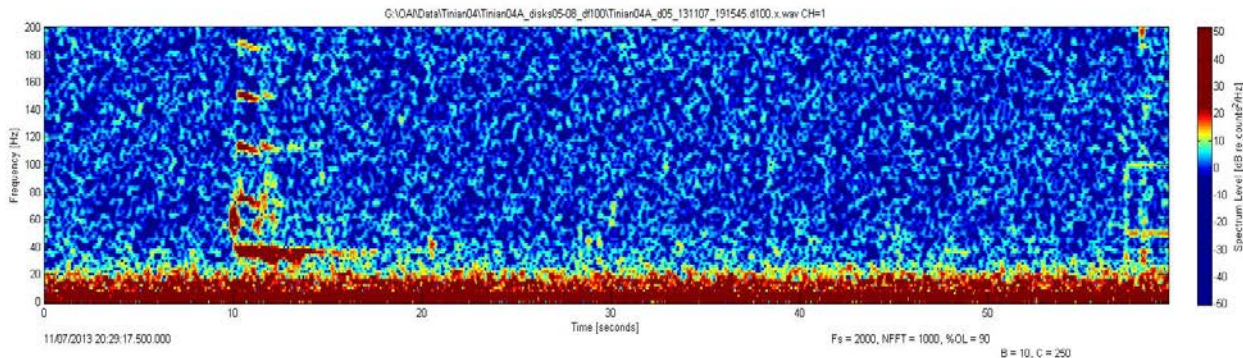


Figure 16. – Spectrogram of unidentified whale “tonal” call within the 2013-14 Tinian HARP dataset. This sound is similar to calls previously recorded from Bryde’s whales in the eastern tropical Pacific (type Be3; Oleson et al. 2003), though is higher frequency and shorter duration. Bryde’s whale calls have not been recorded in the Marianas to allow for comparison to detected sounds and definitive identification.

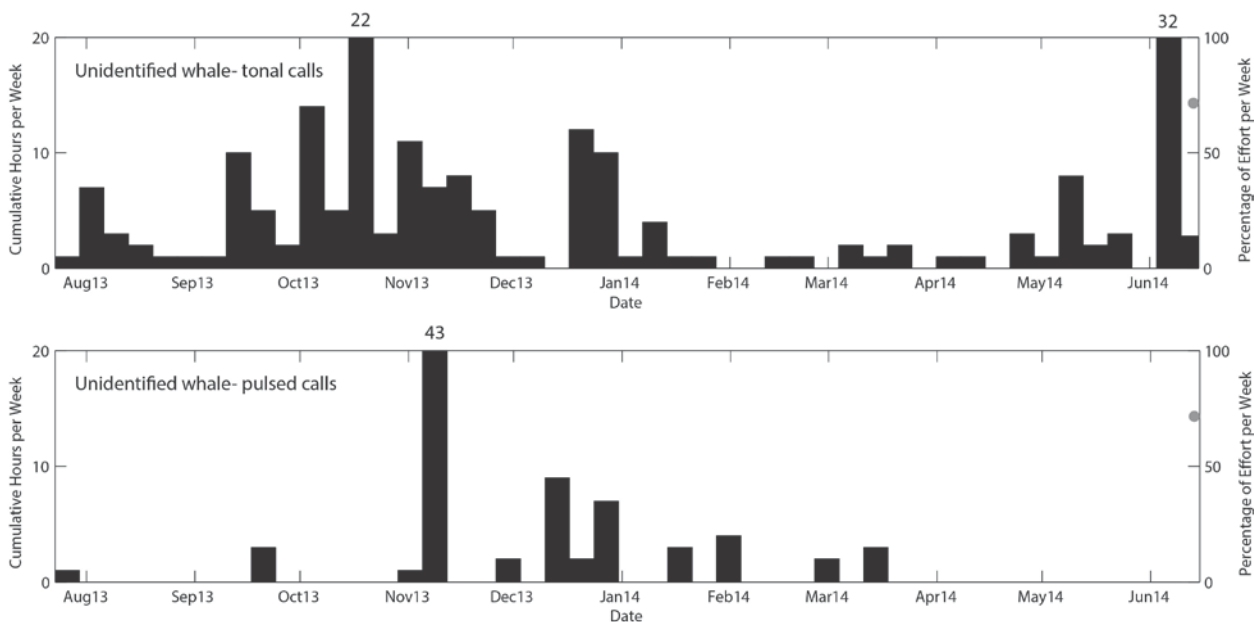


Figure 17. -- Acoustic detections of unidentified whale calls within the 2013-14 Tinian HARP dataset. Baleen whale calls are marked in hourly bins, not as individual calls or calling bouts, such that detection of a single or multiple calls within an hour is counted as one hour of detection. Values noted above the upper axis indicate cumulative hourly counts greater than the axis maximum. Y-axis scaling varies among plots. The light gray dot represents recording effort during the final monitoring week. Recording effort was 71.4% (5 minutes every 7 minutes).

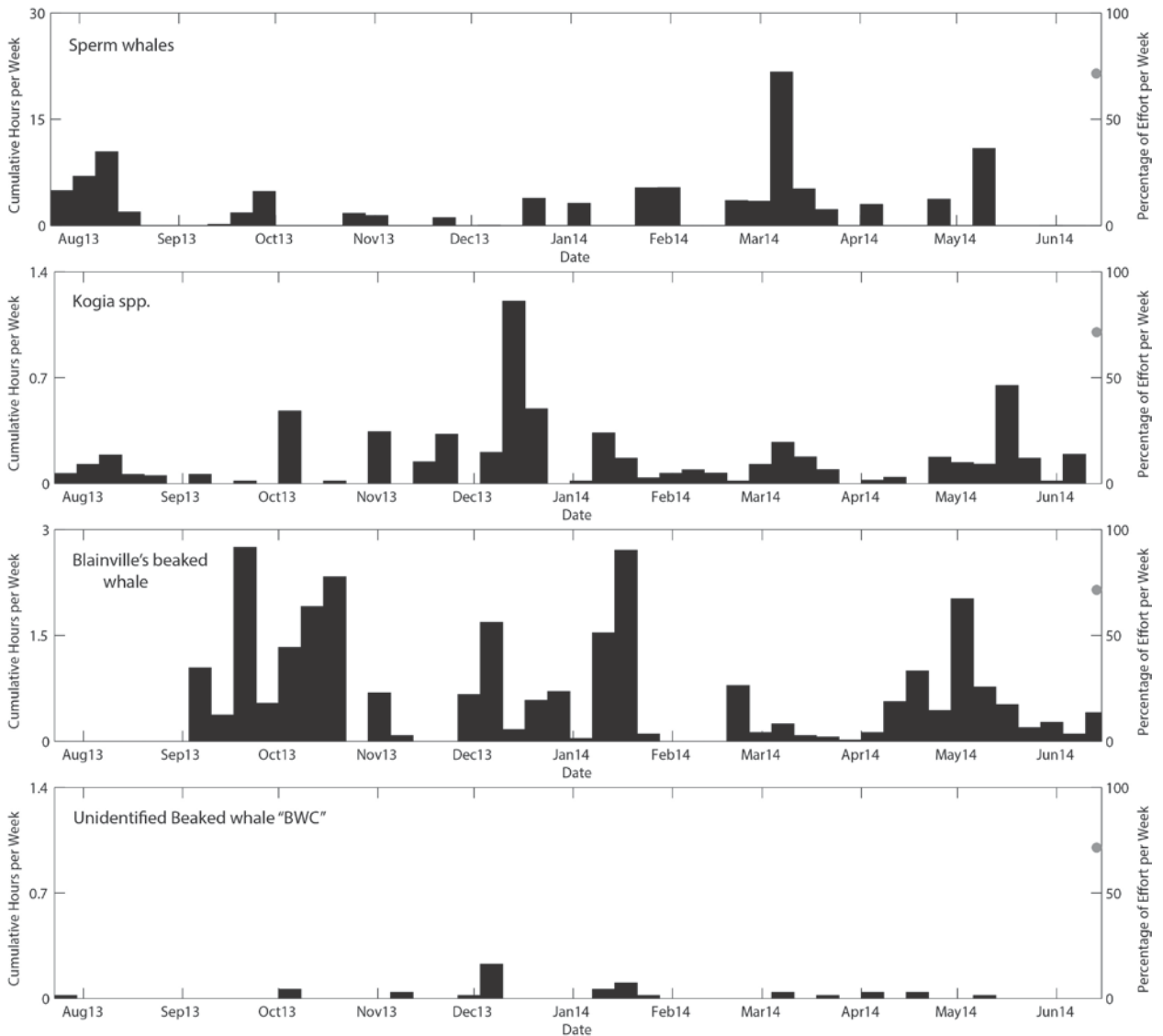


Figure 18.-- Acoustic detections of sperm whales (including *Kogia* spp.) and beaked whales within the 2013-14 Tinian HARP dataset. Sperm whale and *Kogia* occurrence are noted as calling bouts such that cumulative bout duration represents the total amount of time these species were heard during each weekly bin. Beaked whale calls were automatically detected and manually classified to species, and bout duration was measured following these detection and classification steps. BWC refers to the unidentified beaked whale described in Baumann-Pickering et al. (2013). Y-axis scaling varies among plots. The light gray dot represents recording effort during the final monitoring week. Recording effort was 71.4% (5 minutes every 7 minutes).

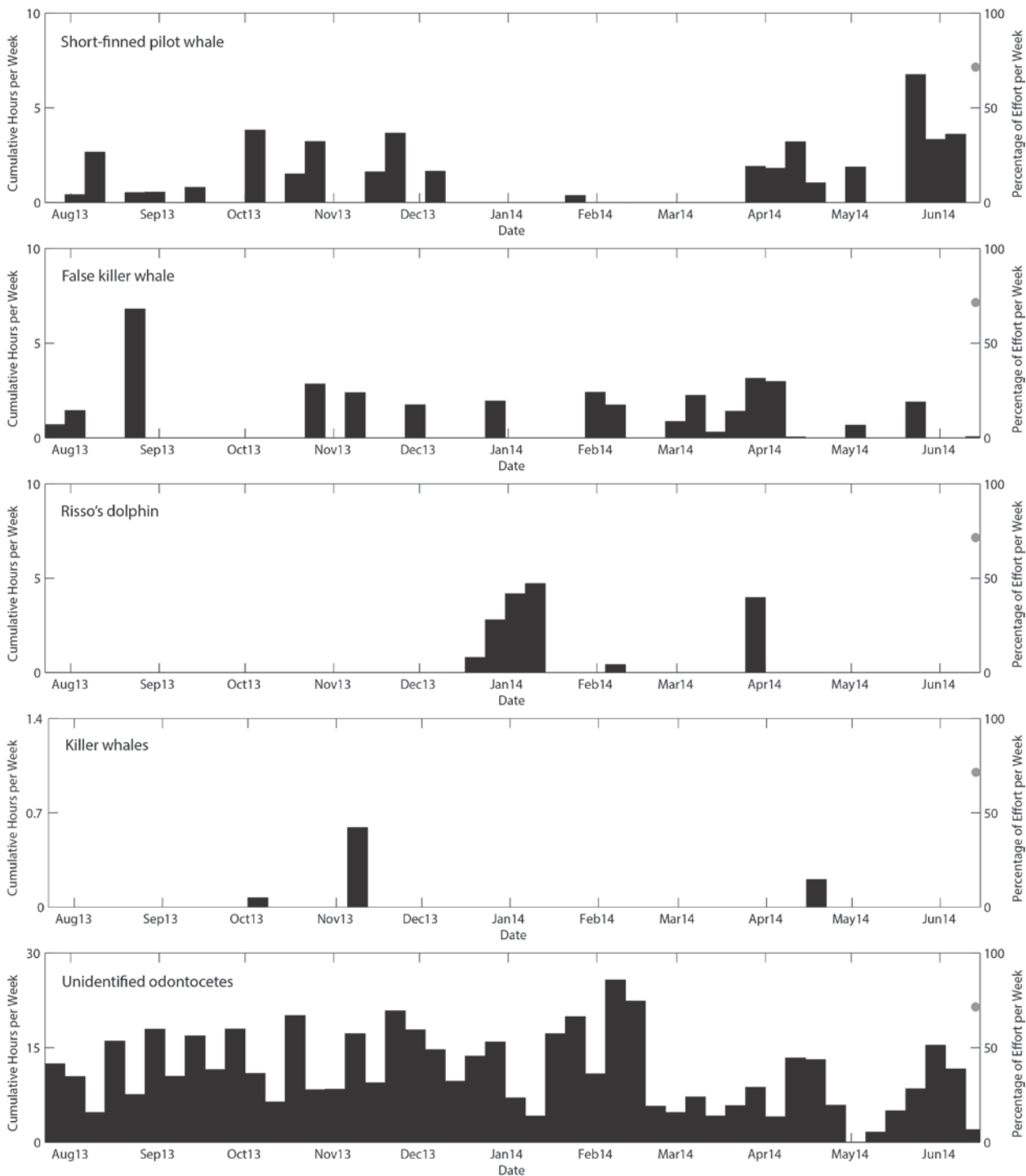


Figure 19. -- Acoustic detections of short-finned pilot whales, false killer whales, Risso's dolphins, killer whales, and unidentified odontocetes within the 2013-14 Tinian HARP dataset. Odontocete species occurrence is noted based on calling bouts such that cumulative bout duration represents the total amount of time each species were heard during each weekly bin. Y-axis scaling varies among plots. The light gray dot represents recording effort during the final monitoring week. Recording effort was 71.4% (5 minutes every 7 minutes).

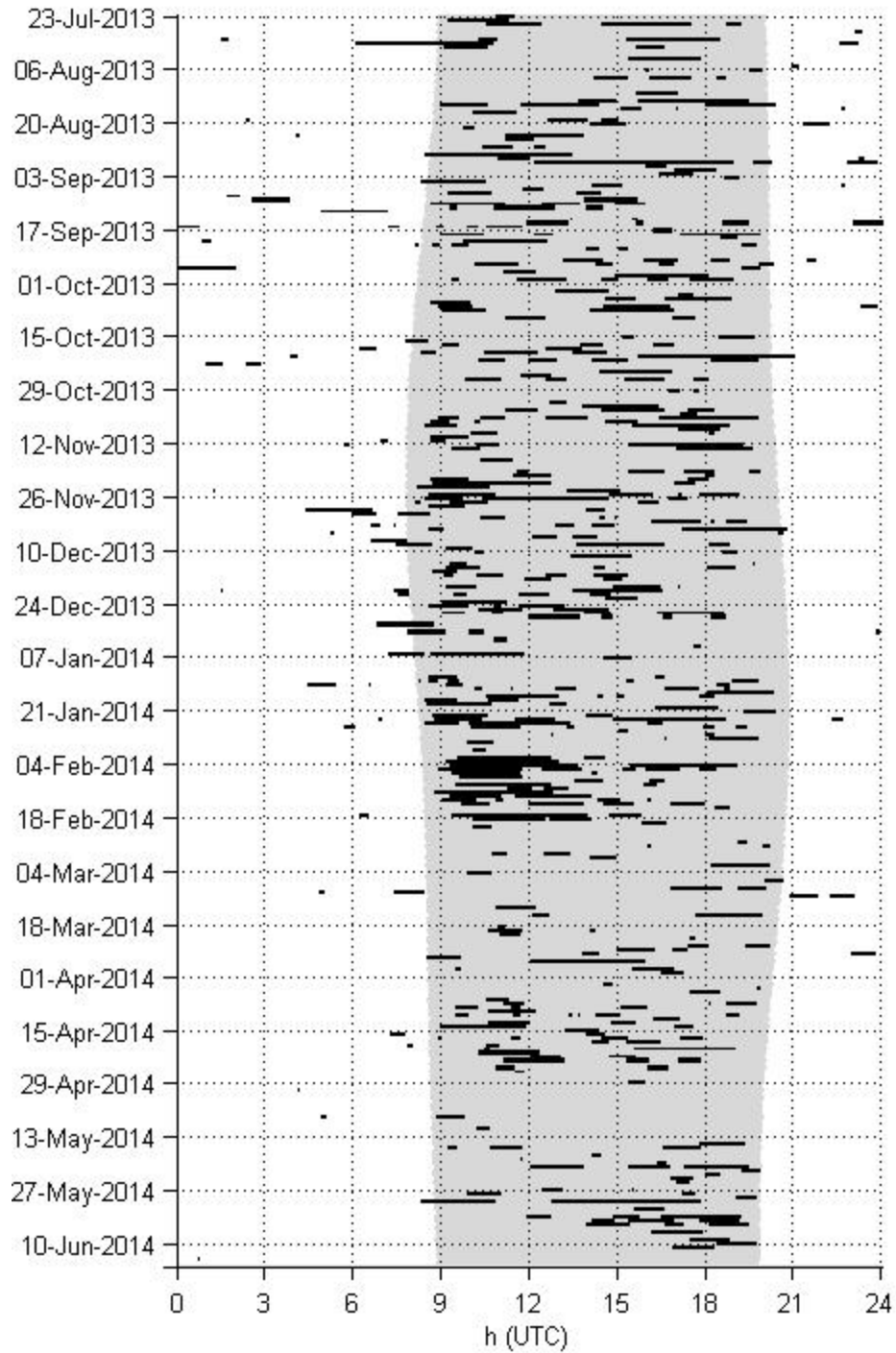


Figure 20. – Occurrence of unidentified odontocete sounds in one minute bins throughout the 2013-14 Tinian HARP dataset. Gray shading indicates nighttime.

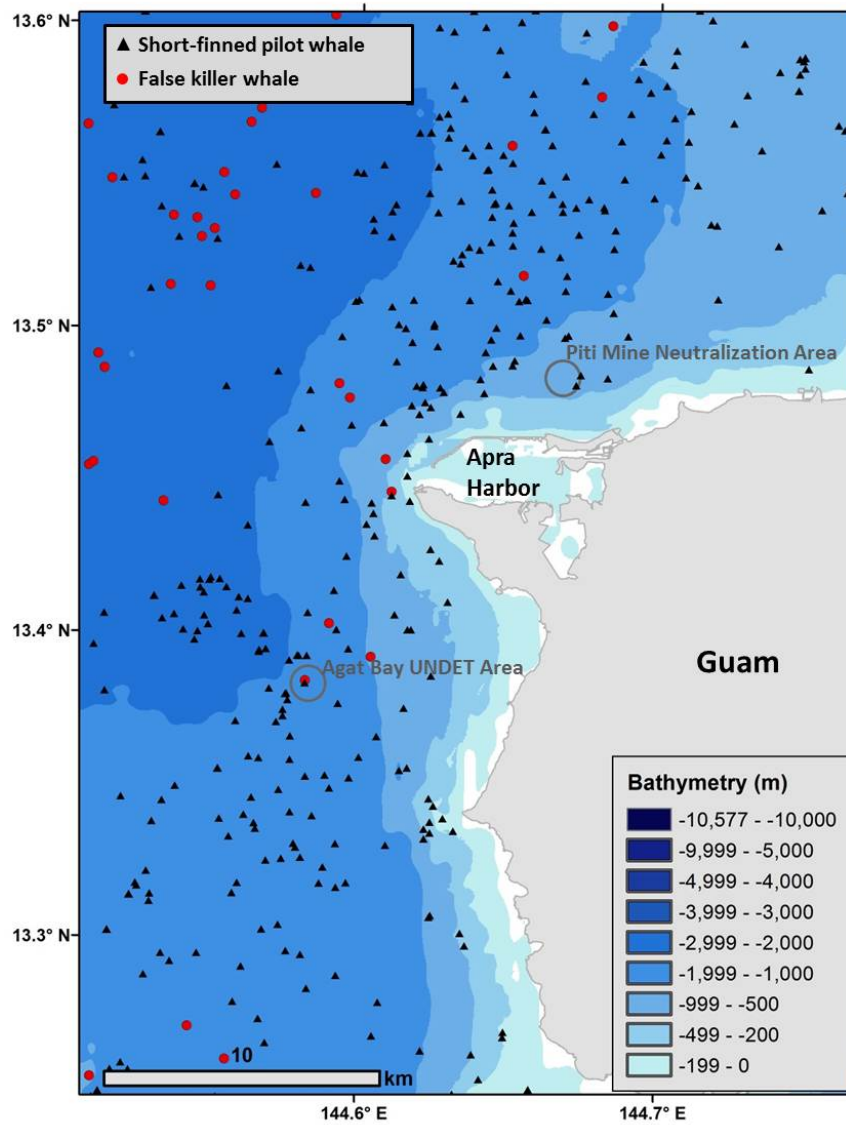


Figure 21.-- Navy underwater detonation and explosive ordnance areas and short-finned pilot whale and false killer whale filtered satellite tag locations. The circles at the Piti Floating Mine Neutralization Area and the Agat Bay UNDET Area represent the 640 m exclusion zone around the detonation site.

Appendix I: Introgressive hybridization of Fraser's dolphin mitochondrial and nuclear DNA into Mariana Islands bottlenose dolphins

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Abstract

We used mitochondrial sequence and nuclear microsatellite loci to examine introgression of Fraser's dolphin DNA into the Mariana Islands population of bottlenose dolphins. By comparing the nuclear genotypes of the Mariana Islands samples to those of 'pure' bottlenose dolphins and Fraser's dolphins, we estimate that the Mariana Islands animals derive approximately 14% of their nuclear ancestry from Fraser's dolphins. The fact that every Mariana Islands sample showed evidence of nuclear introgression, combined with the fact that those exhibiting mitochondrial introgression all share the same Fraser's dolphin haplotype, suggests that there was a single hybridization event far enough in the past to allow Fraser's dolphin nuclear DNA to permeate the population. The Mariana Islands samples exhibited low genetic diversity compared to other bottlenose dolphin populations, suggesting that they represent a small, genetically isolated population.

Introduction

Most species concepts assume a complete lack of interbreeding between species. However, there is increasing evidence that interspecific hybridization is relatively common (Arnold 1992; Bernatchez et al. 1995; Seehausen et al. 2002; Shaw 2002). Introgressive hybridization, in which genetic material from one species persists in the genome of another species following hybridization, can result in gene trees that do not match species trees and seriously mislead phylogenetic studies. This is particularly true in studies based solely on mitochondrial DNA, as introgression of maternally-inherited genomes is predicted to occur far more rapidly and persist longer than introgression of nuclear genomes (Chan and Levin 2005).

Cetacean species are known to exhibit an unusually high rate of interspecific hybridization. Isolated hybridization events have been documented both in wild populations and among captive animals, and do not always involve sister species (Bérubé 2002; Kingston et al. 2009). Introgressive hybridization has long been suspected as a source of taxonomic confusion in the Delphinidae (Kingston et al. 2009). The bottlenose dolphin population in Shark Bay, Western Australia, contains haplotypes

from both common (*Tursiops truncatus*) and Indo-Pacific (*T. aduncus*) bottlenose dolphins, suggesting a hybrid origin for the population (Krützen et al. 2004). The Clymene dolphin (*Stenella clymene*) is believed to have originated as a result of hybridization between spinner dolphins (*S. longirostris*) and striped dolphins (*S. coeruleoalba*) (Amaral et al. 2014).

Martien et al. (2014b) found that five out of 15 common bottlenose dolphin samples collected around the southern islands of the Mariana Archipelago possessed haplotypes characteristic of Fraser's dolphins (*Lagenodelphis hosei*). Photographs confirmed that the samples came from five different individuals, all of which appeared to be morphologically normal bottlenose dolphins. Martien et al. also conducted a search of genetic data available online and found two additional samples collected in China and identified as *T. truncatus* that possess the same Fraser's dolphin haplotype that they detected in the Mariana Islands bottlenose dolphin population.

In this study, we use mitochondrial DNA sequence data and nuclear microsatellite genotype data to further investigate the extent and origin of hybrid ancestry in Mariana Islands bottlenose dolphins. We compare the Mariana Islands samples to bottlenose dolphin samples from elsewhere in the western Pacific and the Hawaiian Archipelago and to Fraser's dolphin samples from throughout the Pacific and Indian Oceans. Our results provide insight into the degree of Fraser's dolphin introgression into the nuclear genomes of the Mariana Islands animals and the evolutionary history of hybridization into the population.

Methods

Samples

The focus of our study was 15 samples collected around the southern islands of the Mariana Archipelago. The samples came from animals that appeared morphologically to be bottlenose dolphins, yet five of them possessed Fraser's dolphin haplotypes (Martien et al. 2014b). We also analyzed 169 bottlenose dolphin samples collected outside the Mariana Archipelago (Figure 1). Most of these (n=159) were collected within the U.S. Exclusive Economic Zone surrounding the Hawaiian Archipelago (Hawai'i EEZ samples) between 1999 and 2013, primarily during research surveys conducted by the Southwest and Pacific Islands Fisheries Science Centers and during dedicated small-boat surveys conducted by Cascadia Research Collective. The remaining bottlenose dolphin samples were biopsies and strandings from other areas of the North Pacific (Figure 1). We consider the Hawai'i EEZ samples to represent 'pure' bottlenose dolphins (i.e., not hybridized with Fraser's dolphins), while the western Pacific samples could, given their proximity to the Mariana Archipelago, exhibit some degree of hybrid ancestry. We therefore only used the Hawai'i EEZ samples in analyses that required *a priori* stratification.

Our sample set also included 47 tissue samples collected from Fraser's dolphins between 1973 and 2009. These samples were primarily from animals stranded in the Philippines, with the remainder consisting of biopsies and strandings from around the Pacific and Indian Oceans (Figure 1). Examining population structure within Fraser's dolphin was not a goal of this study, nor did we have enough samples to stratify the Fraser's dolphin data set. Therefore, in analyses that included the Fraser's dolphin samples, we treated all Fraser's dolphin samples as a single stratum.

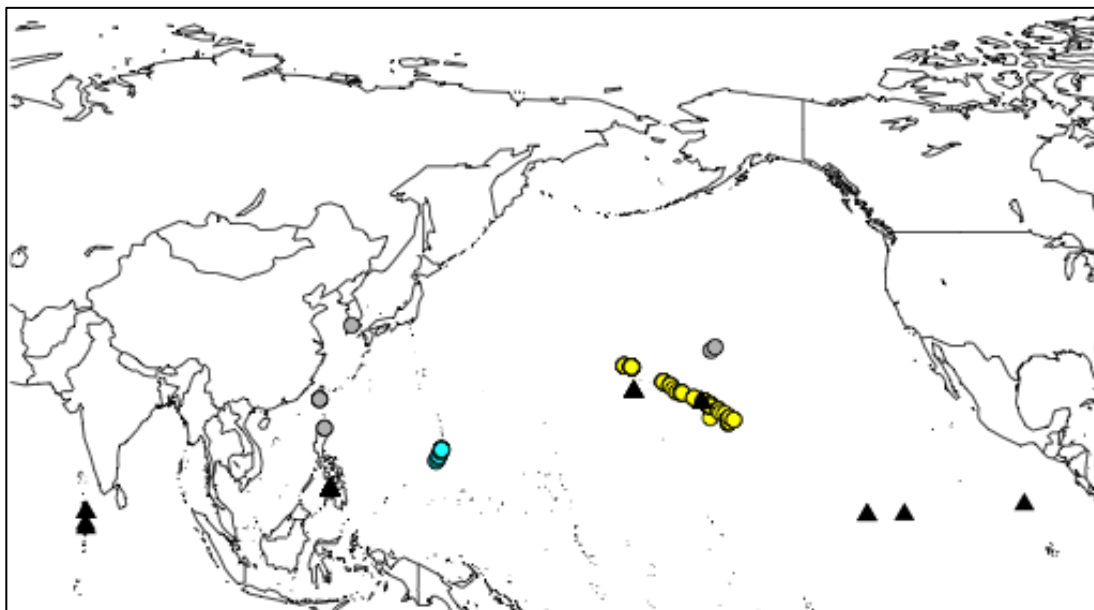


Figure 1. Distribution of bottlenose dolphin (circles) and Fraser's dolphin (black triangles) samples. Bottlenose dolphin samples from CNMI are in blue, those from the Hawai'i EEZ are in yellow, and all other samples are in gray.

Laboratory analyses

DNA extractions were performed using either a silica-based filter purification (Qiaextractor, DX reagents; Qiagen) or a sodium chloride protein precipitation (Miller et al. 1988). Standard protocols were used for PCR amplification, as well as for mitochondrial DNA (mtDNA) sequencing (Saiki et al. 1988; Sambrook et al. 1989; Palumbi et al. 1991). A 400 basepair region of the 5' end of the hypervariable mtDNA control region was amplified using primers D (5'- CCTGAAGTAAGAACCAGATG- 3'; Rosel et al. 1994) and TRO (5'- CCTCCCTAAGACTCAAGG-3'; developed at SWFSC). The PCR cycling profile for mtDNA sequencing consisted of 94 °C for 2.5 min, followed by 35 cycles of 94 °C for 45 sec, 1 min at 56 °C annealing temperature, and 72 °C for 1.5 min, then a final extension at 72 °C for 5 min. Both the forward and reverse strands of the amplified DNA product were sequenced as mutual controls on the Applied Biosystems Inc. (ABI) 3730 DNA Analyzer. All sequences were aligned using Sequencer v4.1 software (Gene Codes Corp., 2000).

We genotyped all samples at fourteen microsatellite loci, all of which were dinucleotide repeats. Ten of the loci (KWM1b, KWM2a, KWM2b, KWM12a, D5, Ttr11, Ttr34, Ttr48, TexVet7, and D08) were also used in Martien et al.'s (2012) analysis. One locus used by Martien et al. (D8) did not amplify reliably in Fraser's dolphins, and so was excluded from our study. In order to increase the precision of our estimates of nuclear ancestry we used four additional loci (Ttr58, TexVet5, EV94, SL125), which were chosen because initial screening showed them to be highly polymorphic in Fraser's dolphins. Control samples from Martien et al.'s study were included on every genotyping plate in order to ensure consistent scoring of alleles across the two studies.

Primer sets for loci KWM1b, KWM2a, KWM2b, and KWM12a were derived from killer whales (*Orcinus orca*; Hoelzel et al. 1998), locus D5 from beluga whales (*Delphinapterus leucas*; Buchanan et al. 1996), loci Ttr11, Ttr34, Ttr48, Ttr58 (Rosel et al. 2005), TexVet5 and TexVet7 (Rooney et al. 1999), locus D08 (Shinohara et al. 1997) were all derived from bottlenose dolphins (*Tursiops sp.*), locus EV94 from Humpback whales (*Megaptera novaeangliae*; Valsecchi and Amos 1996), and locus SL125 spinner dolphins (*Stenella sp.*; Galver 2002). Extracted DNA was amplified using a 25 μ L reaction of 1x PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, and 1.5 mM MgCl₂), 0.15 mM of each dNTP, 0.3 μ M of each primer, 0.5 units of Taq DNA polymerase, and approximately 10ng of DNA. The PCR cycling profile consisted of 90 °C for 2.5 min, followed by 35 cycles of 94 °C for 45 sec, 1 min at annealing temperature, and 72 °C for 1.5 min, then a final extension at 72 °C for 5 min. The optimal annealing temperature was 55 °C for the loci D08, TexVet5, TexVet7, Ttr48, Ttr11, and SL125, 45 °C for loci KWM1b, KWM2a, KWM2b, and KWM12a, 57 °C for loci D5 and Ttr34, 52 °C for locus EV94 and 60 °C for locus Ttr58, respectively.

The amplifications were assessed electrophoretically on a 2 % agarose gel for quality and size before loading onto the ABI 3730 DNA Analyzer. ABI Genemapper v4.0 was used along with an internal standard marker, Genescan-500 ROX, Applied Biosystems Inc., to determine allele fragment size.

Samples were genetically sexed by amplification and Real-Time PCR (Stratagene) of the zinc finger (ZFX and ZFY) genes (Morin et al. 2005).

Data review

Prior to analysis, the mtDNA and nucDNA data sets were reviewed for quality using the standards described in Martien et al. (2014a) and Morin et al. (Morin et al. 2010). This included 10% random replication, re-sequencing of unique haplotypes, having all allele size calls reviewed by two independent genotypers, and eliminating samples deemed to be of poor quality.

For the bottlenose dolphin data set, we assessed each microsatellite locus for deviations from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium as a means of ensuring that the loci were amplifying correctly (e.g., no null alleles) and that we had

not included genetically differentiated populations into a single stratum. We tested for deviations from HWE using tests for heterozygote deficiency (Raymond and Rousset 1995) and exact tests of HWE (Guo and Thompson 1992), as implemented in *strataG* (available upon request from F.I. Archer), a package for the statistical program language R (R Development Core Team 2011). We used the same software to evaluate linkage disequilibrium for each pair of loci using Fisher's method and the Markov chain method. All HWE and linkage disequilibrium tests were conducted using 1,000 dememorization steps, 10,000 batches, 1,000 iterations per batch. The tests were conducted separately for CNMI and the Hawai'i EEZ and combined across the two strata to calculate a global *P*-value for each locus (Fisher 1935). The jackknife procedure described in Morin et al. (2009a) was used to identify samples that were highly influential (i.e., log-odds greater than two) in deviations from HWE. The genotypes identified by the jackknife procedure were removed from the data set. HWE and linkage disequilibrium analyses were not conducted for Fraser's dolphin data set, as there were not enough samples in that data set to stratify into putative populations, and equilibrium is not expected when samples from multiple populations are combined into a single stratum.

Pairs of samples that matched in sex, mtDNA haplotype, and microsatellite genotype were considered duplicate samples. When available, photo-identification data were also used to identify duplicate samples from the same individual. The program DROPOUT (McKelvey and Schwartz 2005) was used to identify additional pairs of samples whose genotypes differed at four or fewer loci. These pairs could represent duplicate samples with genotyping errors. One sample from each duplicate pair was removed prior to analysis. We also used DROPOUT to calculate the probability of two randomly selected individuals sharing an identical genotype.

Analyses

We incorporated into our analyses data published by Martien et al. (2012) on bottlenose dolphins sampled from the main Hawaiian Islands. Martien et al.'s data set included mitochondrial control region sequences from 119 animals and genotypes from 116 animals at ten of the 14 microsatellite loci used in this study. We included the data from Martien et al. in all of our mtDNA analyses. However, we did not include Martien et al.'s nucDNA data since doing so would have precluded us from using some of the loci that were most polymorphic for Fraser's dolphins and therefore most useful for estimating the proportion of Fraser's dolphin ancestry.

We calculated haplotypic diversity (*h*) and nucleotide diversity (π) for the mtDNA data set using *strataG*. These calculations were made both including and excluding the five CNMI individuals that possessed Fraser's dolphin haplotypes, as including haplotypes from two different species in the same stratum results in a strong upward bias in diversity estimates, particularly for nucleotide diversity. The mtDNA diversity analysis was the only analysis for which these individuals were excluded. For the nucDNA data set, we used *strataG* to calculate average number of alleles per locus,

expected and observed heterozygosity, and allelic richness. The nucDNA diversity estimates include all individuals, regardless of haplotype.

We tested the null hypothesis of no population structure between CNMI and the Hawai'i EEZ for both the mtDNA and nucDNA data sets by conducting a χ^2 test (Rolf and Bentzen 1989), as implemented in *strataG*. We estimated the magnitude of genetic differentiation between CNMI and Hawai'i EEZ using the statistics F_{ST} (Wright 1931) and Φ_{ST} (Excoffier et al. 1992) for the mtDNA data set and F_{ST} and F'_{ST} (Meirmans 2006) for the nucDNA data set. To get a sense of the relative nuclear differentiation of the CNMI animals from Fraser's dolphins and all other bottlenose dolphins, we also used the nucDNA data set to estimate pairwise genetic differentiation between the CNMI animals, all non-CNMI bottlenose dolphins, and Fraser's dolphins.

To estimate the proportion of the nuclear ancestry of the CNMI animals that comes from bottlenose and Fraser's dolphins, we used the Bayesian clustering program STRUCTURE (Pritchard et al. 2000; Falush et al. 2003; Hubisz et al. 2009). We first used STRUCTURE to analyze all bottlenose dolphin samples, clustering them into $k=1$ to 6 groups using an admixture model with correlated allele frequencies and no prior information on group membership. We then re-ran the same analysis, but included the Fraser's dolphin samples and examined values of k from 1 to 4.

We next used STRUCTURE to look for individuals that had recent hybrid ancestry by labeling all bottlenose dolphin samples, including those from the CNMI, as bottlenose dolphins and all Fraser's dolphin samples as Fraser's dolphins. We then ran STRUCTURE with the USEPOPINFO option and had the analysis look for immigrant ancestry up two generations in the past, which is the longest time frame the analysis can examine. For this analysis, we assumed that allele frequencies were independent between the two species. Finally, we repeated this analysis but without assigning a species label to the CNMI samples. Thus, the analysis used an uninformative prior as to the species origin of the CNMI samples and estimated the proportion of their nuclear ancestry that came from each species.

All STRUCTURE analyses were run with a burn-in of 100,000 steps and a run length of 500,000 steps. We replicated each run 10 times and averaged the ancestry coefficients of individuals across replicate runs using the CLUMPP algorithm of Jakobsson and Rosenberg (2007). All STRUCTURE and CLUMPP analyses were conducted within the R package *strataG* (available upon request).

Results

Data review

Among the bottlenose dolphin samples, fifteen pairs of replicate samples were identified either genetically and photographically, all of which matched with respect to

sex and mtDNA haplotype. One sample from each pair was therefore excluded from the final data set. In addition, twelve samples were found to be genetically identical to samples included in Martien et al.'s (2012) study. In these cases, the sample used by Martien et al. was excluded, as the newer samples had been genotyped at more loci. In all cases of replicate samples, both within our sample set and between our sample set and that of Martien et al., both samples were collected from the same Hawaiian island.

We excluded eight Fraser's dolphin samples from the mtDNA data set and 22 from the microsatellite data set due to poor sample quality. Only one bottlenose dolphin sample was excluded from the mtDNA data set and five from the nucDNA data set due to poor quality. After all exclusions our mtDNA dataset included 169 bottlenose dolphins and 39 Fraser's dolphins, while the nucDNA data set included 164 bottlenose dolphins and 25 Fraser's dolphins.

The jackknife analysis of the bottlenose dolphin data set identified one individual that was homozygous for a rare allele at locus SL125t and was therefore having a disproportionate effect on HWE at that locus. That individual's genotype at SL125t was treated as missing data for all microsatellite analyses. Three loci were identified as being out of HWE for the Hawai'i EEZ samples, while none were out of HWE in the CNMI samples. However, the Hawai'i EEZ is known to contain multiple populations (Martien et al. 2012). When the HWE analysis was re-run separately for each of the four populations identified by Martien et al., none of the loci were out of HWE. Similarly, while seven pairs of loci were found to be out of linkage equilibrium in the data set overall, none of them were out of equilibrium within any of the populations identified by Martien et al. Therefore, all 14 loci were retained.

Diversity

We resolved 28 unique haplotypic sequences among the bottlenose dolphin samples we analyzed, 13 of which were also identified by Martien et al. (2012). There were seven haplotypes identified within the Hawai'i EEZ by Martien et al. that we did not detect. Thus, the final combined data set included 35 haplotypes, including the single Fraser's dolphin haplotype (LH11) detected in CNMI. All mtDNA analyses were conducted on this combined data set (Table 1). We resolved 26 unique haplotypes among the Fraser's dolphin samples (Table 2). Five Fraser's dolphin samples – three from the Philippines and two from Hawai'i – possessed the same haplotype (Lh11) detected in the CNMI bottlenose dolphin samples.

Both haplotypic and nucleotide diversity of bottlenose dolphins were low in CNMI when the individuals possessing Fraser's dolphin haplotypes were excluded (Table 3). Including these individuals resulted in substantially higher diversity estimates, as would be expected when combining haplotypes from two different species in a single stratum, but haplotypic diversity was still lower than for the Hawai'i EEZ, entire bottlenose dolphin data set as a whole, and the Fraser's dolphin data set. Estimates of nuclear genetic diversity were also lower in CNMI than for any other stratum (Table 4).

Table 1. Bottlenose dolphin haplotype frequencies by location. Sample sizes are given in parentheses. Haplotypes are numbered to be consistent with Martien et al. 2012. Haplotypes marked with an asterisk were only found by Martien et al. (2012), not in the new samples analyzed for this paper. Haplotypes 21-25 have only been detected at Palmyra Atoll, which is not part of our study area, and so are omitted from the table. Haplotype Lh11 is the Fraser's dolphin haplotype detected in five CNMI bottlenose dolphins.

Haplotype	CNMI (15)	Philippines (2)	Taiwan (4)	Korea (1)	Hawai'i EEZ (267)	NE Pacific (2)
1					62	
2					34	
3					23	
4					46	
5					5	
6					30	
7					2	
8				1	2	
9*			1		5	
10*					1	
11*					1	
12					17	
13			1		15	
14*					1	
15*					1	
16*					1	
17					3	
18*					1	
19					6	
20					3	
26					3	
27					1	
28					1	
29						1
30						1
31					1	
32	7	1				
33	1					
34	1	1				
35			1			
36			1			
37					1	
38					1	
39	1					
Lh11	5					

Table 2. Fraser's dolphin haplotype frequencies by location. Sample sizes are given in parentheses.

Haplotype	Maldives (4)	Philippines (25)	Taiwan (3)	Hawai'i (3)	North Pacific (3)	ETP (1)
Lh1		2				
Lh2	1	3	1			
Lh3		1				
Lh4		3				
Lh5						1
Lh6		2				
Lh7		1				
Lh8		1				
Lh9		2				
Lh10		1				
Lh11		3		2		
Lh12		1				
Lh13		1				
Lh14		1				
Lh15				1		
Lh16		1				
Lh17		1				
Lh18	1					
Lh19					1	
Lh20					1	
Lh21					1	
Lh22		1				
Lh23			1			
Lh24			1			
Lh25	1					
Lh26	1					

Table 3. Diversity estimates for the mtDNA data sets.

	Sample Size	Number of Haplotypes	Haplotype Diversity	Nucleotide Diversity
CNMI – no Fraser's haps	10	4	0.533	0.004
CNMI – all	15	5	0.705	0.029
Hawai'i EEZ	267	26	0.874	0.026
All bottlenose dolphins	286	34	0.893	0.022
All Fraser's dolphins	39	26	0.965	0.017

Table 4. Diversity estimates for the nucDNA data sets. Allelic richness was calculated for a minimum sample size of 14.

	Sample Size	Num. alleles	H_e	H_o	A_R
CNMI	14	5.86	0.689	0.658	5.857
Hawai'i EEZ	144	8.79	0.746	0.743	6.332
All bottlenose dolphins	164	9.43	0.752	0.737	6.550
All Fraser's dolphins	25	7.79	0.726	0.737	6.561

Differentiation

CNMI was significantly differentiated from the Hawai'i EEZ in both the mtDNA ($F_{ST} = 0.192$, $\Phi_{ST} = 0.166$, χ^2 p -value < 0.0001) and nucDNA ($F_{ST} = 0.070$, $F'_{ST} = 0.251$, χ^2 p -value < 0.0001) data sets. The magnitude of differentiation in the nucDNA data set, as measured by both F_{ST} and F'_{ST} , was more than double between CNMI and Fraser's dolphins than it was between CNMI and all non-CNMI bottlenose dolphins, and was comparable to the differentiation between Fraser's and bottlenose dolphins (Table 5).

Table 5. Estimates of genetic differentiation between CNMI animals, bottlenose dolphins not from CNMI, and Fraser's dolphins.

Comparison	F_{st}	F'_{st}	χ^2 P value
CNMI vs. bottlenose	0.067	0.245	<0.0001
CNMI vs. Fraser's	0.176	0.605	<0.0001
Bottlenose vs. Fraser's	0.160	0.615	<0.0001

When we used the program STRUCTURE to cluster all bottlenose dolphin samples, including those from CNMI, the model with the highest support was the one with two groups. Under this model, the CNMI samples had an average assignment probability of 91.2% to group 1, the other western Pacific samples had an average assignment probability of 86.1% to group 1, and the Hawai'i EEZ samples had an average assignment probability of 61.3% to group 2 (Figure 2a). When we included the Fraser's dolphin samples in the analysis, the best model was the one with three groups, and the bottlenose dolphin samples were much more clearly separated into a two groups. Under this model, the CNMI had 88.1% assignment to group 1, the western Pacific and Hawai'i EEZ bottlenose dolphin samples had average assignments to group 2 of 73.0% and 95.8%, respectively, and the Fraser's dolphins had an average assignment of 99.3% to group 3 (Figure 2b).

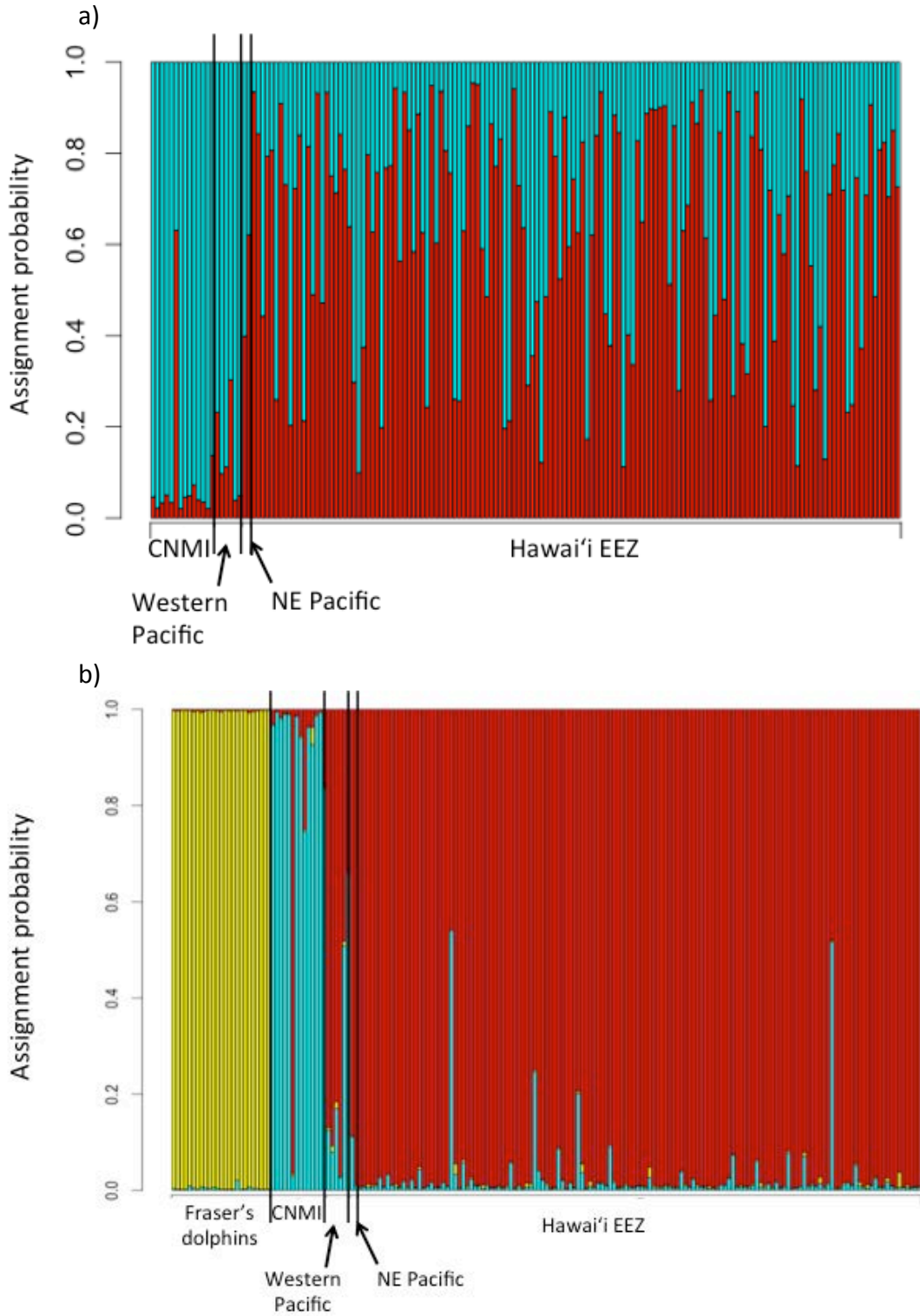


Figure 2. Graphical representation of STRUCTURE results. Each vertical bar represents an individual and is shaded as to the proportion of the individual's ancestry that is attributable to each of the groups defined by STRUCTURE. When only bottlenose dolphins were included in the analysis (a), the model with two groups was favored, while when both bottlenose and Fraser's dolphins were included (b), the model with three groups was favored.

When we used the USERPOPINFO option to look for evidence of recent hybrid ancestry among the CNMI samples, we found that, on average, 5.7% of the nucDNA ancestry of the CNMI samples was derived from Fraser's dolphins. The percent of Fraser's dolphin ancestry increased to 16.1% when we did not use an uninformative prior as to the species origin of the CNMI samples. In contrast, all other bottlenose dolphin samples and Fraser's dolphin samples were estimated to have received, on average, over 99% of their nucDNA ancestry from bottlenose dolphins and Fraser's dolphins, respectively. When the species origin of the CNMI animals was treated as unknown, all but two were estimated to have obtained more than 5% of their nucDNA ancestry from Fraser's dolphins (Table 6).

Table 6. Summary of STRUCTURE results for the CNMI samples.

Sample #	Sex	Haplotype	% ancestry from	
			Fraser's	Bottlenose
104035	M	32	0.062	0.938
104066	F	Lh11	0.079	0.921
104067	M	34	0.187	0.813
104070	M	32	0.041	0.959
108172	M	32	0.128	0.872
108183	M	39	0.064	0.936
108207	F	Lh11	0.147	0.853
108208	F	Lh11	0.255	0.745
116858	F	32	0.103	0.897
116866	M	32	0.077	0.923
116867	M	Lh11	0.526	0.474
116868	M	Lh11	0.317	0.683
116869	M	32	0.238	0.762
116881	M	33	0.028	0.972

Discussion

Our results indicate that the Fraser's dolphin introgression that Martien et al. (2014b) detected into the mitochondrial genomes of CNMI bottlenose dolphins is also present in their nuclear genomes. Nearly all of the CNMI animals, including those with bottlenose dolphin haplotypes, show greater than 5% Fraser's ancestry, with some individuals showing as much as 50% Fraser's ancestry. This result is consistent with either ongoing hybridization or a hybridization event that occurred enough generations in the past to allow the Fraser's dolphin alleles to spread throughout the population.

Though we cannot distinguish between past versus ongoing hybridization based on the nuclear DNA results, the pattern of mitochondrial introgression and photographic data suggest that past hybridization is more likely. Mitochondrial introgression can only occur if a female Fraser's dolphin mates with a male bottlenose dolphin and the resulting offspring then recruits into the paternal bottlenose dolphin population. This is

a low probability event that is unlikely to have happened multiple times. Furthermore, given the relatively high haplotypic diversity we detected in the Fraser's dolphin data set, we would expect to detect more than one Fraser's dolphin haplotype in the CNMI animals if hybridization were ongoing. However, only one haplotype (LH11) was detected, and no Fraser's dolphin haplotypes were found in the other strata. Finally, photographs taken at the time of biopsy show that all individuals, regardless of haplotype, appear to be morphologically normal bottlenose dolphins (Martien et al. 2014b), reducing the likelihood that any are first generation hybrids. Thus, the mitochondrial and photographic data suggest that the widespread nuclear introgression we detected is due to a past hybridization event.

Because the Fraser's dolphin haplotype that we detected in the CNMI animals is identical to haplotypes found in Fraser's dolphins from both Hawai'i and the Philippines, it is not possible to estimate at what point in the past hybridization occurred. It is possible that sequencing the full mitochondrial genome or multiple nuclear genes from Fraser's dolphins and the CNMI animals would reveal mutations unique to the CNMI animals, in which case dating of the hybridization event might be possible. Full mitogenome sequences would also be helpful in pinpointing the Fraser's dolphin source population for the hybridization.

We did not detect Fraser's dolphins in any of the bottlenose dolphin samples from elsewhere in the western Pacific. Furthermore, we were unable to confirm the accuracy of the online genetic data suggesting that two bottlenose dolphins stranded in China possessed the same Fraser's dolphin haplotype detected in the CNMI bottlenose dolphins. It is possible that these represent Fraser's dolphins that were simply mis-identified as bottlenose dolphins. Thus, at this time the hybridization of bottlenose and Fraser's dolphins is not known to extend beyond the Mariana Islands.

The low genetic diversity of the CNMI population in both the mitochondrial and nuclear data sets indicate that it is a small population that does not receive substantial gene flow from neighboring populations. Haplotypic (h) and nucleotide (π) diversity among the CNMI samples is considerably lower than it is among island-associated populations around the main Hawaiian Islands ($h = 0.0779-0.892$, $\pi = 0.018-0.022$; Martien et al. 2012), indicating that the CNMI population is either considerably smaller or more genetically isolated than the Hawaiian populations. Thus, the data suggest that the CNMI animals represent an island-associated population with limited gene flow with offshore populations, though samples from the offshore waters near the Mariana Islands are needed to confirm this conclusion.

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Institute of Environmental and Marine Studies, Silliman University, for use of the Philippines samples and Morgane Lauf for assisting with laboratory processing. Funding for this project was provided by Commander, U.S. Pacific Fleet Environmental Readiness Division, and NMFS Pacific Islands Fisheries Science Center. Samples were collected under the following permits: NMFS MMPA permit 774-1714 issued to the SWFSC and CNMI-DFW permit, license no. 01721-10 (2010 samples); NMFS MMPA permit 14097 issued to the SWFSC and CNMI-DFW permit, license no. 02260-11 (2011 samples); NMFS MMPA permit 15240 issued to PIFSC and CNMI-DFW permit, license nos. 02444-12 and 02694-13 (2012 and 2013 samples).

References

- Amaral, A. R., G. Lovewell, M. M. Coelho, G. Amato, and H. C. Rosenbaum. 2014. Hybrid speciation in a marine mammal: the clymene dolphin (*Stenella clymene*). *PloS one* 9:e83645.
- Arnold, M. L. 1992. Natural hybridization as an evolutionary process. *Annual Review of Ecology and systematics* 23:237-261.
- Bernatchez, L., H. Glémet, C. C. Wilson, and R. G. Danzmann. 1995. Introgression and fixation of Arctic char (*Salvelinus alpinus*) mitochondrial genome in an allopatric population of brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences* 52:179-185.
- Bérubé, M. 2002. Hybridism. Pp. 596-600 in W. F. Perrin, B. Würsig, and J. G. M. Thewissen, eds. *Encyclopedia of marine mammals*. Academic Press, San Diego, CA.
- Buchanan, F. C., M. K. Friesen, R. P. Littlejohn, and J. W. Clayton. 1996. Microsatellites from the beluga whale *Delphinapterus leucas*. *Molecular Ecology* 5:571-575.
- Chan, K. M., and S. A. Levin. 2005. Leaky prezygotic isolation and porous genomes: rapid introgression of maternally inherited DNA. *Evolution* 59:720-729.
- Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479-491.
- Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567-1587.
- Galver, L. M. 2002. *The Molecular Ecology of Spinner Dolphins, Stenella longirostris: Genetic Diveristy and Population Structure*. Pp. 192. University of California, San Diego.
- Guo, S. W., and E. A. Thompson. 1992. Performing the Exact Test of Hardy-Weinberg Proportion for Multiple Alleles. *Biometrics* 48:361-372.
- Hoelzel, A. R., M. Dahlheim, and S. J. Stern. 1998. Low genetic variation among killer whales (*Orcinus orca*) in the Eastern North Pacific and genetic differentiation between foraging specialists. *Journal of Heredity* 89:121-128.

- Hubisz, M. J., D. Falush, M. Stephens, and J. K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* 9:1322-1332.
- Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23:1801-1806.
- Kingston, S. E., L. D. Adams, and P. E. Rosel. 2009. Testing mitochondrial sequences and anonymous nuclear markers for phylogeny reconstruction in a rapidly radiating group: molecular systematics of the Delphininae (Cetacea: Odontoceti: Delphinidae). *BMC Evolutionary Biology* 9:245-263.
- Krützen, M., W. B. Sherwin, P. Berggren, and N. Gales. 2004. Population structure in an inshore cetacean revealed by microsatellite and mtDNA analysis: Bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. *Marine Mammal Science* 20:28-47.
- Martien, K. K., R. W. Baird, N. M. Hedrick, A. M. Gorgone, J. L. Thieleking, D. J. McSweeney, K. M. Robertson, and D. L. Webster. 2012. Population structure of island-associated dolphins: evidence from mitochondrial and microsatellite markers for common bottlenose dolphins (*Tursiops truncatus*) around the main Hawaiian Islands. *Marine Mammal Science* 28:E208-E232.
- Martien, K. K., S. J. Chivers, R. W. Baird, F. I. Archer, A. M. Gorgone, B. L. Hancock-Hanser, D. Mattila, D. J. McSweeney, E. M. Oleson, C. L. Palmer, V. L. Pease, K. M. Robertson, G. S. Schorr, M. Schultz, D. L. Webster, and B. L. Taylor. 2014a. Nuclear and mitochondrial patterns of population structure in North Pacific false killer whales (*Pseudorca crassidens*). *Journal of Heredity* 105:611-626.
- Martien, K. K., M. C. Hill, A. M. Van Cise, K. M. Robertson, S. M. Woodman, L. L. Dolar, V. L. Pease, and E. M. Oleson. 2014b. Genetic diversity and population structure in four species of cetaceans around the Mariana Islands.
- Meirmans, P. G. 2006. Using the AMOVA framework to estimate a standardized genetic differentiation measure. *Evolution* 60:2399-2402.
- Morin, P. A., K. K. Martien, F. I. Archer, F. Cipriano, D. Steel, J. Jackson, and B. L. Taylor. 2010. Applied conservation genetics and the need for quality control and reporting of genetic data used in fisheries and wildlife management. *Journal of Heredity* 10:1-10.
- Morin, P. A., A. Nestler, N. T. Rubio-Cisneros, K. M. Robertson, and S. L. Mesnick. 2005. Interfamilial characterization of a region of the ZFX and ZFY genes facilitates sex determination in cetaceans and other mammals. *Molecular Ecology* 14:3275-3286.
- Palumbi, S. R., A. P. Martin, S. Romano, W. O. McMillan, L. Stice, and G. Grawbowski. 1991. A simple fool's guide to PCR. University of Hawai'i, Honolulu, HI.
- Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

- Rooney, A. P., D. B. Merritt, and J. N. Derr. 1999. Microsatellite diversity in captive bottlenose dolphins (*Tursiops truncatus*). *Journal of Heredity* 90:228-231.
- Rosel, P. E., A. E. Dizon, and J. E. Heyning. 1994. Genetic analysis of sympatric morphotypes of common dolphins (genus *Delphinus*). *Marine Biology* 119:159-167.
- Rosel, P. E., V. Forgetta, and K. Dewar. 2005. Isolation and characterization of twelve polymorphic microsatellite markers in bottlenose dolphins (*Tursiops truncatus*). *Molecular Ecology Notes* 5:830-833.
- Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA-Polymerase. *Science* 239:487-491.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning, a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Seehausen, O., K. Egbert, M. V. Schneider, L. J. Chapman, C. A. Chapman, M. E. Knight, G. F. Turner, J. J. M. van Alphen, and R. Bills. 2002. Nuclear markers reveal unexpected genetic variation and a Congolese-Nilotic origin of the Lake Victoria cichlid species flock. *Proceedings of the Royal Society of London Series B-Biological Sciences* 270:129-137.
- Shaw, K. L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. *Proceedings of the National Academy of Sciences* 99:16122-16127.
- Shinohara, M., X. Domingo-Roura, and O. Takenaka. 1997. Microsatellites in the bottlenose dolphin *Tursiops truncatus*. *Molecular Ecology* 6:695-696.
- Valsecchi, E., and W. Amos. 1996. Microsatellite markers for the study of cetacean populations. *Molecular Ecology* 5:151-156.
- Wright, S. 1931. Evolution in mendelian populations. *Genetics* 16:97-159.

Appendix II: Mitogenome phylogeography of short-finned pilot whales in the North Pacific, with reference to the Mariana Islands

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Abstract

Short finned pilot whales (SFPW) are a globally distributed temperate and tropical species. At least two morphotypes have been described in the western Pacific, and recent genetic analysis based on the mitochondrial control region indicates that those two types may be geographically segregated. Low diversity in the mitochondrial DNA, typical of social odontocetes, has made it difficult to determine the evolutionary significance and patterns of SFPW diversity. We have sequenced complete mitochondrial genomes from 99 SFPWs to infer evolutionary relationships and patterns in the Pacific. Results indicate that there are three major groups in the pilot whale phylogeny, corresponding to the two known morphotypes (called Naisa and Shiho based on original descriptions in Japan), and a third, widely distributed group that spans the range of the other two groups in the Pacific. These results suggest evolutionary divergence of multiple types of pilot whales. Global analysis of mitochondrial genomes and nuclear DNA is needed to determine the extent and patterns of differentiation among these types.

Introduction

Although short-finned pilot whales (SFPWs) are described as a single species throughout their range, two morphotypes have been described around Japan (Kasuya et al. 1988). Based on their strong morphological and genetic differences, it has been proposed that these morphotypes may constitute separate subspecies. There is also evidence of a possible third morphologically and genetically distinct type around Japan (hereafter referred to as “stock 3”), though this evidence is weaker (Kasuya et al. 1988, Oremus et al. 2009). Although it has been suggested that the Japanese morphotypes have distributions corresponding to different habitats in the Pacific (e.g., different water temperature; Kasuya et al. 1988), little is known about the actual distributions because we have little data on morphology across the Pacific. It has been proposed that that the control region haplotypes may be diagnostic of the morphotypes (Oremus et al. 2009). Diagnosability cannot be fully tested, as we do not have morphological data from most specimens, but has been shown to be true for a subset of the samples near Japan. If the control region haplotypes are diagnostic, we can infer the distribution of morphotypes based on DNA sequences. Genetic data from pilot whales outside of Japan can be used as a proxy to infer the distributions of morphotypes based on unique mitochondrial DNA haplotypes identified in the animals of known morphotype.

Mitochondrial DNA (mtDNA) sequence data from all SFPWs collected in the Mariana Islands have been previously analyzed to investigate population structure of SFPWs in the Marianas (Martien et al. 2014). Within the Marianas, there was evidence of genetic differentiation between island groups, though this may reflect familial or social structure rather than population level differentiation. A majority of the Mariana Islands samples had haplotypes (A1, A2) previously found to be common in the South Pacific, Indian and Atlantic Oceans, though two samples had haplotypes similar to that identified in the Japanese “stock 3”, which is intermediate to the Naisa and Shiho types and more similar to samples from the Atlantic and Indian Oceans (Oremus et al. 2009, Martien et al. 2014). A subset of the Mariana Islands samples were incorporated into a study aimed at determining the global distribution of the types described in Japan (Van Cise et al. Submitted). This latter study has revealed that the Shiho type is restricted to northern Japan and the eastern Pacific, while the Naisa type occurs in Hawai’i and the western and South Pacific, in addition to southern Japan.

However, the low genetic diversity in SFPWs is limiting our ability to resolve the relationships between these haplotypes and groups based on control region sequence alone. Phylogenetic relationships of unique control region haplotypes cannot be confidently determined with these short sequences. Some common haplotypes are widespread, suggesting genetic continuity, but this could be simply due to lack of diversity in the short control region. Previous studies have shown that globally common control region haplotypes in killer whales resolved to phylogenetically and geographically distinct ecotypes (and potentially subspecies or species) when longer DNA sequences were used (Morin et al. 2010). To improve our resolving power for SFPWs, we have sequenced the full mitochondrial genome of 99 SFPWs from across the Pacific and into the Indian Ocean in order to further examine the SFPW phylogeography and taxonomy.

Methods

Tissue samples were obtained from stranded dead animals and by remote biopsy of free-swimming short-finned pilot whales from locations across the Pacific Ocean and in the Indian Ocean, and stored in the Southwest Fisheries Science Center (SWFSC) tissue collection, either frozen at -80°C without preservative, or preserved in 20% DMSO saturated with NaCl, or in ethanol. DNA was extracted from tissue samples using either a silica-membrane method (Qiextractor® DX reagents, Qiagen, Valencia, CA, USA), or a simple salt-precipitation procedure (Miller et al. 1988). Detailed sample information is in Table 1, and sample locations are shown in Figure 1.

Mitogenome sequences were sequenced using multiplexed DNA libraries for capture enrichment and next-generation sequencing as described by Hancock-Hanser et al. (2013), using a previously published short-finned pilot whale mitogenome sequence (Accession No. HM060333; Morin et al. 2010) to design the capture-enrichment

microarray. After capture enrichment, libraries were amplified and sequenced using single-end 100 base-pair (bp) sequencing on an Illumina HiSeq2000 Analyzer.

Assembly of reads to the reference mitogenome was done using custom scripts (Dryad data repository doi:10.5061/dryad.cv35b) in the R computing environment (R Development Core Team 2011) to iteratively run publicly available analysis packages for quality filtering (FASTX toolkit; http://hannonlab.cshl.edu/fastx_toolkit/), assembly (BWA; Li & Durbin 2009), multiple alignment (MAFFT; Katoh et al. 2005), and SNP detection (GATK; DePristo et al. 2011, Nielsen et al. 2011). The reference mitochondrial sequence (accession HM060333) was modified to improve assembly coverage at the “ends” of the linearized mitogenome by adding 40bp from each end to the opposite end (so that reads could map across the artificial break-point of the linearized sequence). All sequences were aligned and visually inspected in the program Geneious (V. 6.0.5, Biomatters, Auckland, New Zealand), and indels and unique variants were verified in the BAM files. Previously published control region sequences were aligned to the mitogenome sequences to identify the mitogenome haplotypes that contained previously known control region haplotype sequences.

We used the Bayesian phylogenetic approach implemented in BEAST 2 (Bouckaert et al. 2014) to estimate the tree based on the full, unpartitioned mitogenome sequences for 58 unique haplotypes identified from the 99 samples plus two previously published sequences (Morin et al. 2010, Vilstrup et al. 2011). We used a coalescent prior for the tree, with a strict clock. Posterior distributions of parameters were estimated using Markov chain Monte Carlo simulation, with samples drawn every 10^3 steps over a total of 10^7 steps. The first 10% of samples were discarded as burn-in, with the remaining samples checked for acceptable convergence and mixing. We used the program PopArt (<http://popart.otago.ac.nz>) to generate a median joining network (MJN; Bandelt et al. 1999) of the haplotypes.

Results

We obtained 99 complete mitochondrial genomes from the 101 samples sequenced. There were 56 unique sequences (haplotypes) among our new sequences, and two haplotypes from two previously published short-finned pilot whale mitogenomes (Morin et al. 2010, Vilstrup et al. 2011). Phylogenetic analysis of the 58 haplotypes yielded the unrooted phylogenetic tree shown in Figure 2. The median joining network is shown in Figure 3, with haplotype nodes colored to indicate whether the haplotypes contained the control region sequences previously identified for each of the three putative types of SFPW's (Oremus et al. 2009, Van Cise et al. Submitted).

Discussion

Although about 40% of all mitogenome haplotypes in this study did not contain control region sequences that have previously been found in morphologically identified Japanese morphotypes, the phylogenetic tree indicates that there are three major

clades that correspond to the three described types, and that the geographic range of each is limited but overlapping with at least one of the other types. Clade 1 corresponds to the Naisa type in the central and western Pacific. Clade 2 contains all samples of the poorly known “stock 3”, but also closely related haplotypes that span the Pacific. Little is known about “stock 3”, but the wide geographical distribution of related haplotypes suggests that these could represent a widely distributed pelagic type that is separate from the two described morphotypes. Clade 3 corresponds closely to the Shiho type in the eastern Pacific and North Japan.

The distributions of mitogenome haplotypes (Figure 1a-b) for the three morphotypes or stocks of SFPW's are consistent with the distributions of control region haplotypes described previously (Oremus et al. 2009, Van Cise et al. Submitted). Figure 1c-d uses the relationships inferred from the complete mitochondrial phylogeny to show the distribution of haplotypes from each of the 3 clades. Based on the mitogenome clade distributions, we confirm that the clade containing the Shiho type was found only in the eastern Pacific, and the clade containing Naisa type was found in Hawai'i, the western/southern Pacific, and the Indian Ocean. The three mitogenome haplotypes that contained the single control region sequence previously found in the putative “stock 3” from offshore southern Japan were found in the Mariana Islands, Samoa, and New Zealand. The clade containing this “stock 3” type was found across the tropical Pacific and in New Zealand. While we know little about this putative stock, it appears that it may be limited to the western and southern Pacific. The Mariana Islands fall within the geographic ranges of both the Naisa type (Van Cise et al. Submitted) and the putative “stock 3” from southern Japan (Clade 2, Figure 1d), and had a diverse set of haplotypes that were unique to these samples (i.e., not found anywhere else), but that were phylogenetically linked to others in both clades 1 and 2 (Figure 2).

In order to determine whether the mitochondrial genome phylogeny clades represent genetically distinct ecotypes or subspecies of SFPWs in the Pacific, further work is needed to expand the sampling of SFPWs globally, and to match haplotypes to morphologically identified whales. Additional analysis of the phylogenetic tree using sequences from other cetaceans could also be used to estimate the approximate time of divergence between these three groups.

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MMPA permit 14097 issued to the SWFSC and CNMI-DFW permit, license no. 02260-11 (2011 samples); NMFS MMPA permit 15240 issued to PIFSC and CNMI-DFW permit, license nos. 02444-12 and 02694-13 (2012 and 2013 samples).

Figures and Tables

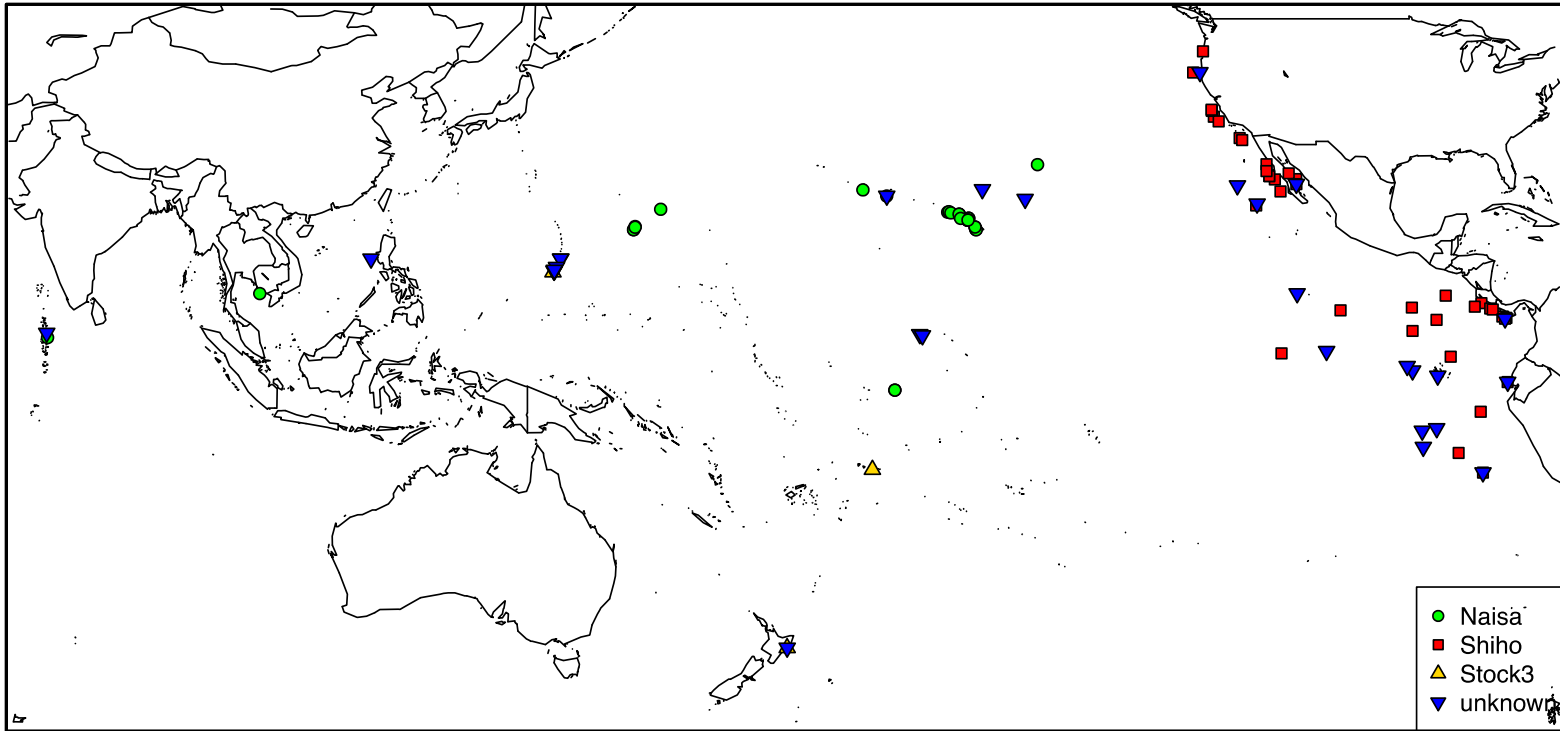
Table 1. Sample information

SWFSC ID	haplotype	Broad strata	Fine strata	Long CR haplotype	Short Standard Haplotype	Type	phylogeny clade
1297	mtGen02	NPAC	CALIFORNIA	E3	E1	Shiho	3
1685	mtGen02	NPAC	CALIFORNIA	E3	E1	Shiho	3
1739	mtGen14	NPAC	CALIFORNIA	19	E1	unknown	3
1864	mtGen04	NPAC	CALIFORNIA	E3	E1	Shiho	3
4629	mtGen15	ETP	GULF OF CALIFORNIA	E3	E1	Shiho	3
4642	mtGen04	ETP	GULF OF CALIFORNIA	E3	E1	Shiho	3
4682	mtGen16	ETP	GULF OF CALIFORNIA	9	9	unknown	3
4683	mtGen02	ETP	MEXICO IFS	E3	E1	Shiho	3
4694	mtGen02	NPAC	CALIFORNIA	E3	E1	Shiho	3
4986	mtGen02	NPAC	NPAC IFS	E3	E1	Shiho	3
5766	mtGen02	NPAC	CALIFORNIA	E3	E1	Shiho	3
7618	mtGen04	ETP	BAJA	E3	E1	Shiho	3
8752	mtGen02	NPAC	CALIFORNIA	E3	E1	Shiho	3
9850	mtGen17	INDIA	INDIA	A1	A1	unknown	1
9871	mtGen18	INDIA	INDIA	K	K	Naisa	1
11454	mtGen19	ETP	ETP IFS	E3	E1	Shiho	3
11496	mtGen20	ETP	ETP IFS	2	2	unknown	2
11515	mtGen21	ETP	BAJA	E3	E1	Shiho	3
11526	mtGen02	ETP	BAJA	E3	E1	Shiho	3
11873	mtGen22	ETP	EL SALVADOR	E3	E1	Shiho	3
11943	mtGen05	ETP	COSTA RICA	E3	E1	Shiho	3
11954	mtGen06	ETP	PANAMA	E3	E1	Shiho	3
11977	mtGen23	ETP	CLIPPERTON IS.	5	5	unknown	3
11985	mtGen24	ETP	ECUADOR	2	2	unknown	2
12009	mtGen25	ETP	ECUADOR	6	6	unknown	3
12029	mtGen26	ETP	ECUADOR	E3	E1	Shiho	3
12030	mtGen07	ETP	ECUADOR	7	7	unknown	3
12095	mtGen02	ETP	PANAMA	E3	E1	Shiho	3
13367	mtGen27	PHILLIPINES	PHILLIPINES	14	14	unknown	1
16047	mtGen08	ETP	COSTA RICA	E3	E1	Shiho	3
16079	mtGen28	ETP	ETP IFS	E3	E1	Shiho	3
16167	mtGen06	ETP	ETP IFS	E3	E1	Shiho	3
17977	mtGen02	ETP	BAJA	E3	E1	Shiho	3
17981	mtGen29	ETP	BAJA	E2	E1	Shiho	3
18191	mtGen02	ETP	ETP IFS	E3	E1	Shiho	3
18261	mtGen08	ETP	COSTA RICA	E3	E1	Shiho	3
18293	mtGen30	ETP	PANAMA	E3	E1	Shiho	3
18298	mtGen31	ETP	PANAMA	2	2	unknown	2
18941	mtGen01	HI	LANAI	J	J	Naisa	1
18953	mtGen01	HI	LANAI	J	J	Naisa	1
23968	mtGen32	CAMBODIA	CAMBODIA	K	K	Naisa	1
25546	mtGen02	NPAC	OREGON	E3	E1	Shiho	3
30435	mtGen33	HI	NWHI	J	J	Naisa	1
30439	mtGen01	HI	NWHI	J	J	Naisa	1
30442	mtGen34	HI	NWHI	12	12	unknown	1
30535	mtGen35	HI IFS	HI IFS	11	11	unknown	2
33294	mtGen36	NEW ZEALAND	NEW ZEALAND	A1	A1	unknown	1
33295	mtGen37	NEW ZEALAND	NEW ZEALAND	C	C	Stock3	2
33798	mtGen01	HI	LANAI	J	J	Naisa	1
33813	mtGen38	HI	LANAI	J	J	Naisa	1
33814	mtGen09	HI	LANAI	J	J	Naisa	1
33851	mtGen01	HI	OAHU	J	J	Naisa	1
33852	mtGen01	HI		J		Naisa	1

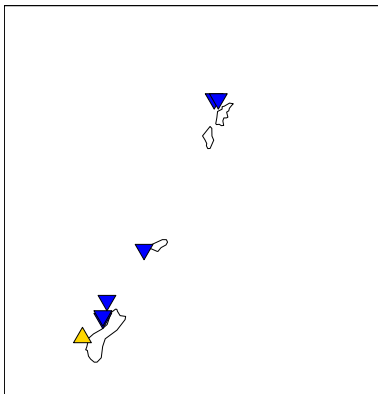
33861	mtGen03	HI	OAHU	J	J	Naisa	1
33879	mtGen03	HI	OAHU	J	J	Naisa	1
33916	mtGen01	HI	OAHU	J	J	Naisa	1
33941	mtGen01	HI		J		Naisa	1
33981	mtGen03	HI	KAUAI	J	J	Naisa	1
37746	mtGen02	ETP	ETP IFS	E3	E1	Shiho	3
37752	mtGen39	ETP	ETP IFS	E3	E1	Shiho	3
37753	mtGen40	ETP	ECUADOR	E3	E1	Shiho	3
37766	mtGen41	ETP	ETP IFS	10	10	unknown	3
37772	mtGen42	ETP	ETP IFS	2	2	unknown	2
37781	mtGen43	ETP	ETP IFS	E3	E1	Shiho	3
37783	mtGen44	ETP	ETP IFS	8	8	unknown	3
37788	mtGen45	ETP	ETP IFS	E3	E1	Shiho	3
37884	mtGen05	ETP	COSTA RICA	E3	E1	Shiho	3
37896	mtGen46	ETP	MEXICO IFS	3	3	unknown	2
37907	mtGen02	ETP	BAJA	E3	E1	Shiho	3
38312	mtGen47	ETP	MEXICO IFS	E1	E1	Shiho	3
38314	mtGen48	ETP	MEXICO IFS	2	2	unknown	2
45934	mtGen01	HI		J		Naisa	1
49063	mtGen49	KIRIBATI	KIRIBATI	2		unknown	2
49070	mtGen50	KIRIBATI	KIRIBATI	20		unknown	1
51029	mtGen01	HI		J		Naisa	1
55234	mtGen01	HI		J		Naisa	1
55239	mtGen01	HI		J		Naisa	1
67152*	mtGen12	ETP	ETP IFS	10	10	unknown	3
67165*	mtGen13	ETP	ETP IFS	10	10	unknown	3
74708	mtGen51	HI_IFS	HI_IFS	K	K	Naisa	1
78786	mtGen52	KIRIBATI	KIRIBATI	K		Naisa	1
78787	mtGen53	KIRIBATI	KIRIBATI	K	K	Naisa	1
79766	mtGen54	SAMOA	SAMOA	C	C	Stock3	2
79992	mtGen01	HI		J		Naisa	1
88594	mtGen03	HI		J		Naisa	1
92242	mtGen09	HI		J		Naisa	1
102494	mtGen01	HI		J		Naisa	1
104024	mtGen10	MARIANA_Is.	CNMI	A2		unknown	1
104027	mtGen10	MARIANA_Is.	CNMI	A2	A1	unknown	1
104051	mtGen55	MARIANA_Is.	CNMI	18		unknown	2
104055	mtGen11	MARIANA_Is.	CNMI	A1		unknown	1
104078	mtGen11	MARIANA_Is.	CNMI	A1		unknown	1
108166	mtGen56	MARIANA_Is.	CNMI	17		unknown	1
112652	mtGen01	HI		J		Naisa	1
112653	mtGen01	HI		J		Naisa	1
113653	mtGen01	HI		J		Naisa	1
114348	mtGen57	HI_IFS	HI_IFS	2		unknown	2
114352	mtGen01	HI		J		Naisa	1
114354	mtGen01	HI		J		Naisa	1
114565	mtGen03	HI		J		Naisa	1
116839	mtGen58	MARIANA_Is.	CNMI	C		Stock3	2

* previously published, (Morin et al. 2010, Vilstrup et al. 2011)

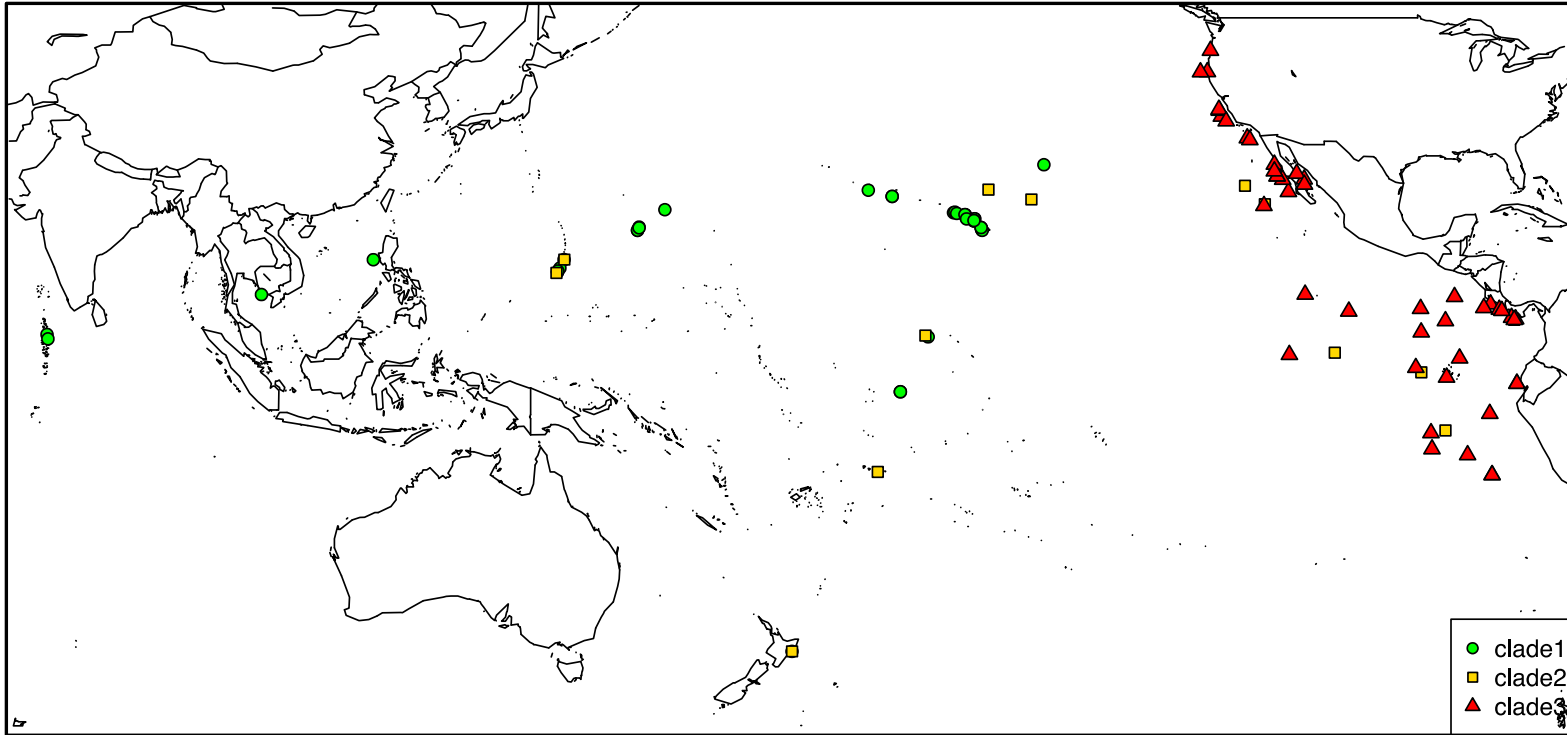
Figure 1a. Sample locations and types. The color indicates whether the haplotype contains the control region sequences previously identified by Oremus et al. (2009) as being found in the northern Japan (“Shiho”, Red), southern Japan (“Naisa”, Green), or putative third southern (stock 3) stocks (putatively containing Control Region haplotype C, Yellow). Blue triangles = haplotypes not previously identified from one of the types or stocks.



1b. Samples from the Mariana Islands. Symbols and colors identify types as in 1a.



1c. Samples showing samples colored by clades from the phylogenetic tree (Fig. 2).



1d. Samples from the Mariana Islands. Symbols and colors identify types as in 1c.

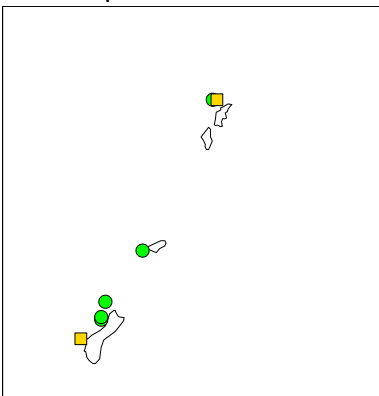


Figure 2. Phylogenetic tree of 58 mitogenome haplotypes. Colors are as described in Figure 1a.

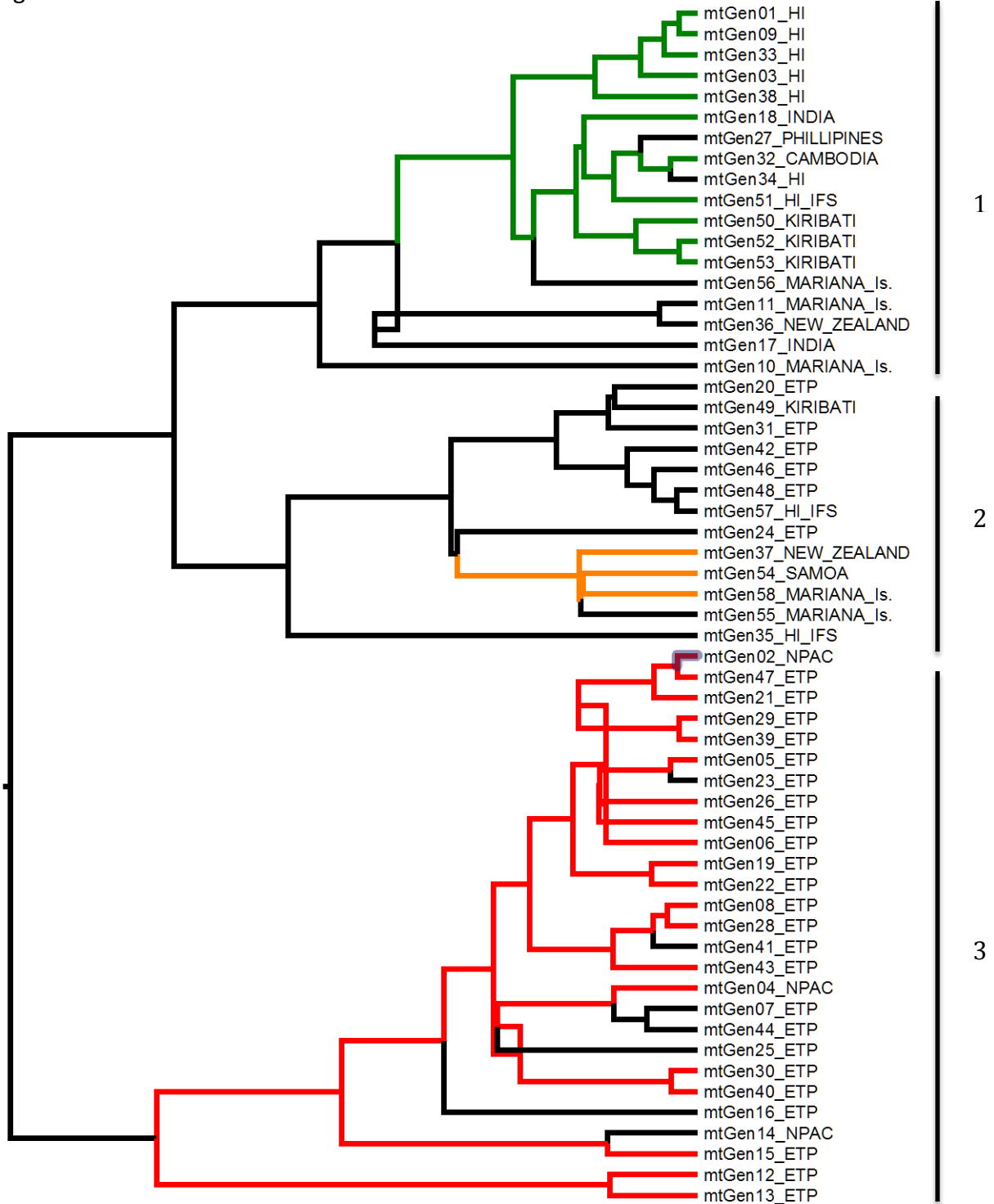
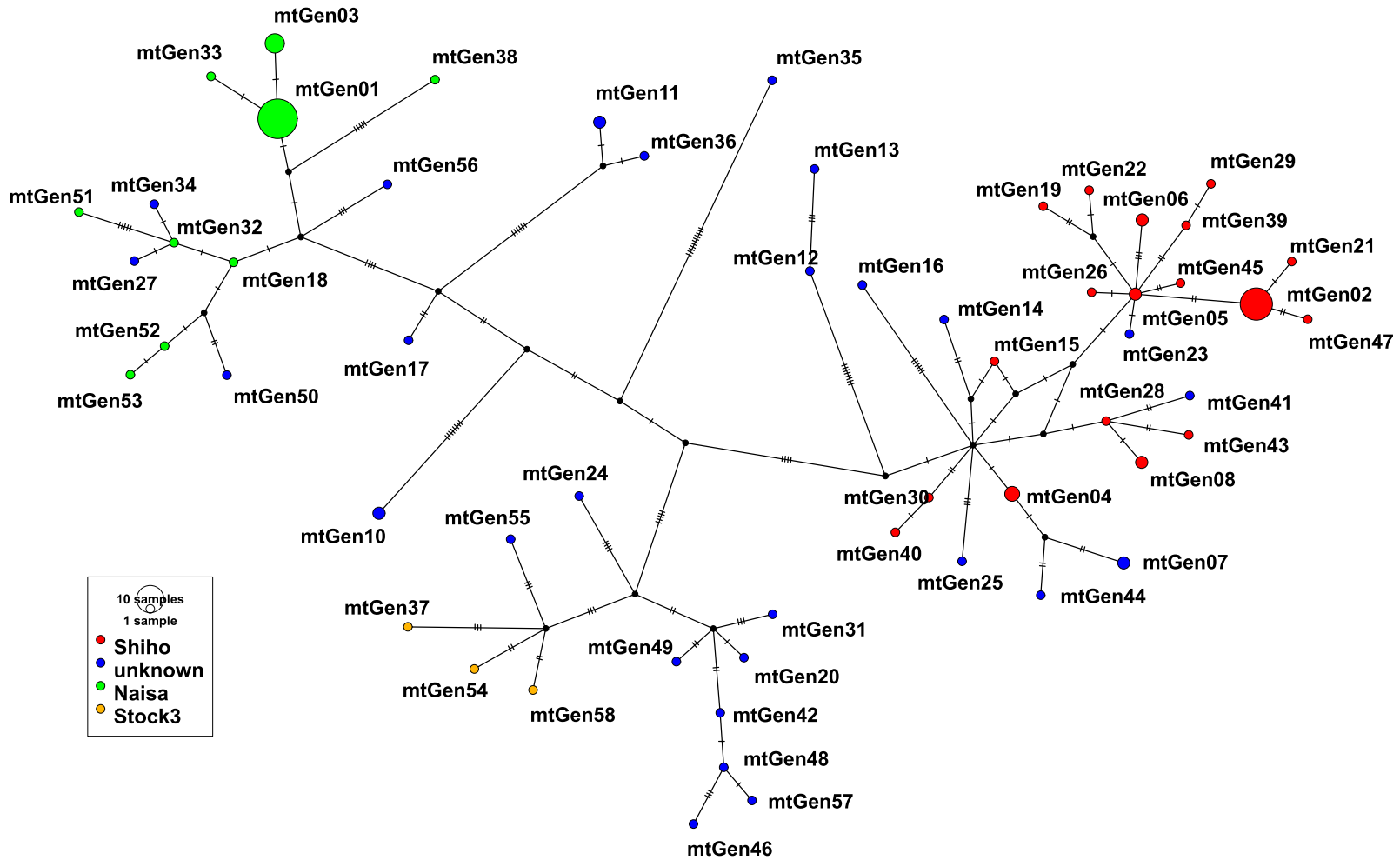


Figure 3. Median joining network of mitogenome haplotypes. The size of the circle is proportional to the number of samples with that haplotype. Colors are as described as in Figure 1a.



References

- Bandelt, H. J., P. Forster and A. Rohlf. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16:37-48.
- Bouckaert, R., J. Heled, D. Kuhnert, et al. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput Biol* 10:e1003537.
- Depristo, M. A., E. Banks, R. Poplin, et al. 2011. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics* 43:491-498.
- Hancock-Hanser, B., A. Frey, M. Leslie, P. H. Dutton, E. I. Archer and P. A. Morin. 2013. Targeted multiplex next-generation sequencing: advances in techniques of mitochondrial and nuclear DNA sequencing for population genomics. *Molecular Ecology Resources* 13:254-268.
- Kasuya, T., T. Mayashita and F. Kasamatsu. 1988. Segregation of two forms of short-finned pilot whales off the Pacific Coast of Japan. *The Scientific Reports of the Whales Research Institute* 39:77-90.
- Katoh, K., K. Kuma, T. Miyata and H. Toh. 2005. Improvement in the accuracy of multiple sequence alignment program MAFFT. *Genome Informatics* 16:22-33.
- Li, H. and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25:1754-1760.
- Martien, K. K., M. C. Hill, A. M. Van Cise, et al. 2014. Genetic diversity and population structure in four species of cetaceans around the Mariana Islands. NOAA Technical Memorandum NMFS, NOAA-TM-NMFS-SWFSC-536.
- Miller, S. A., D. D. Dykes and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* 16:1215.
- Morin, P. A., F. I. Archer, A. D. Foote, et al. 2010. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. *Genome Research* 20:908-916.
- Nielsen, R., J. S. Paul, A. Albrechtsen and Y. S. Song. 2011. Genotype and SNP calling from next-generation sequencing data. *Nature Reviews Genetics* 12:443-451.
- Oremus, M., R. Gales, M. L. Dalebout, et al. 2009. Worldwide mitochondrial DNA diversity and phylogeography of pilot whales (*Globicephala* spp.). *Biological Journal of the Linnean Society* 98:729-744.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Van Cise, A., K. K. Martien, P. A. Morin, R. W. Baird, N. M. Hedrick and K. M. Robertson. Submitted. Redrawing the map: mtDNA provides new insights into the distribution and diversity of short-finned pilot whales in the Pacific Ocean. *Marine Mammal Science*.
- Vilstrup, J. T., S. Y. Ho, A. D. Foote, et al. 2011. Mitogenomic phylogenetic analyses of the Delphinidae with an emphasis on the Globicephalinae. *BMC Evol Biol* 11:65.

