ROUGH-TOOTHED DOLPHIN (*STENO BREDANENSIS*)

PIGMENTATION PATTERNS ACROSS THE HAWAIIAN ISLANDS

by

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ABSTRACT

Rough-toothed Dolphin (*Steno bredanensis*)
Pigmentation Patterns across the Hawaiian Islands

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Rough-toothed dolphins (*Steno bredanensis)* are a common dolphin found in multiple oceans worldwide and are ubiquitous in the waters around the Hawaiian Islands. Photo-identification is presently used as a predominant research technique with cetaceans, including *S. bredanensis,* a delphinid whose pigmentation is unique to the individual level. Employing a catalogue of over 2,000 rough-toothed dolphins from the islands of Hawai'i, O'ahu, and Kaua'i/Ni'ihau, common pigmentation patterns were identified and categorized. This analysis focused on the cape and dorsal fin areas, based on the prevalence of pigmentation in these areas, and their use as individual identification factors. Rough-toothed dolphin photos were scored based on the percent pigmentation cover in these areas, in 25% cover increments. Individual dolphins were grouped based on which of the three islands they were associated with. For both patterns tested (dorsal fin and cape pigmentation), there was an association of island and pigmentation pattern (using a χ^2 test of independence), although the association was stronger for the dorsal fin pattern. This study indicates that there are geographic differences in pigmentation patterns in Hawaiian rough-toothed dolphins, something that has been shown to be true in various other delphinids. Hawaiian rough-toothed dolphins may therefore be genetically distinct by island. However, more research is needed to determine how pigmentation and geographic association are linked, not only in rough-toothed dolphins but in other delphinids as well.

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Chapter 1: Introduction

Whales and dolphins, hereafter cetaceans, are the sentinels for the health of our oceans. They often indicate the strength of an ecosystem or the biodiversity of that system (Roman et al. 2014). Systems that support large mammals like cetaceans must contain an abundance of prey resources in order to sustain them (Roman et al. 2014). Researchers also often use cetaceans when attempting to understand the impacts that humans have on the environment and animals around them—particularly in the areas of sound pollution, noise pollution, chemical pollution, and fishing pressure. There is a lot of importance placed on pigmentation as a means for individual identification of cetaceans, despite there being little research on patterns of pigmentation in general.

Pigmentation is one of the most distinctive features of many cetacean species, including the rough-toothed dolphin (*Steno bredanensis;* Rosso et al. 2008). In cetaceans, pigmentation and photo identification (photo-ID) are used to keep track of population statuses, ages of individual organisms, and separate stocks that do not mingle (*i.e.*, insular and pelagic; Baird et al. 2003). However, despite pigmentation being vital in photo-ID studies, there is only a small amount of research on their marks, if they are genetic, or how they vary (Oremus et al. 2012). In numerous species of delphinids, gaps exist in the current literature about how pigmentation varies between genetically distinct groups (Oremus et al. 2012). If pigmentation patterns are similar within populations but different between populations, there may be implications for management of different stocks of rough-toothed dolphins.

My research on rough-toothed dolphin pigmentation in the Hawaiian Islands provides insight into how external morphological features can vary both within and between populations. The result of this research contributes to the limited body of knowledge on pigmentation in cetaceans, and specifically rough-toothed dolphins. Because pigmentation is commonly used as an identifying feature in photo-ID research, being able to use photographs taken for identification purposes and understand more about a specific individual, like their main geographic association, would be useful for future research (Baird et al. 2013; Rosso et al. 2008; Hammond et al. 1990). Photo-ID research in its current form is a time intensive form of research—it tends to require a lot of time matching photos of individuals against a known catalog. It is also intensive in the field, as it involves time spent in a boat or from shore and relies a lot on chance and word of mouth to find animals. Any way to make photo-ID matching more productive would be welcome, particularly to the marine mammalogy community.

I investigated whether there is a difference in pigmentation between rough-toothed dolphins sighted in Kaua'i/Ni'ihau, O'ahu, and Hawai'i Island. Rough-toothed dolphins as a species and a population in Hawai'i have their population structure separated into populations based on where they were originally sighted, including populations at Hawai'i, O'ahu, and Kaua'i/Ni'ihau, the islands focused on herein (Baird et al. 2003). Although rough-toothed dolphins are not managed like a commercial fish species, they are protected under the Marine Mammal Protection Act, and understanding if there are distinct stocks is vital to preserving genetic diversity (Baird 2016). Furthermore, other delphinids may have pigmentation that indicates community association, and there are multiple threatened cetacean species found worldwide that this study could apply to.

Chapter 2: Literature Review

Introduction

My research is on pigmentation in rough-toothed dolphins (*Steno bredanensis)*, and how patterns in their pigmentation could indicate population structure. My research focused on rough-toothed dolphins in the Hawaiian Islands, so I will broadly describe the ecosystem they live in and the life history of the dolphins. Cetacean scientists use pigmentation as a means of identification for multiple reasons. First, pigmentation is generally constant throughout the life of many delphinids and can provide a more reliable identification method than notches in the dorsal fin, which are more ephemeral (Bichell et al. 2017; Baird et al. 2003; Hammond et al. 1990). Later in this review, I will go into the importance of pigmentation in rough-toothed dolphins and how it relates to previous research of cetaceans.

Cetaceans in Hawai'i

The Hawaiian Islands are one of the most biodiverse island chains on the planet (Gillespie et al. 2008). Not only does the land support a variety of endemic flora and fauna, but marine species use the oasis in the middle of the Pacific as a protective haven. For instance, Humpback whales use the tropical waters of the Hawaiian Islands as calving grounds before beginning yearly migrations to Alaska to feed. In addition, the Hawaiian Islands are home to cetaceans that are native to the islands or spend their time in the open ocean near the main island chain. The islands are a hotspot for cetacean diversity because of the variety of niches available including deep water, coastal, pelagic, and shallow habitats (Baird 2016). Some of the cetacean species found in the Hawaiian Islands include: rough-toothed dolphins, common dolphins, pilot whales, melon-headed whales, false killer whales, and pygmy killer whales, among others (Baird 2016). In addition to hosting a diverse cetacean system, Hawai'i supports unique species in coral reefs and multiple commercial fisheries, as well as the largest marine national monument in the United States, Papahānaumokuākea. As we seek to understand and limit human impacts on these systems, watching for how cetaceans react to changes could be one of the most important pathways for research in the future.

Rough-toothed dolphin

The rough-toothed dolphin (*Steno bredanensis)* occurs worldwide in tropical and subtropical waters. They generally live in the open ocean, making research into population structure, ecology, and other life history characteristics very limited (Albertson 2012). The entire species is managed as a single stock, although recent research has proposed the management of Atlantic and Pacific stock separately (at a minimum), due to genetic differentiation (Albertson et al. 2012). The rough-toothed dolphin has multiple populations around the Hawaiian Islands (Baird et al. 2008; Alberston 2012), particularly around Kaua'i and Ni'ihau (Baird et al. 2003). They are the fifth most-commonly seen species overall in an odontocete survey of the Hawaiian Islands (Baird et al. 2003).

Despite being recorded as mostly inhabiting deep waters, rough-toothed dolphin prey on mahi-mahi and squid—species that are not deep-water associated (Albertson et al. 2012). Rough-toothed dolphins are present worldwide in many different environments and tend to exhibit some degree of philopatry (tendency to remain near where born) and the low haplotype diversity indicates a highly matrilineal social structure (Albertson et al. 2012; Oremus et al. 2008). Group sizes vary for rough-toothed dolphins, tending towards smaller groups around oceanic islands and larger groups in pelagic waters (Albertson et al. 2012). Stable matrilineal groups tend to lead to quicker genetic differentiation—with little gene flow between populations.

Recently research has been conducted around tropical islands, showing that rough-toothed dolphins have high site fidelity—they will stay around the same area, not often venturing far from their home (Baird et al. 2008). In the Hawaiian Islands, rough-toothed dolphins are generally found near the islands of Kaua'i/Ni'ihau, and near the big island, Hawai'i (Baird et al. 2008). Based on sampling of the islands, there has been found to be only a 2% dispersal rate between islands, with a very high resighting rate for the island of Hawai'i (Baird et al. 2008). This may indicate that there are specific island-associated populations of rough-toothed dolphins around the Hawaiian Islands, although there is limited research specifically in this region. However, research in another island chain, French Polynesia, has indicated separate insular and pelagic populations of rough-toothed dolphins (Oremus et al. 2012).

In French Polynesia, despite previous research noting that rough-toothed dolphins are a pelagic species, there are groups of dolphins that exhibit site fidelity in the Society Islands (Miyazaki and Perrin 1994; Oremus et al. 2012). There was also an indication of demographic partitioning between two of the French Society islands, Mo'orea and Raiatea (Oremus et al. 2012). There was also a genetic analysis conducted in this study—the researchers found that the most dominant haplotype for the Mo'orea groups did not occur at Raiatea, and vice versa (Oremus et al. 2012). In addition, the two sample island groupings were found to be genetically distinct (Oremus et al. 2012). A similar result was found earlier with Gray's spinner dolphins in the same region (Oremus et al. 2007). The Hawaiian Islands are a similar ecosystem to the Society Islands and may present the same level of genetic differentiation between populations associated with individual islands. Although this study did not go into morphological or pigmentation differences in rough-toothed dolphins in the area, it provides evidence that there may be unique, possibly genetically diverse small insular populations around the Hawaiian Islands, which may have conservation implications.

Threats Faced

Despite the rough-toothed dolphin's worldwide status as "Least Concern" on the IUCN Red List, the small amount of research about their population structure and the variety of threats they face elicit some uncertainty in the literature. In Hawai'i, rough-toothed dolphins face threats from fishery interactions, particularly fishermen shooting the animals for trying to take fish from fishing lines (Baird 2016). In addition, rough-toothed dolphins can be caught in longline fishing nets and face other general boat interactions (Miyazaki and Perrin 1994). For instance, research found that rough-toothed dolphins modified their behavior when approached by boats, avoiding them 18% of the time off Hawai'i and 38% of the time off of Kaua'i/Ni'ihau (Baird et al. 2008).

Finally, sound pollution is a threat that rough-toothed dolphins face, particularly from large-scale military sonar implements (Fourney et al. 2017). In the Hawaiian Islands, the United States Navy uses mid-frequency sonars in military practices, which puts multiple species of cetaceans in the area at possible risk for adverse reactions, and at worst stranding events (Fourney et al. 2017). With the possibility of small island-associated populations with a relatively small area of suitable habitat, moving away from loud noises becomes increasingly difficult (Fourney et al. 2017). If rough-toothed dolphins have genetically distinct smaller populations, they need to be managed as such, and not as one singular stock. This could be vital if more threats arise in the face of climate change.

Photo-Identification

Photo-identification (photo-ID) is a common method of research for biologists, particularly those that work with cetaceans. In the last 20 years camera technology has become much more precise, increasing the use of photo-ID as a research tool. Photo-ID is often used from aerial surveys, boat surveys, and shore-based observations. For cetaceans, photo-ID is used to identify individual animals, generally with photos taken from boats. There are a variety of methods for matching photos, including using notches in fins, scars, pigmentation patterns, color, and others that are usually unique to the species being studied (Hammond et al. 1990). Recently, computer programs have been created to match significantly quicker, but these are not available for every species, particularly those with limited research (Hillman et al. 2003; Kniest et al. 2010).

With photo-ID studies, researchers can conduct a variety of different analyses vital to understanding cetacean population structure. Researchers use photo-identification to track the lives of individual animals over extended research surveys. This can help in understanding movement of animals, niche partitioning, human interaction, and even basic biological questions like age (Baird et al. 2003; Baird et al. 2008; Mayr and Ritter 2005). In addition, photo-identification is important for understanding survivability in mark-recapture studies—to assess tagging or biopsies and their effects on the study animals (Gendron and De la Cruz 2012). By taking pictures and seeing how one animal changes throughout its life, scientists can better understand the species, and how to gauge age and sex in unknown individuals. Cetacean photo-ID is used in nearly all studied species, including humpback whales, spotted dolphins, killer whales, and gray whales (Baird et al. 2013; Rosso et al. 2008; Hammond et al. 1990). Using pigmentation as a method for long term photo-ID is common; in bottlenose dolphins, researchers and inexperienced students were shown to make accurate matches based on pigmentation approximately 90% and 60% of the time, respectively (Bichell et al. 2017). Photo-identification has been one of the most common research methods for cetaceans and should continue to be for the foreseeable future.

Pigmentation

Pigmentation is often used in photo-ID studies for cetaceans and other animals. One of the first cetacean species with recognizable pigmentation patterns that are used to identify community associations is the killer whale (*Orcinus orca).* In British Columbia resident orcas and transient killer whales have distinct pigmentation patterns (Sugarman 1984; Baird and Stacey 1988). Despite every killer whale having a unique individual saddle patch, orcas that were sighted in the same pod often shared pigmentation patterns. For instance, resident British Columbia orcas had all five patterns present, while transients had only the smooth and bump saddle patch, not the other three (vertical notch, horizontal notch, and hook; Baird and Stacey 1988). There was a significant difference between the saddle patch patterns in northern residents and transients, as well as southern residents and transients (Baird and Stacey 1988). This indicates that saddle patch pigmentation is heritable at least to a certain degree because residents and transients do not interbreed (Baird and Stacey 1988).

The differences in ecotypes and populations of killer whales is a topic often debated to the present day, with many scientists seeing the morphological and ecological differences between residents, transients, and offshore orcas as grounds for separation into different species (Hoelzel et al. 1991; Baird and Stacey 1988; LeDuc 2008; Foote et al. 2016). Rough-toothed dolphins and killer whales are both delphinids—they share the same family and could share this similar pigmentation characteristic. Although rough-toothed dolphins do not have a saddle patch, nor as much research into their community ecology, the pigmentation swath beneath their dorsal fin could have similar patterns as the killer whales.

Another species with variation in pigmentation that was been researched significantly is the harbor porpoise (*Phocoena phocoena).* A photo study of harbor porpoise from a variety of locations worldwide showed that, on a variety of color morphs, unique variations occurred for each of the locations (Koopman and Gaskin 1994). These pigmentation morphs included eye patch, cape shape, and ventral stripe, among others (Koopman and Gaskin 1994). The differences in these morphs indicate that pigmentation and color morphology is genetic and possibly predictive of location or community association, even in species like the harbor porpoise which do not form tight-knit matrilineal groups like rough-toothed dolphins. In this study, the researchers used pigmentation differences from all over the body of the animal, particularly since differences should be more pronounced when looking at worldwide species differences as opposed to in one island chain (Koopman and Gaskin 1994).

More recently, photo-identification via pigmentation has been for blue whales (*Balaenoptera musculus*). Gendron and De la Cruz (2012) proposed a new method of photo identification for blue whales, using categories for the dorsal fin shape as well as patterns of pigmentation. Blue whales have similar pigmentation to rough-toothed dolphins, an amorphous area of pigmentation on the dorsal flank area, to the left and right of the dorsal fin. The authors categorized blue whale pigmentation into five categories, focused on the coloration of light and dark gray on the flank (Gendron and De la Cruz 2012). Although the authors did not make any assessments about which pigmentation types were more common by sighting location, their method of pattern categories is a useful way to look at animals like rough-toothed dolpins that have the gray-on-gray pigmentation type.

Finally, another tropical dolphin, the striped dolphin (*Stenella coeruleoalba*, that also occurs in the Hawaiian Islands), was studied using multiple morphological variables. In the northwestern Mediterranean Sea, flank pigmentation, forehead white patch, and others were analyzed (Rosso et al. 2008). Using these variables, the authors found that in larger group sizes, there was a larger pigmentation distance—that is, a larger dissimilarity between dolphins in the groups (Rosso et al. 2008). This was only in a small region, the northwestern Mediterranean, so there was little comparison between groups, but the pigmentation distance indicates there could be a genetic component to pigmentation morphology (Rosso et al. 2008). As with the harbor porpoise study, Rosso et al. (2008) looked at morphological variables across the entire body of the animal, which is more difficult than just the dorsal fin and accompanying region.

Population Assessment

Many cetaceans, including the rough-toothed dolphin, have global distributions, resulting in difficulties when attempting to assess the populations. For instance, killer whales have many different populations, including transients, residents for different regions of the world (*e.g.*, Southern Residents in the San Juan Island region of Washington state), and they also have separate ecotypes, morphologies that are distinct based on region (Foote et al. 2016). Killer whales are one of the most studied delphinids, and the body of knowledge on others is significantly smaller. In some of these other cetaceans, such as bottlenose dolphins, nearshore and offshore populations have been shown to be genetically distinct as well as morphologically distinct (Hoelzel et al. 1998). Morphological variation describes a wide variety of phenotypes, from the shape of the rostrum to where a color starts on the animal to size and so on. There are an infinite number of morphometrics that can be collected on a single animal.

For rough-toothed dolphins in the Hawaiian Islands there has been no research into the morphological distinctions between either insular or pelagic individuals, as well as between populations near the different islands. Bottlenose dolphins in the Hawaiian Island chain are genetically distinct between the main groups, which may indicate that rough-toothed dolphin groups are as well (Baird et al. 2016). The significance of morphologically distinct populations indicates a potential for genetic differentiation. However, intraspecific morphological variation can be indicative of other life history aspects, from anti-predator defenses to diet variation (Bolinick et al. 2011). The morphological variation of individual pigmentation may seem like a small aspect of overall morphology, but even small variations can indicate important components of a species' life history.

Conclusion

Pigmentation is a ubiquitous component of the morphology of many cetaceans, and research has shown that it can be indicative of genetics and community association. In cetaceans alone, pigmentation has been shown to have a genetic basis, as well as be indicative of groupings in animals where that research has been conducted. Because of the importance of photo-identification in cetacean studies and pigmentation in photo-identification, increasing the knowledge base of the importance of pigmentation.

Chapter 3: Methods

Data for this project was taken in the form of photographs from Cascadia Research Collective. The photographs were taken from 2005-2017 in the main Hawaiian Island chain, in various field efforts to capture estimates and identify individual Hawaiian cetaceans. While in the field, researchers used boat-based surveying with recorded transect lines; however, those transect lines varied based on weather, season, and what the target animal was. A variety of boats were used, generally a medium-sized rigid-hulled zodiac with researchers scanning for animals at four points on the boat including a lookout over the bow, two on the starboard and port sides, and one at the aft end of the boat. The person piloting the boat was not responsible for sighting individuals for safety reasons. Researchers were equipped with binoculars for sighting animals, as well as DSLR cameras for taking species identification photos.

When an animal was sighted, generally by a spout in the distance, the boat would navigate as close to the location as possible and begin recording an encounter. By labeling each different encounter with specific species present, categorization of photographs is easier when returning to the lab. The animals were followed as closely as possible, with focus generally on getting a good angle shot for photo-ID purposes. In some circumstances, biopsies were taken or tags were placed, however not on every encounter and this was noted in the record of the encounter. Metrics such as GPS location, weather, Beaufort sea scale, group size, and activity were also recorded at the start of the encounter, and periodically updated while staying with the group, approximately every thirty minutes. The boat would end an encounter when it was decided that a majority of the individuals had been photographed, or another species appeared within sighting distance. During any encounter, researchers who were not photographing remained scanning the horizon for other animals.

Good angle photos were the focus of the photography, which referred to angling the boat parallel to animals about to surface and show their dorsal fins, in the case of the rough-toothed dolphin. This allowed photographers to get the best view of the identifiable features. Care was taken also to avoid glare on the fin from the sun, since that can make identification difficult. Particular care was taken to obtain a clear identifying photo for a tagged or biopsied animal, to make sure researchers knew which individual was being tagged. Some photographs have also come into the catalog from other sources, such as whale watching vessels. Because there is limited need for a randomized design if the photos received from outside sources are of acceptable quality they are included in the catalog.

When in the field, thousands of photos are taken for one encounter. When the photos are returned to the lab to be analyzed, the photos will be assigned both to an encounter, based on the timestamps and species present, as well as the photographer, which is indicated by a photo taken of the individual prior to the encounter. When faced with the initial deluge of photos, researchers will first remove unusable photos, of which there are many, then internally match the photos within the encounter, assigning each unique individual a temporary ID which can later be used to take through the full catalog. The goal is to get a distinct and clear photo of each side of the dorsal fin and as much of the cape area (the area with unique pigmentation) as possible. Any photos that show more of the body are helpful as well, since more identifying pigmentation can be seen. One of the challenges with photos of rough-toothed dolphins (and many other odontocetes) is that they travel in large groups—so any one photo may have four or five dolphins in it. The isolation of dorsal fins takes a significant amount of photo manipulation and effort. During this process, researchers can also assign sex to individuals, and a calf to a mother, if there is a prominent neonate close in the presence of another dolphin.

Once an encounter has been sifted through, a "Best of" file is created for each temporary ID, so that ID can be searched against the catalog. Often, not just the perfect angle photos are included—some that show different angles, a distinct scar, more pigmentation, or any other defining feature could be useful for someone attempting to identify the animal. These photos can also help if the dolphin is already in the catalog but has a new feature such as a notch or a cookie-cutter shark bite—which should be noted for future identification. In addition, the best quality photos—those used in the catalog—are assigned a quality number from 1 to 5. In my study I will only be using photos of quality 3 and above, which usually means that pigmentation is visible and can be coded. If for some reason all or most of the pigmentation is not visible in a photo, that photo was not included in the analysis.

A negative match generally results in a new ID number being added to the catalog; however, all identifications are initially checked, generally by someone less experienced, then independently matched by two senior researchers. If a match is confirmed, any photos from the new encounter that would aid in future identification are added into the full catalog, and all photos from the encounter are placed in the individual ID's file, where all photos they are in are stored. Generally, a new photo is only added to the catalog if it is of better quality than the older photos, or is of a different side, or if there is a significant change to the fin. In order to prevent the catalog from being unwieldy, photos are often changed out as better photos become available; however, generally the very first and very last sighting are always included, which allows for a quick picture of how long we have seen an individual. If an individual was seen at multiple island groups, generally rare in the catalogue, the most common place sighted will be used.

For the purposes of my study, I chose multiple patterns that I would look for when going through the catalog of individuals, based on patterns I saw multiple times in the catalog (Table 1). Examples of dolphins that have each pattern are shown following Table 1 (Figures 1-5).

Table 1: Pigmentation patterns and the code that is associated with those patterns. Each rough-toothed dolphin individual with quality photos was analyzed for these codes.

Pigmentation Pattern	Code
Pigmentation does not extend to dorsal fin	A
Pigmentation covers approximately 50-75% of cape	B1
Pigmentation covers approximately 75-100% of cape	B2
Pigmentation covers approximately 25-50% of cape	B3
Hole in pigmentation	C
Wispy	D
Pigmentation covers 25-50% of dorsal fin	E1
Pigmentation covers 50-75% of dorsal fin	E2
Pigmentation covers 0-25% of dorsal fin	E3
Pigmentation reaches below midline	G

Figure 1: Pigmentation does not extend to dorsal fin (Code A) in a rough-toothed dolphin, catalog ID HISb0142, Cascadia Research Collective.

Figure 2: Pigmentation covers 75-100% of cape (Code B2) in rough-toothed dolphin HISb0148.

Figure 3: Pigmentation covers approximately 25-50% of cape (Code B3) in rough-toothed dolphin HISb0748.

Figure 4: Hole in pigmentation (Code C) in rough-toothed dolphin HISb0828.

Figure 5: Pigmentation reaches below midline (Code G) in rough-toothed dolphin HISb0871.

Figure 6: Pigmentation covers 25-50% of cape and pigmentation does not extend to dorsal fin (Codes A and B3) in rough-toothed dolphin HISb1082. Injury seen below midline is likely due to a cookie-cutter shark.

The individual photos were coded using an adapted method that involves subjective scoring of the photographs by one scorer who has experience with photo-ID of the species (Chen et al. 2018; Brown et al. 2015). Photographs taken in the Hawaiian Islands of rough-toothed dolphins were coded by quartiles (0-25%, 25-50%, 50-75%, 75-100%) based on 1) the cape cover pigmentation (the area of the animal directly beneath the dorsal fin), and separately, 2) dorsal fin cover pigmentation. The other coding was on a presence/absence basis. For instance, if an individual had a no dorsal fin pigmentation, and between 75-100% cape cover pigmentation, a 1 was noted for those two codes, with zeroes for the rest. This allowed for the calculation of the proportion of codes in each island (Hawai'i, O'ahu, and Kaua'i/Ni'ihau). After coding the photographs, analysis of codes based on location (near which island the encounters occurred) was conducted. Due to limited samples, only the codes for cape cover pigmentation and dorsal fin pigmentation were analyzed. A χ^2 test for independence was used as the primary statistical analysis, employing an α of 0.10 for statistical significance. R (R Core Team 2020) was used to conduct the χ^2 test, and the package 'corrplot' (Wei and Simko 2017) was used to plot results.

Chapter 4: Results

The number of *S. bredanensis* individuals in the different categories of cape cover pigmentation was not independent of the island association (Fig. 7, $\chi_4^2 = 74.75$, $p < 0.001$). The highest proportion of *S. bredanensis* individuals with 75%-100% cape cover pigmentation were found in Kaua'i/Ni'ihau (60.4% of individuals found there), with a lesser proportion at O'ahu (47%), and the smallest proportion at Hawai'i (42%). The highest proportion of individuals with 25-50% cape cover were found in Hawai'i (26.4%), then O'ahu (19%), then Kaua'i/Ni'ihau (9%).

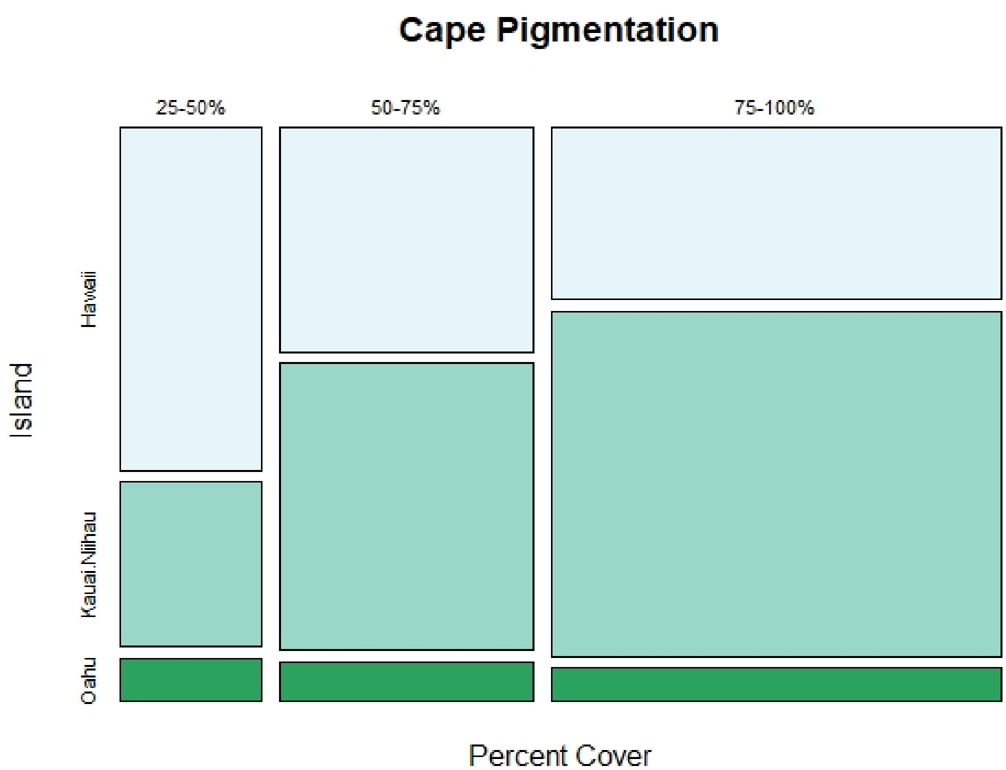

Figure 7: Cape cover pigmentation on *S. bredanensis*, represented as the proportion of individuals in each pigmentation category from Hawai'i ($n = 512$), Kaua'i/Ni'ihau ($n = 716$), and O'ahu ($n = 83$). All *S. bredanensis* individuals observed in this had at least 25% cape cover pigmentation, therefore the category '0-25%' is not shown.

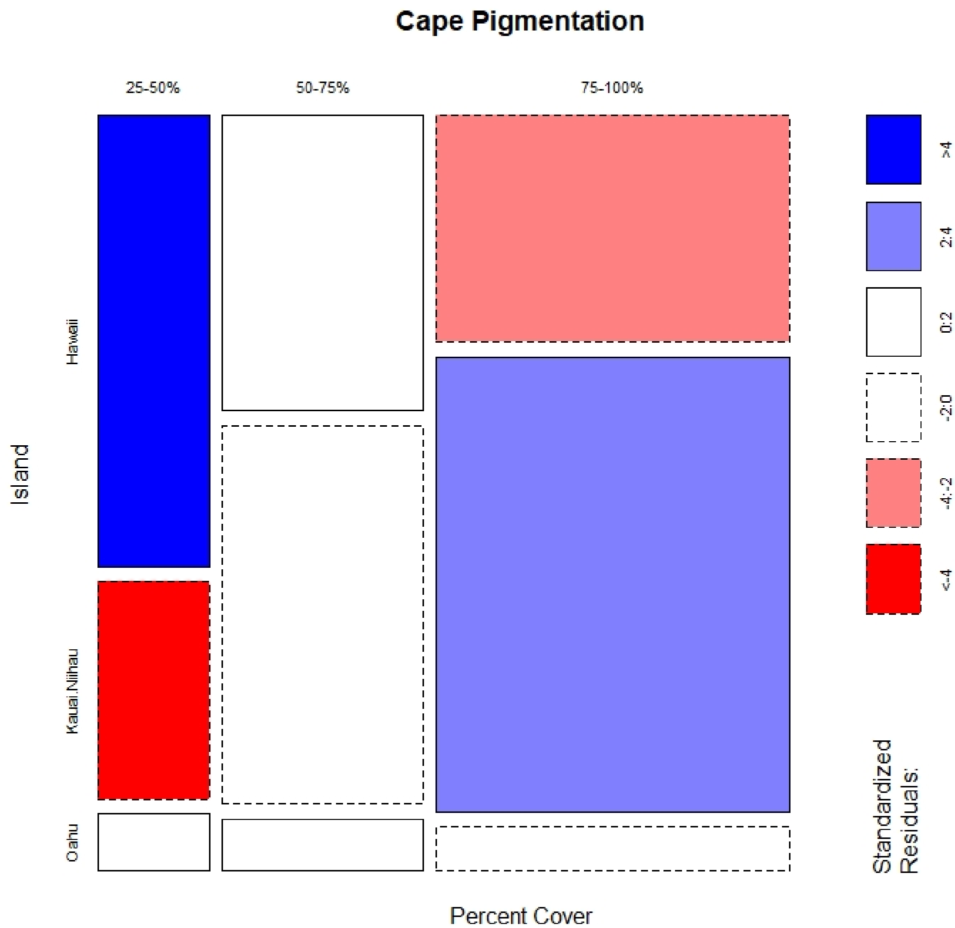

Figure 8: Standardized residuals from the χ^2 test of independence on cape cover pigmentation category and island of residence for *S. bredanensis*. Residuals measure the strength of the difference between observed and expected counts.

By examining the standardized residuals from the χ^2 test of independence, it is clear that the significant result is due to 1) more individuals from Hawai'i in the 25-50% pigmentation category, and more individuals from Kaua'i/Ni'ihau in the 75-100% category, than expected by chance alone, and 2) fewer individuals from Kaua'i/Ni'ihau in the 25-50% pigmentation category, and fewer individuals from Hawai'i in the 75-100% category, than expected by chance alone (Fig 8). Note that O'ahu had much fewer individual *S. bredanensis* photographed and the observed counts did not vary much from expected. In addition, the 0-25% cape cover pigmentation category is not shown because all *S. bredanensis* individuals observed had at least 25% of cape cover.

Dorsal Fin Pigmentation

The number of *S. bredanensis* individuals in the different categories of dorsal fin pigmentation was also not independent of the island association (Fig. 9, $\chi_6^2 = 10.66$, $p < 0.09$). O’ahu had the highest proportion of dorsal fins without pigmentation (49% of individuals found there), then Hawai’i and O’ahu at the same proportion (38%). For 25-50% pigmentation cover, all three island areas were very similar, with Hawai’i at the highest proportion (16%), then Kaua’i/Ni’ihau (15%), then O’ahu (11%). 50-75% dorsal fin pigmentation was the reverse of that, with O’ahu (8%), then Hawai’i (5%), then Kaua’i/Ni’ihau (4%), following the same order as the no visible pigmentation. Finally, there were very few 75-100% dorsal fin cover codes, and none at O’ahu. Hawaii had largest proportion of individuals with 75-100% pigmentation (0.8%), then Kaua’i/Ni’ihau (0.8%).

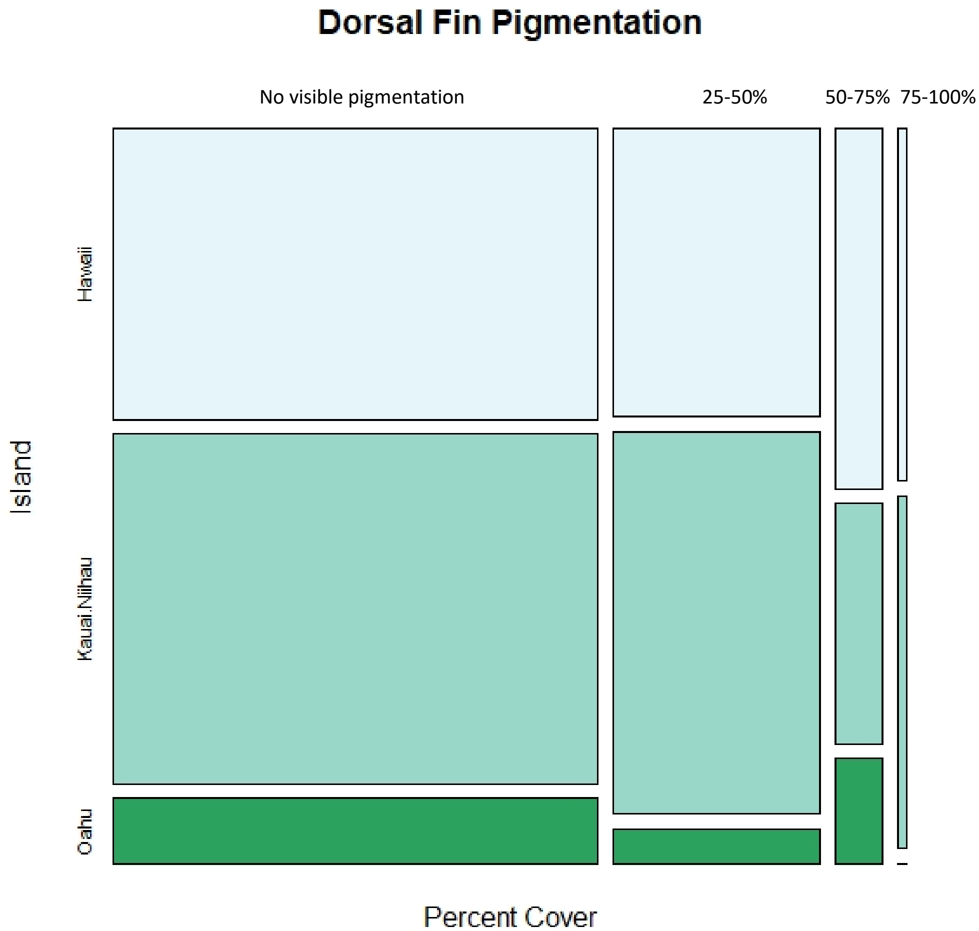

Figure 9: Dorsal fin pigmentation on *S. bredanensis*, represented as the proportion of individuals in each pigmentation category from Hawai'i (*n* = 512), Kaua'i/Ni'ihau (*n* = 716), and O'ahu (*n* = 83).

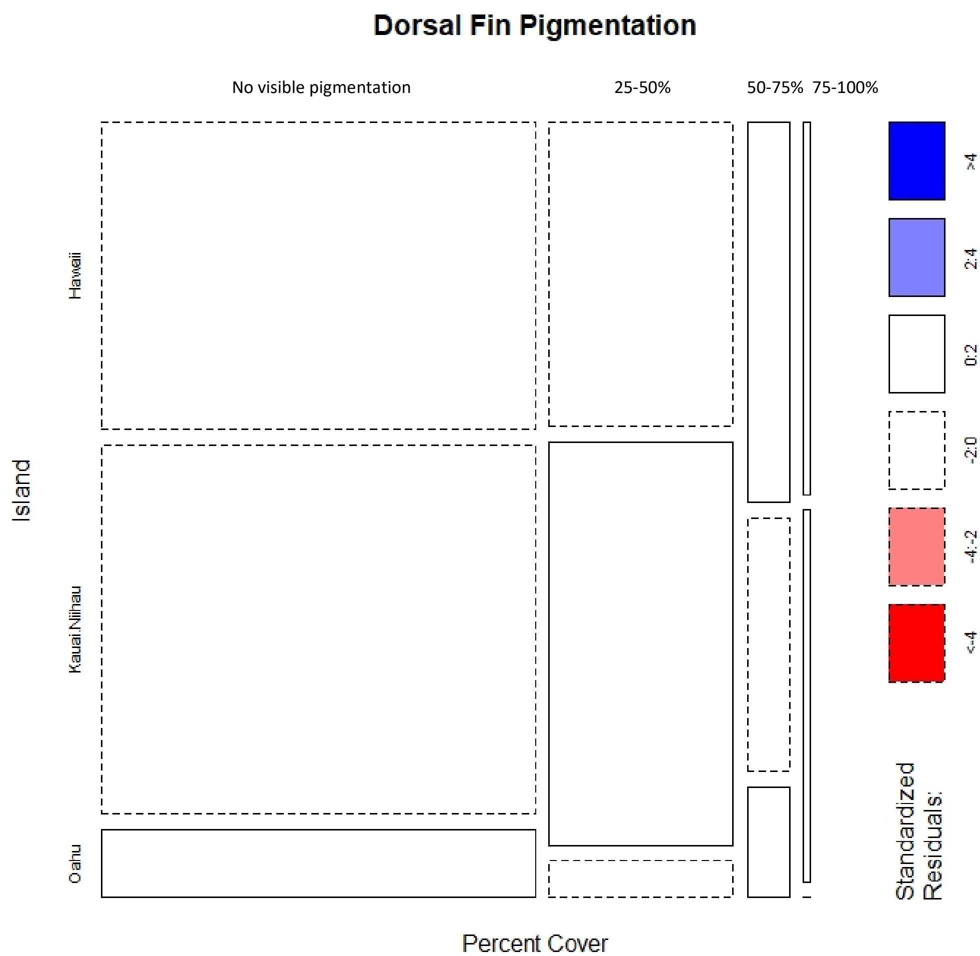

Figure 10: Standardized residuals from a χ^2 test on the relationship between dorsal fin pigmentation cover and island of residence for *S. bredanensis.* Residuals measure the strength of the difference between observed and expected counts.

The significant result for dorsal fin pigmentation is due to 1) less individuals from Hawai'i and Kaua'i/Ni'hau in the no visible pigmentation category than expected by chance alone (Fig 10). In addition, there are significantly fewer individuals from Hawai'i and O'ahu in the 25-50% pigmentation category than expected by chance alone (Fig 10). Finally, Kaua'i/Ni'ihau had less than expected individuals in the 50-75% dorsal fin cover category. For the 75-100% category there was a small number of individuals from all three islands (and none from O'ahu), and no significant differences in expected and observed counts.

Chapter 5: Discussion

Pigmentation patterns, along with dorsal fin notches, are used to identify individual rough-toothed dolphins to assess population sizes and site fidelity of individuals. When looking at a catalog of individual dolphins, having more information about what the markings might indicate could only be helpful for researchers. When looking at cape cover pigmentation and dorsal fin pigmentation, two defining characteristics of rough-toothed dolphins, there were significant differences by island grouping within the Hawaiian Islands.

Figure 11: Map of the Hawaiian Islands (U.S. Geological Survey 1996).

For cape cover pigmentation, an interesting pattern emerged. In terms of geographical distance, from east to west, the islands go Hawai’i, O’ahu, then Kaua’i/Ni’ihau (Figure 11). Two of the codes, 75-100% cover and 25-50% cover, followed a geographical pattern—that is, the largest proportion of codes for 75-100% cover were in Kaua’i/Ni’ihau, then O’ahu, then Hawai’i. The pattern was flipped for 25-50%, as well. This leads to the thought that if pigmentation does have a genetic component, and *S. bredanensis* has high site fidelity with limited genetic drift between islands, the largest differences in pigmentation would be between the furthest islands (Oremus et al. 2012).

Geography is a large predictor for genetic drift, especially between islands (Barton 1996). Due to the proximity of the islands, some genetic interchange makes sense, but differentiation may indicate both that the individuals exhibit high site fidelity and that there may be individual populations. The differences in cape cover pigmentation could indicate that the *S. bredanensis* population in the Hawaiian Islands are actually a few different genetically distinct populations, perhaps around Hawai'i, O'ahu, and the Kaua'i/Ni'ihau area. The results here show that looking at cape cover warrants more research.

For other cetaceans, geography as a predictor of different pigmentation forms has been observed previously. For instance, orca whale (*O. orca)* ecotypes in the Pacific Ocean, in addition to having unique body morphologies, have significantly different saddle patch pigmentation patterns, which is in a similar area of the body to the rough-toothed dolphin cape pigmentation (Emmons et al. 2017). Like rough-toothed dolphins, not only are the saddle patches individually unique, but geographically unique groups have patterns that are unique to that ecotype (Emmons et al. 2017). For orcas, ecotypes also include differences in food selection, and can represent much larger geographical distances than the Hawaiian Islands; however, this indicates that in other cetaceans, specific pigmentation patterns for distinct groups of the same species have been studied (Evans et al. 1982). The significance of pigmentation patterns and their connection to islands in *S. bredanensis* shows that even though the distance is small, differentiation may be occurring in this population.

However, the other code observed, dorsal fin pigmentation, did not follow this same pattern. There were significant differences in which islands had the highest percentage of fin cover as with cape cover. As a whole, there was less pigmentation on the dorsal fin than on the cape area of the dolphin. It was not uncommon to see capes with greater than 75% cover, but that was rare for dorsal fin cover, and overall, the percentages were smaller. The dorsal fins had more barriers to coding accurately as well, because light and reflection off the water often made the photos more difficult to discern for pigmentation cover.

In dorsal fin pigmentation, there were significant differences in codes for no visible pigmentation (0-25%) and 25-50% cover (Figure 9). O’ahu had the highest percentage of 0-25% pigmentation, and Kaua’i/Ni’ihau as well as Hawai’i were close to the same percentage. The dorsal fin proportions did not follow the same pattern as the cape cover pigmentation, so more research would be helpful to determine what these differences might indicate. The fact that there are significant differences could indicate that, similarly to the cape cover, there are differences in pigmentation between the three island populations studied here.

Differences in dorsal fin pigmentation related to distance have also been seen in Australian humpback dolphins, over a smaller overall distance than most studies of pigmentation in dolphins (Brown et al. 2015). Though the populations in that study encompassed a larger distance than the rough-toothed dolphins in Hawai’i, significant differences in dorsal fin pigmentation are present in both species (Brown et al. 2015). Dorsal fin pigmentation differences by population in both Australian humpback and rough-toothed dolphins indicate that other delphinids could have pigmentation differences as well. In addition, the fact that the Hawaiian rough-toothed dolphins show significant differences despite geographically close islands is a strong indication that pigmentation is a characteristic that differentiates rapidly. More in-depth research could provide more information about rough-toothed dolphins and pigmentation, particularly genetic research. Obtaining enough data to compare dolphins that have both been photographed and gene sequenced will take significant research effort but would result in greater clarity on the connection between unique pigmentation patterns and genetics.

In this study, the sample of quality photos was small, especially of *S. bredanensis* individuals from O'ahu. There were only 83 photos that were able to be coded in O'ahu, compared with 512 individuals in Hawai'i and 716 individuals in Kaua'i/Ni'ihau. With better cameras and more research effort, there will be more high-quality photos that can be coded. In addition, being able to use more precise software to estimate percent cover would also be helpful and may reinforce the significant differences that were found here more clearly. As well, any pigmentation differences that are genetic would be more pronounced between populations that are farther apart by distance—for instance, rough-toothed dolphins from Mo'orea and Raiatea compared with the dolphins in the Hawaiian Islands. Comparing photographs from those areas may either clarify or further muddy the question of are these pigmentation patterns genetic and are there specific patterns or frequency of patterns associated with some islands.

In addition, there are many other characteristics that could be intriguing to link with pigmentation. When photographing rough-toothed dolphins, it is sometimes possible to determine neonates, that is young individuals, and connect them with their mothers. Seeing if there are similarities between neonates and their mothers in pigmentation could provide more clarity as to whether pigmentation patterns are genetic. If both the mother and neonate have 25-50% cape cover, for instance, that could indicate a strong genetic tie to pigmentation. In addition, comparing pigmentation patterns between individuals with known gender could illuminate more about pigmentation, and if pigmentation is significantly different between males and females within the same population.

For delphinids, the fact that at least two pigmentation patterns are variable by location opens up many avenues of research. Many cetaceans, particularly dolphins of a similar size to rough-toothed dolphins, have pigmentation in both the cape and dorsal fin area. Because photo-identification is such a ubiquitous research method, there are many photos that can be coded in a way similarly to how they were coded here. As mentioned earlier, delphinids that have populations in geographically distant locations could provide insight into whether what the data showed here related to cape cover and dorsal fin pigmentation is true for different delphinid species.

Chapter 6: Conclusion

Pigmentation is one of the most visible and accessible metrics used to identify and compare rough-toothed dolphins. In the population in the Hawaiian Islands, pigmentation has been used to identify individual rough-toothed dolphins by Cascadia Research since 2005 to track population movement and conduct research. Coding quality photos for specific types of pigmentation, including cape cover pigmentation and dorsal fin pigmentation, showed some significant differences by island associated with (Hawai’i, Kaua’i/Ni’ihau, O’ahu). However, due to the limited quantity of photographs that could be coded, the results only provide a window into how pigmentation is connected to location.

In Hawaiian rough-toothed dolphins, both dorsal fin pigmentation and cape cover pigmentation were not independent of island association. The percent pigmentation cover varied more than would have been expected by chance alone, for both patterns, between the three island areas. In a species with documented high site fidelity, finding significant differences between locations in a key physical characteristic, pigmentation, indicates a lot about potential population differentiation between the three island areas. Despite the Hawaiian Islands occupying a relatively small geographical space, rough-toothed dolphins from O’ahu, Hawai’i, and Kaua’i/Ni’ihau have pigmentation characteristics that differ between these areas, which, with more research, could be used in the future to predict community associations, and potentially populations.

References

Albertson, G. R. (2012). *Worldwide Phylogeography and Local Population Structure of the Rough-Toothed Dolphin* (Steno bredanensis). Ph.D Oregon State University.

Barton, N. H. (1996). Natural selection and random genetic drift as causes of evolution on islands. *Phil. Trans. R. Soc. Lond. B., 351*, 785–795.

Baird, R. W. (2016). The lives of Hawaii's dolphins and whales: natural history and conservation. *University of Hawai'i Press, Honolulu, HI*.

Baird, R. W., & Stacey, P. J. (1988). Variation in saddle patch pigmentation in populations of killer whales (*Orcinus orca*) from British Columbia, Alaska, and Washington State. *Canadian Journal of Zoology*, *66*, 2582–2585.

Baird, R. W., Mcsweeney, D. J., Webster, D. L., Gorgone, A. M., & Ligon, A. D. (2003). Studies of odontocete population structure in Hawaiian waters: Results of a survey through the main Hawaiian Islands in May and June 2003. *Report Prepared under Contract# AB133F-02-CN-0106*.

Baird, R. W., Webster, D. L., Mahaffy, S. D., McSweeney, D. J., Schorr, G. S., & Ligon, A. D. (2008). Site fidelity and association patterns in a deep-water dolphin: Rough-toothed dolphins (*Steno bredanensis*) in the Hawaiian Archipelago. *Marine Mammal Science*, *24*(3), 535–553.

Bichell, L. M. V., Krzyszczyk, E., Patterson, E. M., & Mann, J. (2017). The reliability of pigment pattern-based identification of wild bottlenose dolphins. *Marine Mammal Science*, *34*(1), 113-124.

Brown, A., Bejder, L., Parra, G., Cagnazzi, D., Hunt, T., Smith, J., & Allen, S. (2015). Sexual Dimorphism and Geographic Variation in Dorsal Fin Features of Australian Humpback Dolphins, *Sousa sahulensis*. *Advances in Marine Biology*, *73*, 273-314.

Caro, T., Beeman, K., Stankowich, T., & Whitehead, H. (2011). The functional significance of colouration in cetaceans. *Evolutionary Ecology*, *25*, 1231-1245.

Chen, B., Jefferson, T.A., Wang, L., Gao, H., Zhang, H., Zhou, Y., Xu, X., Yang, G. (2018). Geographic variation in pigmentation patterns of Indo-Pacific humpback dolphins (Sousa chinensis) in Chinese waters. *Journal of Mammalogy*, *99*(4), 915-922.

Emmons, C., Hard, J., Dahlheim, M., Waite, J. (2018). Quantifying variation in killer whale (*Orcinus orca*) morphology using elliptical Fourier analysis. *Marine Mammal Science*, *35* (1), 5-21.

Evans, W., Yablokov, A., & Bowles, A. (1982). Geographic variation in the color pattern of killer whales (*Orcinus orca*). *Reports of the International Whaling Commission. 32*, 687–694.

Forney, K. A., Southall, B. L., Slooten, E., Dawson, S., Read, A. J., Baird, R. W., & Brownell, R. L. (2017). Nowhere to go: Noise impact assessments for marine mammal populations with high site fidelity. *Endangered Species Research*, *32*(1), 391–413.

Gendron, D., & De la Cruz, A. U. (2012). A new classification method to simplify blue whale photo-identification technique. *Journal of Cetacean Resource Management*, *12*(1), 79–84.

Gillespie, R.G., Claridge, E.M. and Roderick, G.K. (2008). Biodiversity dynamics in isolated island communities: interaction between natural and human-mediated processes. *Molecular Ecology*, *17*, 45-57.

Hammond, P. S., Mizroch, S., & Donovan, G. (1990). Individual Recognition of Cetaceans: Use of Photo-Identification and Other Techniques to estimate Population Parameters. *Reports of the International Whaling Commission (Special Issue 12)*.

Hoelzel, A. R., & Dover, G. A. (1991). Genetic differentiation between sympatric Killer whale populations. *Heredity*, *66*(2), 191–195.

Koopman, N. H., & Gaskin, D. E. (1994). Individual and geographical variation in the pigmentation patterns of the harbour porpoise, *Phocoena phocoena*. *Canadian Journal of Zoology*, *72*, 135–143.

Miyazaki, N., Perrin, W.F., (1994). Rough-toothed dolphin *Steno bredanensis* (Lesson, 1928). In: Ridgway, S.H., Harrison, R. (Eds.), Handbook of Marine Mammals. Academic Press, San Diego, CA. 1–21.

Oremus, M., Poole, M. M., Albertson, G. R., & Baker, C. S. (2012). Pelagic or insular? Genetic differentiation of rough-toothed dolphins in the Society Islands, French Polynesia. *Journal of Experimental Marine Biology and Ecology*, *432–433*, 37–46.

Oremus, M., Poole, M. M., Steel, D., & Baker, C. S. (2007). Isolation and interchange among insular spinner dolphin communities in the South Pacific revealed by individual identification and genetic diversity. *Marine Ecology Progress Series*, *336*, 275–289.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Roman, J., Estes, J.A., Morissette, L., Smith, C., Costa, D., McCarthy, J., Nation, J., Nicol, S., Pershing, A. and Smetacek, V. (2014), Whales as marine ecosystem engineers. *Frontiers in Ecology and the Environment*, *12,* 377-385.

Rosso, M., Moulins, A., & Würtz, M. (2008). Colour patterns and pigmentation variability on striped dolphin *Stenella coeruleoalba* in north-western Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom*, 88(6), 1211-1219.

Sugarman, P. (1984). *Field guide to the orca whales of greater Puget Sound and southern British Columbia*. Whale Museum (Moclips Cetological Society).

U.S. Geological Survey. (1996). Map of Hawaiian Islands (edited by Geographic Guide – Travel). http://www.geographicguide.com/oceania-maps/hawaii.htm

Wei, T & Simko, V. (2017). R package "corrplot": Visualization of a Correlation Matrix (Version 0.84). Available from https://github.com/taiyun/corrplot.