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Describing the Ecology of Cryptic Marine Megafauna and the Threats to their Survival

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Describing the Ecology of Cryptic Marine Megafauna and the Threats to their Survival

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by

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Thank you for supporting me in all that I do, Mom, Clair, Heather, Chris, Bella, Sophie, and Brett. Professora Carita Feliz: you’re the best. I love you all.

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ABSTRACT

Describing the Ecology of Cryptic Marine Megafauna and the Threats to their Survival

by

Lindsey Eleanor Peavey

Marine megafauna are large, long-lived, highly mobile, and feed below the surface, making much of their ecology mysterious to humans. They are also exposed to a number of human-caused threats of varying magnitude across their ranges, which are of particular concern for endangered species. Because of their cryptic nature, quantitative estimates such as their roles as consumers across disparate oceanic food webs are lacking. I use multiple non-invasive approaches such as stable isotope analyses, genetics, expert surveys, and cumulative impacts modeling to describe the ecology and conservation priorities of sea turtles and marine mammals for case-study populations that lack estimates. In my dissertation, I discuss how my findings enhance our understanding of megafauna ecology, and how these integrative approaches may advance the ways in which we prioritize research and management strategies to meet population recovery objectives.

I. Olive Ridley Sea Turtle Opportunistic Generalism and Oceanic Foraging Habitat Inferred from Stable Isotopic Compositions in the Eastern Pacific Ocean

Nitrogen stable isotopic compositions have been widely used to gain insight into trophic dynamics, especially of highly mobile aquatic predators whose behavior and dietary preferences are difficult to directly observe and measure. Olive ridley sea turtles (*Lepidochelys olivacea*) are oceanic consumers distributed across >3 million km^2 of the tropical and subtropical eastern Pacific Ocean, and their open ocean trophic ecology has not yet been empirically described. Individuals migrate through and feed within biogeographic regions where varying nutrient cycling regimes result in phytoplankton with distinct $\delta^{15}N$ values that are inherited by the turtles. Here, we have used bulk tissue and compound specific isotopic results to demonstrate that olive ridleys in the eastern Pacific are opportunistic omnivores, and we hypothesize that their trophic role remains constant with ontogenesis. We observed some variation in niche structure across food webs, but little variation in median trophic position (~3.15±0.26) across the entire eastern Pacific seascape, revealing that during their 2006 breeding season olive ridleys occupied the same generalist consumer function across their range. The relatively productive region of the Costa Rica Dome was the only exception; during our study's time frame individuals fed at a trophic position approximately 0.36 (median) higher there than in any other oceanographic sub-region sampled. This suggests that in high productivity areas, like the Costa Rica Dome, intra-individual variation can decrease. Whereas generalism and flexibility in foraging, nesting, and migration (i.e., lack of precise migrating corridors) suggest olive ridleys in the eastern Pacific may be resilient to the impacts of climate change, their low metabolism, narrow thermal niche and temperature-dependent sex determination suggest they may still be quite vulnerable to environmental change unless they are able to undergo adaptation.

A. Introduction

Mobile marine consumers can have differing and variable foraging strategies. Generalists opportunistically feed upon a dynamic array of items across space and time, and often from multiple basal nutrient and/or trophic levels (omnivory). Prey and/or foraging habitat selection can be determined by a number of influences on phenotype, physiology, or community structure. Examples include intra- and interspecific competition, prey species composition and prey availability, energy requirements, imminent threats, chemical cues (Hay, 2009), environmental shocks, nutrient pulses or shifts, and human exploitation of the consumer, its prey, and/or its competitors. Specialists feed on a small number of prey items and are subject to the same selection determinants as generalists, but have developed morphological and/or behavioral traits that restrict their foraging plasticity. Both types of consumers are important in regulating food web dynamics such as complexity (Kondoh, 2003; Gellner and McCann, 2011), nutrient cycling (Kitchell et al., 1979; McManamay et al., 2011), and stability (Vandermeer, 2006; Alcántara and Rey, 2012). A consumer's trophic role in an ecosystem can change across space (e.g., habitat) and time (e.g., with growth) due to aforementioned influences, or it can remain constant despite those influences. Estimated tropic position of a consumer within a food web combined with other aspects of its ecology (e.g., niche width, mobility, fasting) can provide evidence to infer foraging strategy.

Stable isotopic compositions have been used extensively to gain insight into the trophic ecology especially of highly mobile aquatic predators whose behavior and dietary preferences are difficult to directly observe and measure (Fry, 1988; Olson et al., 2010). Carbon and nitrogen isotopic compositions vary across ecosystems and are tracers of metabolic and biogeochemical processes. Reactions and trophic transfers alter the ratio of rare to common isotopes in environments and organisms, known as isotopic fractionation. For example, nitrogen that is integrated and stored in consumer tissues is enriched in ^{15}N relative to its food, a trophic discrimination that reverberates through an entire food web such that top predators have the highest $^{15}N/^{14}N$ ratios (e.g., $\delta^{15}N$ values) (DeNiro and Epstein, 1981; Fry, 1988; Cabana and Rasmussen, 1996). Trophic discrimination is reasonably predictable and can be accounted for with a discrimination factor (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Bradley et al., 2014). If examining multiple consumers within the same aquatic food web over a given time period, trophic discrimination is the major form of nitrogen variation that requires consideration. However, different nitrogen cycling regimes across different regions of the same habitat or in different aquatic ecosystems (lake, river, estuary, salt marsh, seagrass, coral reef, kelp forest, upwelling zones, open ocean, etc.) result in variation in phytoplankton or baseline $\delta^{15}N$ values (Hobson, 1999; Vander Zanden and Rasmussen, 2001), as reflected in the dissolved nitrogen pools of resources feeding the primary producers (Peterson and Fry, 1987). Thus, spatially discrete, local food webs with measurably divergent basal $\delta^{15}N$ values are isotopically distinct—natural variation that and can be exploited for comparative landscape-scale (100s-1000s+ km) ecological studies (Hobson et al., 2012). In practice, $\delta^{15}N$ values of the same tissue from several individuals of the same consumer population across multiple food webs allows for characterization of local nitrogen sources and trophic roles (Thomas and Crowther, 2014). It is also possible to describe variation of $\delta^{15}N$ values (or another stable isotope) over any time scale of interest in one discrete food web, or across several food webs.

For large-scale studies, it is important to consider both natural variations of basal $\delta^{15}N$ values as well as variation across trophic levels. Often trophic studies using isotopic compositions measure $\delta^{15}N$ and $\delta^{13}C$ values in “bulk” (whole) tissue. Bulk values reflect a composite view of isotopes assimilated into a tissue, one that combines the ratios stored in all component organic compounds (e.g., protein amino acids) that make up that tissue. Many marine studies have used bulk tissue isotopic compositions to infer the diet composition, niche width and/or trophic interactions of cryptic consumers among diverse taxa (e.g., fish (Jennings et al., 2002; Cunjak et al., 2005), sharks (Estrada et al., 2003; Carlisle et al., 2012), seabirds (Thompson et al., 2015), sea turtles (Vander Zanden et al., 2010; Lemons et al., 2011; Allen et al., 2013), mammals (Burns et al., 1998; Hobson and Schell, 1998; Schell, 2000), and squid (Ruiz-Cooley and Gerrodette, 2012). Niche width is *n*-dimensional hypervolume that describes the ecological space a consumer occupies in a food web based on habitat and prey choices (Hutchinson, 1957), and is a particularly accessible description of a species’ position within and across food webs.

A consumer’s isotopic niche width characterizes carbon and nitrogen integrated from diet based on $\delta^{15}N$ and $\delta^{13}C$ values (Hutchinson, 1957; Carrascal et al., 1994; McDonald, 2002; Newsome et al., 2007; Yeakel et al., 2016). Bearhop et al. (2004) first introduced isotopic niche analysis as a robust approach to estimate niche width, as substitute for, or compliment to, conventional, often invasive, dietary analyses. While such bulk tissue isotopic studies are quite useful and have made substantial headway in filling trophic ecology knowledge gaps for mobile marine consumers, they are constrained by the inability to identify the root of variation in bulk tissue $\delta^{15}N$ and $\delta^{13}C$ values, particularly across a broad geographic range that may experience varying nutrient cycling regimes (Bowes & Thorp 2015).

Amino acid compound-specific nitrogen isotopic analyses (AA-CSIA) can be used to overcome the constraints of bulk tissue analysis. There are two types of amino acids, ones whose $\delta^{15}N$ values change little with trophic transfers and record the nitrogen isotopic baseline, known as "source" amino acids (e.g., phenylalanine), and ones whose $\delta^{15}N$ values increase with trophic transfers (i.e., trophic enrichment) and reflect the consumer's trophic position within a food web, known as "trophic" amino acids (e.g., glutamic acid) (McClellan and Montoya, 2002; Popp et al., 2007). $\delta^{15}N$ values of the two types of amino acids therefore can provide information at a finer resolution, sufficient for parsing influences of source nitrogen and trophic status, beyond the capabilities of bulk tissue analysis alone (McClellan and Montoya, 2002; McCarthy et al., 2007; Popp et al., 2007; Chikaraishi et al., 2009; Lorrain et al., 2009; Chikaraishi et al., 2010). The geographic variability among primary producers (i.e., source values) coupled with N cycling information (e.g., N* (Pennington et al. 2006), or nitrogen isoscapes (Olson et al. 2010)) can help identify the geographic foraging location of mobile consumers. Being able to distinguish between these two sources enables researchers to understand if variation in bulk $\delta^{15}N$ values in consumer tissue is explained by foraging location or strategy.

For example, only after measuring both bulk values and completing AA-CSIA, Seminoff et al. (2012) was able to conclude that the variation of $\delta^{15}N$ in Pacific leatherback sea turtle (specialists) skin could be attributed to the different baseline δ^{15}N values of the eastern versus western foraging regions of the Pacific Ocean. Vander Zanden et al. (2013) used these complimentary analyses to reveal that although Caribbean green sea turtles (*Chelonia mydas*) of the same breeding population feed in several different foraging aggregations with different bulk primary producer and consumer δ^{15}N values, they maintain the same foraging strategy (i.e., trophic position) in each. These examples and others (e.g., yellowfin tuna: Lorrain et al. 2015) demonstrate the efficacy of comparing different cryptic foraging subpopulations of the same turtle species to learn how their trophic status does or does not change over time and space.

Likewise, here we apply bulk stable isotope analysis and AA-CSIA to an elusive open ocean forager whose trophic ecology has not yet been empirically investigated. Olive ridley sea turtles (*Lepidochelys olivacea*) foraging in the eastern Pacific provide an ideal case study because individuals forage across an impressively large range spanning over 3 million km^2 (Olson et al., 2010) and in many distinct biogeographic regions with different baseline δ^{15}N values (e.g., in Longhurst provinces 5, 7, 8, 35, 36, & 37 (VLIZ, 2009)) (Pennington et al., 2006). The eastern region of the tropical and subtropical Pacific Ocean is oceanographically dynamic but has persistent and predictable areas of upwelling, warm pools, cold tongues, and boundary currents (Fiedler and Talley, 2006) that make up spatially explicit food webs. Thus, δ^{15}N isotopic values are systematically distributed across the region's oceanographic features reflecting their different nutrient cycling regimes, such as nitrogen fixation and denitrification.

Olive ridleys are listed as "vulnerable" on the International Union for Conservation of Nature's Red List of Threatened Species (Abreu-Grobois and Plotkin, 2008) as their abundance is *at least* an order of magnitude lower than their historical abundance. Individuals in the population span the waters of ten countries in Pacific Latin America, each having jurisdiction over the management and protection of individuals that occur within their state boundaries. Olive ridleys in the eastern Pacific were commercially overharvested to collapse in the 1960's but have since received considerable conservation attention, especially on nesting beaches, and subsequent significant increase in population size (Plotkin et al., 2012). The eastern Pacific olive ridley breeding population is of particular conservation concern currently because of the high incidences of interaction with fishing gear (Dapp et al. 2013, Lewison et al. 2014).

Olive ridleys are presumed opportunistic generalist omnivores that can feed in both benthic and pelagic habitats (Bjorndal, 1997; NMFS and USFWS, 1998; Robins et al., 2002; Whiting et al., 2007; Wedemeyer-Strombel et al., 2015). From approximately June through December females nest on eastern Pacific beaches from Mexico (Lopez-Castro & Rocha-Olivares 2005) south to Ecuador, with sporadic nesting in Peru (Lopez-Castro and Rocha-Olivares, 2005; Kelez et al., 2009). Although this breeding population is one of the more abundant sea turtle populations in the world, estimated at 1.39 million individuals (Eguchi et al., 2007), we have little empirical information regarding individual foraging behavior because apart from when nesting, they spend their lives in the open ocean where sampling is challenging (Polovina et al., 2003; Plotkin, 2010). Individuals of both sexes and all ages are understood to be nomadic and dispersed across the eastern Pacific anywhere from a few meters (neritic) up to thousands of kilometers (pelagic/oceanic) from shore (Plotkin, 2010); presumably foraging at-will with recorded maximum dive depth of 200 m and duration of 180 min for the species (Polovina et al., 2003; McMahon et al., 2007; Whiting et al., 2007).

In the western Pacific, telemetry evidence has suggested that at least some olive ridleys show fidelity to patchy but predictable suitable foraging habitat (Whiting et al. 2007).

The seemingly divergent foraging behavior of two isolated breeding populations of the same species provokes several questions regarding the magnitude of individual variation, and what it could reveal about the ecologic role this generalist plays in disparate ocean basins and in distinguishable habitats, both benthic and oceanic. Such observed individual variation for mobile marine consumers has been described elsewhere as individual specialization (Bolnick et al., 2002), including for sea turtles (Vander Zanden et al., 2010; Thomson et al., 2012). In this study, we quantitatively describe niche width variation and estimate the trophic positions of adult olive ridleys foraging in disparate oceanic food webs in the eastern Pacific over a large geographic scale. We discuss our findings as they relate to persistent but dynamic oceanic foraging habitats, conservation implications of potentially unique open-ocean foraging areas, and the olive ridley's resiliency to climate and prey changes.

B. Materials and Methods

1. Study Region and Sub-regions

The study region spans the tropical and subtropical eastern Pacific Ocean, extending from ~30° N (Gulf of California) to ~12° S (Peru Current), and ~76° W (west coast of the Americas) to ~115° W. We examined isotopic variation of olive ridley aggregations according to two different theoretical ways to partition isotopic niches, one based on biogeochemical Longhurst provinces (VLIZ 2009), and one based on the distinct and persistent oceanographic features described in Fiedler & Talley (2006). We grouped samples into three Longhurst provinces, 7 ("Coastal – Central American Coastal Province"), 8

(“Coastal – Chile-Peru Current Coastal Province”), and 35 (“Trades – North Pacific Equatorial Countercurrent Province”) (VLIZ 2009) (Figure 1a). Alternatively, we grouped samples into five distinct sub-regions based on the following oceanographic features: the Gulf of California (GC), the North Equatorial Current (NEC), the Eastern Pacific Warm Pool (EPWP), the Costa Rica Dome (CRD), and the Peru Current (PC) (Figure 1b). Spatial analyses across sub-regions were limited to individuals within the boundaries under our assumption that sub-regions represent unique food webs with varying basal levels of isotopic resources.

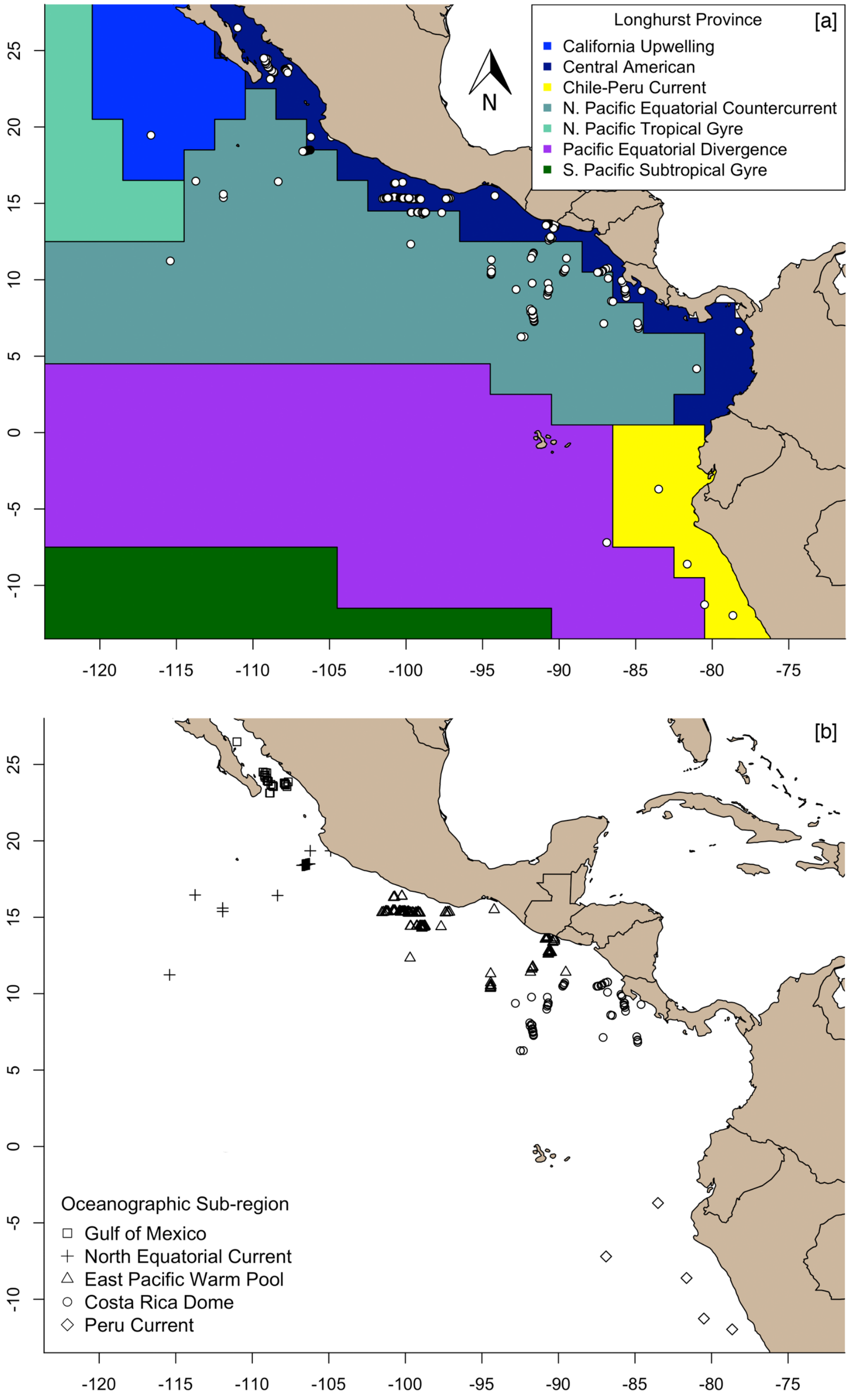

Figure 1. Adult olive ridley turtles samples represented as symbols and aggregated according to [a] Longhurst province (VLIZ 2009) and [b] oceanographic region (Fiedler & Talley 2006).

2. Sample Collection and Preparation

Between August and December 2006, olive ridley sea turtles were opportunistically hand-captured from the National Oceanographic Atmospheric Administration R/V *David Starr Jordan* during the *Stenella* Abundance Research (STAR) cruise. Life history data and tissue samples were collected from each turtle before their release. Morphometric information was collected for all turtles, and putative sex was recorded for mature individuals (approximately straight carapace length ≥ 56 cm) based on external morphology—reproductively mature male turtles have long tales, females have short tales. For individuals sampled within the GC (n = 29), NEC (n = 36), EPWP (n = 192), and CRD (n = 63) sub-regions [alternatively: Longhurst province 7 (n = 172), Longhurst province 8 (n = 21), and Longhurst province 35 (n = 151)], epidermis ("skin") samples were collected using a razor blade from the dorsal neck surface and immediately frozen at -80°C, later transported on ice and archived at the Southwest Fisheries Science Center (La Jolla, CA, U.S.A.). Each skin sample was split for dual archiving (stable isotopes and genetics) and stored at -20°C until laboratory analysis. All turtles were released unharmed in roughly the same location where they were captured.

Prior to stable isotope measurement, samples were thawed and rinsed with distilled water, freeze dried for one 8-hour cycle, and lipid-extracted using an Accelerated Solvent Extractor (ASE 200) according to previously published methods (Lemons et al. 2011, Allen et al. 2013). Whole skin samples (~2mm in length) were split for two types of measurements: bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values, and compound specific nitrogen isotope analysis of amino acids (AA-CSIA). All samples were analyzed for bulk tissue stable isotope values, however due to cost and time restraints, only 4-14 samples in each sub-region were processed for AA-CSIA.

Olive ridleys in the Peru Current were sampled opportunistically during a different sampling effort that took advantage of turtles incidentally captured by Peruvian longline fishing vessels. Using a 2-mm biopsy punch, skin samples were taken from the dorsal neck surface of adult olive ridleys in 2003 (n = 3), 2004 (n = 5), 2008 (n = 10), and 2009 (n = 4), preserved with salt, and archived at -20°C at the Southwest Fisheries Science Center (La Jolla, CA U.S.A.) until laboratory analysis. These samples were lipid extracted and analyzed for bulk tissue stable isotope values as described in Kelez (2011) and Arthur et al. (2014); AA-CSIA was completed for five samples from 2008 and 2009 using the same methods described below and in Arthur et al. (2014).

3. Bulk Tissue Analysis

For GC, NEC, EPWP, and CRD 2006 samples (n = 320), 0.7-1 mg of skin was homogenized with a razor blade and loaded into tin capsules. Samples were analyzed by a Costech Instruments elemental combustion system (ECS4010) coupled to a continuous-flow Thermo Finnigan MAT Delta Plus XL isotope ratio mass spectrometer in the Stable Isotope Laboratory at the University of Florida, Gainesville in 2011. Bulk isotope values are reported in standard delta notation (δ) in parts per thousand (‰): $\delta^{H}X = ([R_{\text{sample}}/R_{\text{standard}}] - 1)(1000)$, where the superscript 'H' is the mass of the rare isotope, '*X*' is the element of interest, and *R* is the ratio of the rare *X* isotope to the common *X* isotope (Fry 2006). $R_{standard}$ were air (atmospheric N_2) and Vienna Pee Dee Belemnite (VPDB) for $\delta^{15}N$ and $\delta^{13}C$, respectively. Continuous calibration was completed using USGS40 (L-glutamic acid: $\delta^{15}N = -4.52‰$ and $\delta^{13}C = -26.39‰$) with an average precision of 0.07‰ for $\delta^{15}N$ and 0.10‰ for

$\delta^{13}C$. To ensure accuracy, 1-3 blind sample duplicates were run per 30 samples with an average standard deviation of 0.14‰ for $\delta^{15}N$ and 0.27‰ for $\delta^{13}C$.

4. AA-CSIA

Of the 320 samples from 2006 used for bulk tissue stable isotope analysis, a subset of 26 2-10mg samples were dried and homogenized with a mortar and pestle and/or razor blade. Samples making up the subset were chosen to cover the widest geographic area of each sub-region: GC (n = 6); NEC (n = 6); EPWP (n = 10); CRD (n = 4); and alternatively Longhurst province 7 (n = 14), Longhurst province 8 (n = 5), and Longhurst province 35 (n = 12).

Samples were prepared (hydrolysis and derivatization) and analyzed for compound-specific isotopic composition of amino acids at the Biogeochemical Stable Isotope Laboratory at the University of Hawaii at Manoa using the instruments and protocols detailed in Décima *et al.* (2013). Samples were hydrolyzed with 0.5ml of 6N HCl and heated for 70 minutes at 150ºC. They were then dried with N_2 at 55ºC. Samples were re-dissolved with 1ml of 0.01N HCl and filtered using VWR 25mm Polyethersulfone filters with 0.2µm pore size. Amino acids were eluted with 4ml 2N NH_4OH and dried with N_2 at 80ºC. Cation exchange was then performed using Grace Alltech GracePure solid phase extraction cation 6mL columns (Grace ALLTECH #5141488, now discontinued).

Next, 0.5ml of 0.2N HCl were added to each sample and heated at 110ºC for 5 minutes, then dried with N_2 at 55ºC. 2.5ml of 4:1 C_3H_8O and CH_3COCl mixture were added to each sample and heated at 110ºC for 60 minutes for esterification, then dried with N_2 at 60ºC. 600µl CH2Cl2 and 200µl $C_4F_6O_3$ were added to each sample and heated at 100ºC for 15 minutes for trifluoroacetylation. 2ml of Phosphate-buffer (KH_2PO_4 + H=Na_2HPO_4 in Milli-Q water, potential Hydrogen [pH] 7) and 1ml of $CHCl_3$ were added to N_2-dried samples, and shaken for one minute to isolate amino acids into the $CHCl_3$ fraction. Phosphate-buffer and $CHCl_3$ fractions were separated via a 600g centrifuge for 5 minutes. The $CHCl_3$ layers were retained. Amino acid isolation was repeated to separate any residual amino acids remaining in the Phosphate-buffer fractions. $CHCl_3$ was evaporated with N_2 and then trifluoroacetylation was repeated. Samples were frozen at -20ºC until analysis.

Just before analysis each sample was dried with N_2 at room temperature and 250-500µl of $C_4H_8O_2$ was added. $\delta^{15}N$ isotopic compositions of 13 amino acids (alanine, glycine, valine, serine, leucine, isoleucine, proline, glutamic acid, phenylalanine, lysine, tyrosine, and norleucine (Nor) and aminoadipic acid (AAA) measured against internal Nor/AAA standards) in each sample were identified and quantified in triplicate using a mass spectrometer (Thermo Scientific Delta^{Plus}V or MAT 253 interfaced with a Trace GC/GCIII) (see Hannides et al. 2009 for further mass spectrometry details). Every block of three sample measurements was bookended by a suite of amino acids with known $\delta^{15}N$ (alanine, threonine, isoleucine, proline, glutamic acid, and phenylalanine). Suite/samples were co-injected with norleucine (Nor) and aminodipic acid (AAA) of known $\delta^{15}N$ to serve as internal standards and control for errors due to sample loss, injection variations, and variability in dilution preparations. Injections consisted of 1ul air, the appropriate volume of Nor/AAA standard, another 1ul of air, followed by the appropriate volume of external suite standard or sample (5:1 ratio of Nor/AAA to standard suite or sample). During injections, background interference was manually corrected as needed (e.g., ensuring background levels were the same before and after each injection), but manual corrections were kept as minimal as possible. Sample $\delta^{15}N$ values for 11 amino acids were corrected for accuracy as needed using regression (typically $R^2>0.9$) of either the Nor/AAA or suite standards. Accuracy was maintained to within 1‰ of the known value, and the average standard deviation of δ^{15}N across all 2006 samples and amino acids was 0.75‰.

The Peru and Hawaii (referenced, see Discussion) samples included in this study were prepared and analyzed separately but in the same lab and with the same protocol at the University of Hawaii at Manoa with an average standard deviation of δ^{15}N across all samples and amino acids of 0.56‰ and 0.63‰, respectively.

5. Isotopic Niche Area

Only sub-regions GC, NEC, EPWP, and CRD [alternatively Longhurst 7 and Longhurst 35] had sufficient individual measurements of bulk tissue δ^{13}C and δ^{15}N values to estimate the isotopic niche width. Standard niche width ellipse and convex hull areas were estimated using maximum likelihood, and Markov chain Monte Carlo (MCMC) credible intervals were generated to calculate uncertainty around ellipse estimates using Stable Isotope Bayesian Ellipses in R (SIBER) functions (Jackson et al. 2011) in the Stable Isotope Analysis in R (SIAR) package (Parnell et al. 2008, Parnell et al. 2010) in R (http://www.r–project.org/). Probability of size differences between ellipses were calculated by comparing pairs of draws from the posterior MCMC distributions.

6. Trophic Position Estimations

Chikaraishi et al. (2009) presented a universal algorithm based on the differences in δ^{15}N values of specific amino acids that can estimate the trophic position (TP) of a variety of aquatic organisms. Although the δ^{15}N values of multiple representative source (e.g., glycine, serine, phenylalanine, etc.) and trophic (e.g., alanine, proline, glutamic acid, etc.) amino acids can be used to estimate TP (e.g., (Hannides et al., 2009; Sherwood et al., 2011;

Décima et al., 2013; Hannides et al., 2013; Bradley et al., 2015; Décima et al., 2015)), to date most studies use just two, phenylalanine and glutamic acid (e.g., (Chikaraishi et al., 2009; Chikaraishi et al., 2010; Dale et al., 2011; Seminoff et al., 2012; Vander Zanden et al., 2013). Bradley et al. (2015) and Nielsen et al. (2015) show that increasing the number of amino acids (i.e., weighted mean) used to estimate marine teleost TPs results in a more precise estimates across taxa and trophic levels, as compared to prey items and/or stomach contents. Per these recommendations we explored both approaches but found negligible difference, so present the most simplistic estimate here. Based on Chikaraishi et al. (2009) algorithm, we used δ^{15}N values for either phenylalanine (Phe) or weighted mean of source amino acids [glycine, serine, tyrosine, lysine, Phe], and glutamic acid (Glu) or weighted mean of trophic amino acids [alanine, valine, leucine, isoleucine, proline, Glu] to estimate olive ridley TP in each sub-region. The following TP equation shows Glu and Phe as placeholders but were replaced with weighted means to compare approaches. Estimates of ^{15}N enrichment factors among amino acids in primary producers ($\beta_{\text{Glu-Phe}}$ = 3.6 ‰) and for each trophic level ($\Delta_{\text{Glu-Phe}}$ = 5.7 ‰) used were recommendations of Bradley et al. (2014). Others suggest $\Delta_{\text{Glu-Phe}}$ = 7.6 ‰ (Chikaraishi et al., 2009), 6.6 ‰ (Nielsen et al., 2015), or other values (see Lorrain et al. (2009), Dale et al. (2011), etc.), however we chose to use 5.7 because it resulted in TPs more consistent with TP estimates from stomach contents:

$$TP = \frac{\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - \beta}{\Delta} + 1$$

Due to the small AA-CSIA sample sizes, we did not assume that TP estimates are normally distributed, and thus used the Kruskal Wallis non-parametric statistical test to compare TP distribution across sub-regions. Exploratory analysis of δ^{15}N probability densities, median TP, and confidence intervals of each sub-region prompted a pooled pairwise comparison (Mann-Whitney U test) of trophic position estimates between the Costa Rica Dome and all others. All statistical tests were performed in R (R Core Team 2013). We treated sub-region as a blocking factor in the Mann-Whitney rank sum test and alpha was set at 0.05 for both tests.

7. Residence Assumption

The eastern Pacific is a dynamic basin and the boundaries of features fluctuate on a seasonal basis, or in response to climatic events such as El Niño. As our sampling effort was completed over one season (Aug-Nov) during a ‘normal’ year (2006), we assume that the feature boundaries that we base the oceanographic sub-region categorizations upon remained consistent during the study time frame.

Our study design hinges on the assumption that stable isotope values reflect diet assimilation from the local food web in which the turtle was sampled. To justify this assumption, we estimate the sample scale as follows. The smallest sub-region by geography is the Costa Rica Dome. Based on Fiedler (2002) and the minimum and maximum latitude and longitude of our samples grouped within the Costa Rica Dome, we estimate the area of our Costa Rica Dome-associated samples to have an 800 km N-S diameter and a 1000 km W-E diameter. In reality, the actual oceanographic feature is larger than the convex hull of the samples. Estimated olive ridley daily swim speed is 15 to 40 km, dependent on behavior. At the high end of the range, Block *et al.* (2002) suggest that high speeds are likely burst-speeds aided by currents, and Polovina *et al.* (2003) reported that olive ridley speeds in Hawaii doubled when “riding” the North Equatorial Current. Via telemetry Whiting et al. (2007) recorded olive ridley movements of up to 37 km over 23 days between nesting, and post-nesting migration swim speeds between Australia beaches and foraging areas of 0.87-

1.54 km h^{-1}. The same study hypothesized that observed slowing of swim speeds indicated foraging, and several instances of slowing during migration were recorded for two of the tagged migrating olive ridleys. Similarly, McMahon et al. (2007) recorded frequent foraging during migration in Australian waters. For olive ridleys studied in the eastern Pacific via telemetry, Plotkin (2010) observed swim speeds of 0.41-1.69 km h^{-1} for females and 0.33-1.33 km h^{-1} for males and concluded that adults swim and presumably forage continuously while not nesting. Given this information, we estimate that if an adult olive ridley were swimming in the eastern Pacific an average of 0.94 km h^{-1}, it would have to swim continuously without rest for 35.5 days to traverse our estimated N-S diameter of the Costa Rica Dome. While these are rough calculations, we state them to justify our assumption that the potential travel radius within the time frame captured by ^{15}N-assimilation from diet in turtle skin (40-50 days (Reich et al. 2008, Thomas & Crowther 2014)) remains within each sub-region, and that there is no spatial autocorrelation between sub-regions.

C. Results

Similar to Olson et al. (2010), we observed a general pattern of ^{15}N enrichment with increasing latitude, with the lowest latitude sample (4.17 N) $\delta^{15}N = 11.85‰$ and the highest latitude sample (26.48 N) $\delta^{15}N =15.56$ (Figure 2). $\delta^{15}N$ probability density plots according to Longhurst province (Figure 3a) do not show a clear enrichment pattern, whereas density plots according to oceanographic sub-region do show the latitude pattern (Figure 3b).

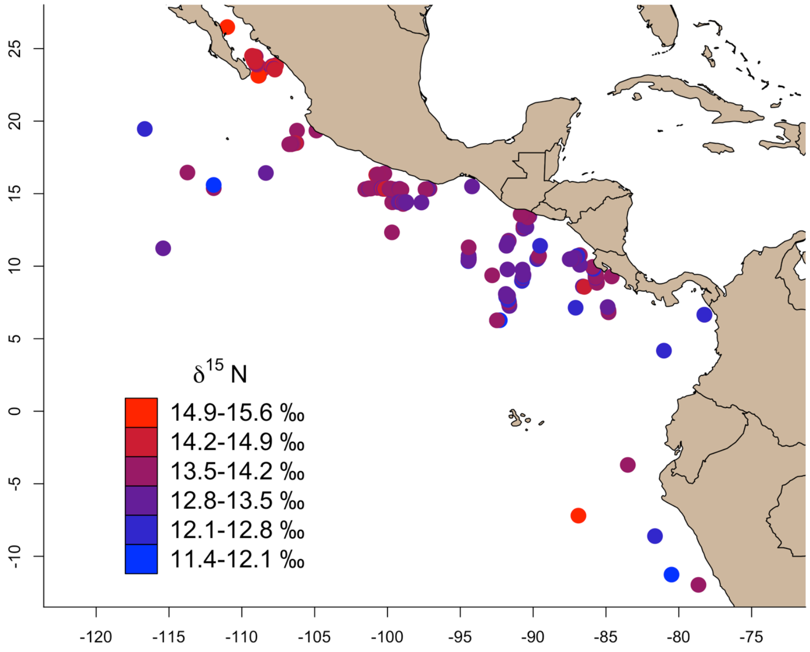

Figure 2. The gradient of δ^{15}N values across the study area range from 11.36‰ to 15.56‰, and in general δ^{15}N increases with latitude.

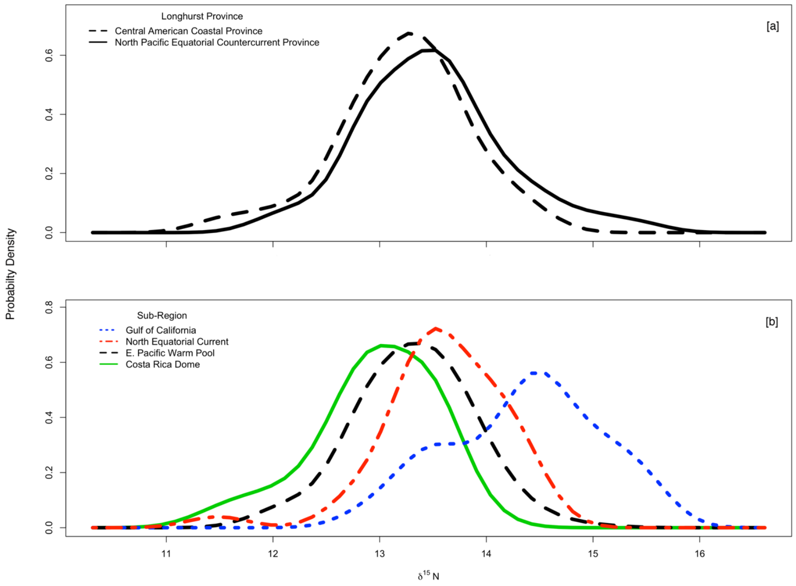

Figure 3. δ^{15}N density plots showing variation across [a] Longhurst provinces, and [b] oceanographic sub-regions. [b] illustrates ^{15}N enrichment with latitude.

Standard ellipse areas representing isotopic niches are plotted in Figure 4a&b and show a population-wide (n = 346) δ^{13}C range of -17.08‰ to -14.51‰ and a δ^{15}N range of 11.36‰[1] to 15.56‰. Maximum likelihood sub-region ellipse area estimates (with small sample size corrections in parentheses) are as follows: Longhurst 7 = 0.658(0.663)‰^{2}, Longhurst 35 = 0.933(0.938)‰^{2}, Costa Rica Dome = 0.5(0.51)‰^{2}, East Pacific Warm Pool = 0.56(0.57)‰^{2}, Gulf of California = 1.04(1.08)‰^{2}, North Equatorial Current = 0.744(0.77)‰^{2}. Pairwise comparison of posterior draws from Bayesian credible intervals around the maximum likelihood standard ellipse area estimates found (Figure 4c&d):

- 99.67% Central American province ellipse < North Pacific Equatorial Countercurrent province ellipse;
- 99.99% and 99.26% probabilities that East Pacific Warm Pool ellipse < Gulf of California and North Equatorial Current ellipses, respectively;
- 99.99% and 96.94% probabilities Costa Rica Dome ellipse < Gulf of California and North Equatorial Current ellipses, respectively;
- 89.2% probability Gulf of California ellipse > North Equatorial Current ellipse; and
- 99.99% probability East Pacific Warm Pool ellipse < Costa Rice Dome ellipse.

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[1] Some Peruvian Current samples lack sample location information. The minimum across all samples is 8.76‰, from a Peruvian Current sample missing lat/long information.

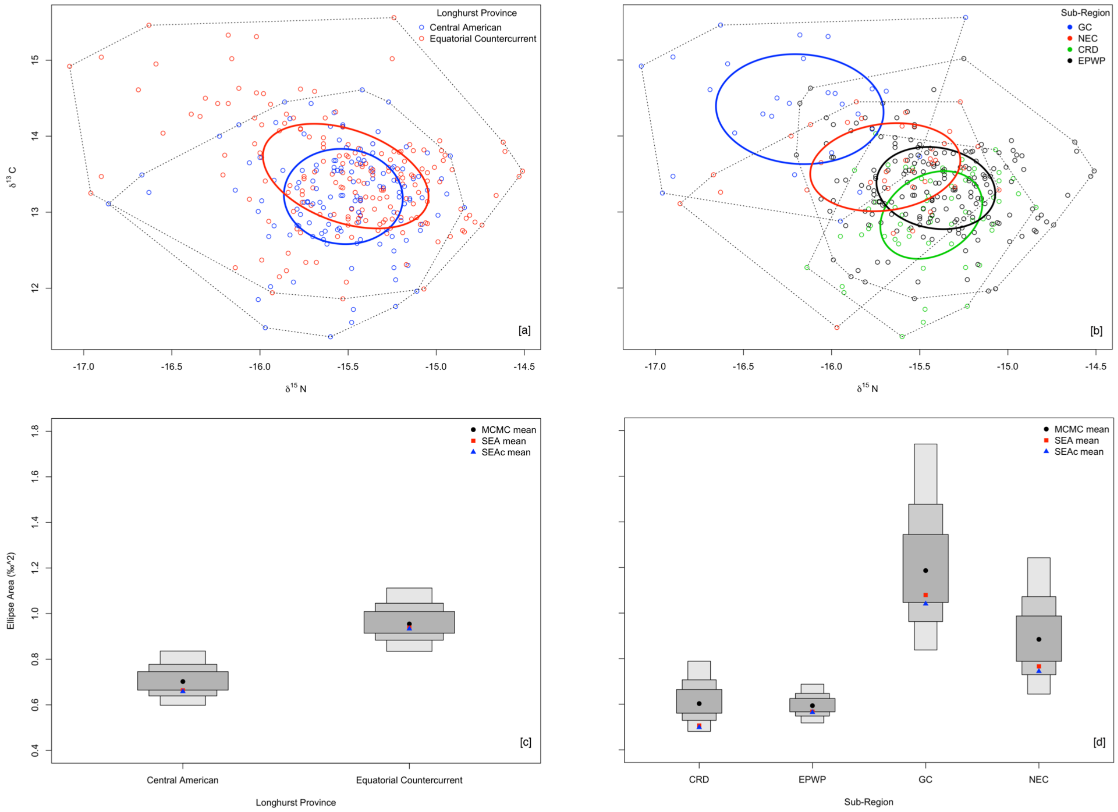

Figure 4. Maximum likelihood standard ellipse area estimation is plotted for [a] Longhurst provinces 7-Central American and 35-North Pacific Equatorial Countercurrent; and [b] oceanographic sub-regions the Gulf of California, North Equatorial Current, East Pacific Warm Pool, and the Costa Rica Dome. Plotted in [c] and [d] are the Bayesian inference credible intervals around the Markov chain Monte Carlo mean ellipse area, standard ellipse area (Rands et al. 2010) estimates, and the small sample size-corrected standard ellipse (SEAc) estimates for the Longhurst provinces and oceanographic sub-regions, respectively.

Median trophic position ($\text{TL}_{\text{Glu/Phe}}$) across the entire eastern Pacific seascape was 3.15 ±0.26 SD (Figure 5). When comparing sub-regions we see no significant difference in $\text{TL}_{\text{Glu/Phe}}$ across Longhurst provinces (χ^2 (2) = 1.8252, p-value =0.4015, α = 0.05), and no significant difference across oceanographic regions GC, NEC, EPWP, CRD, and PC (χ^2 (4) = 5.5215, p-value =0.2378, α = 0.05). However, we do see a difference in $\text{TL}_{\text{Glu/Phe}}$ between the Costa Rica Dome and all other oceanographic sub-regions pooled (W = 88, p-value

=0.04836, $\alpha = 0.05$). Individuals in the Costa Rica Dome were feeding at a 0.36 $TL_{Glu/Phe}$ higher (median) than in other oceanographic sub-regions.

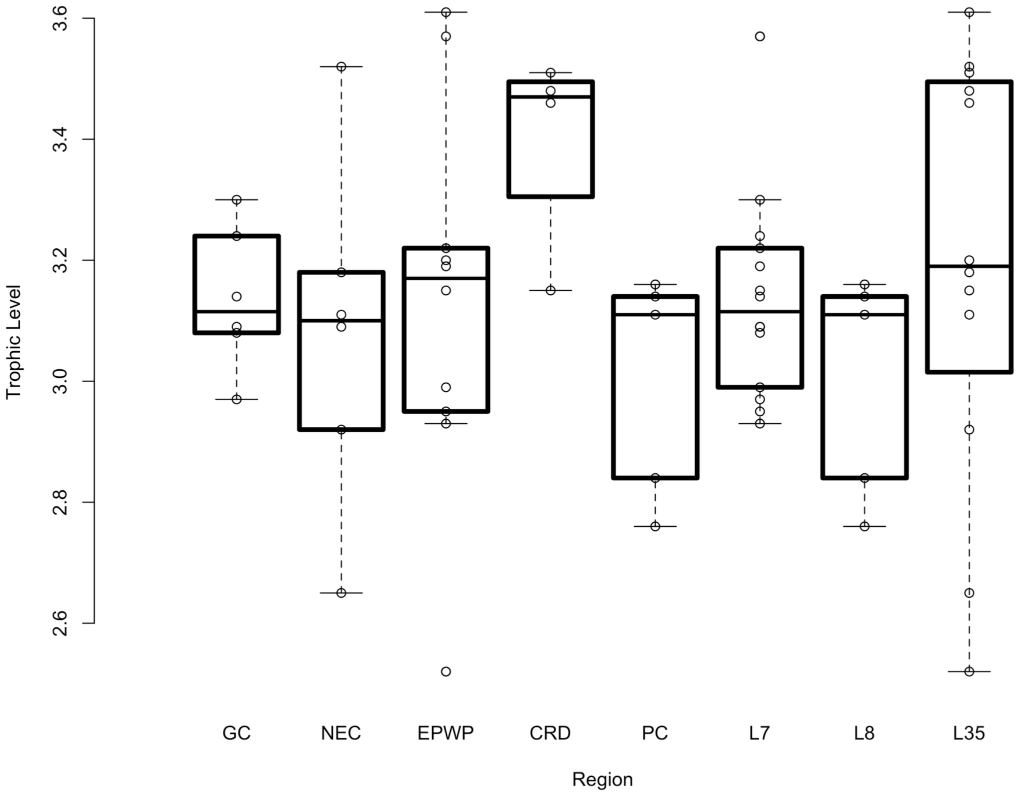

Figure 5. Boxplots showing individual trophic position ($TL_{Glu/Phe}$) estimates as open circles, median trophic position as the dark band within each box, first and third quartiles as the lower and upper box sections, respectively, and the minimum and maximum estimates as whiskers for each sub-region. Sub-region labels are as follows: GC = Gulf of California, NEC = North Equatorial Current, EPWP = East Pacific Warm Pool, CRD = Costa Rica Dome, PC = Peruvian Current, L7 = Central American Coastal, L8 = Chile-Peru Current, L35 = North Pacific Equatorial Countercurrent.

D. Discussion

To understand and preserve the ecological functions of species in marine food webs we need cost-effective tools to study consumers across their foraging ranges. For highly mobile consumers, their range can be tens to thousands of square kilometers and can include temporally dynamic habitats. For sea turtles, most empirical research studies are focused on nesting beaches, and therefore often limited to accessible reproductive females, and/or hatchlings. This study's large sample size and inclusion of oceanic individuals of a variety of sizes (ages) and both sexes of olive ridley sea turtles foraging across much of their range in the eastern Pacific has advanced our understanding of this breeding population's trophic role in oceanic food webs. Here, we measured the δ^{13}C and δ^{15}N values of 346 olive ridley skin samples during one season (Aug-Nov 2006) to estimate isotopic niche—a proxy for ecological niche. Further, to investigate if trophic role varied across space we completed AA-CSIA of a subset of 31 samples grouped by two different theoretical ways to define unique ecosystems in the eastern Pacific Ocean, Longhurst provinces and persistent oceanographic features.

Grouping foragers by Longhurst province did not prove to be insightful. These biogeochemical province boundaries are defined according to physical forces that regulate the distribution of phytoplankton in oceans. Oceans were divided first by biome (polar, westerlies, trade-winds, and coastal boundary zone), and secondarily by environmental parameters (e.g., bathymetry, chlorophyll *a* concentration, surface temperature, and salinity) that distinguish the area as unique (Reygondeau et al. 2013). While these ecological partitions make good sense and seem useful in guiding large-scale biogeochemical studies concerned with nutrient cycling such as isotopic analyses, they are static and quite large compared to the sub-regions we defined based on the persistent oceanographic features described in Fiedler & Talley (2006). Although they may be valid for other seascape-scale investigations, in our study we do not believe they sufficiently capture the nuance of biotic and abiotic differences between oceanographic features, such as thermocline depth and strength. Specifically, provinces 7 and 35 span many degrees of latitude encompassing several local nutrient cycling regimes, and therefore blurring their influences. However we did find it insightful to compare individuals grouped by five oceanographic sub-regions, presumably unique food webs, and so we focus our discussion and conclusions on comparisons across oceanographic sub-regions, not Longhurst provinces.

Olson et al. (2010) explained the causes of ^{15}N enrichment of both copepods and yellowfin tuna with latitude in the eastern Pacific and concluded that the pattern is maintained in all levels of a trophic chain. In short, upwelled nitrate is drawn down at the equator, but as latitude increases denitrification signals dominate.

This study has enhanced our understanding of the open-ocean foraging ecology of the eastern Pacific olive ridley, and in the following paragraphs we discuss the implications of our findings.

1. Empirical evidence for olive ridley omnivory and generalism across the eastern Pacific seascape

Anecdotal observations and stomach content analyses have suggested that adult eastern Pacific olive ridleys are generalist omnivores (Bjorndal 1997, NMFS & USFWS 1998, Holt et al. 1999, Wedemeyer-Strombel et al. 2015). The population range of $\delta^{13}C$ [-17.08‰, -14.51‰] and $\delta^{15}N$ [11.36(8.76)‰, 15.56‰] suggests prey resources come from a variety of primary producers (basal carbon sources), and from primary and secondary trophic levels. Ellipse area estimates suggest that the olive ridley isotopic niche is larger in the Gulf of California and the North Equatorial Current than in the Costa Rice Dome or East Pacific Warm Pool (Figure 4b), however the credible intervals (uncertainty) around the estimates of the two larger sub-region area estimates are much wider than the two smaller sub-regions (Figure 4d).

One explanation as to why the olive ridleys in the Gulf of California have a broader niche (1.08‰^{2}) compared to other sub-regions is that the majority of turtles were sampled in the entrance zone. There, basal resources, particularly N inputs, come from a mix of enriched (denitrified) subsurface water from the Eastern Tropical North Pacific (transported via the California Undercurrent) and tropical surface water from the west; large cell diazotrophy; N_2 fixation in the euphotic zone[2]; and terrestrial sources (sediments, guano, runoff). Upwelled nitrate $\delta^{15}N$ values in the entrance zone have measured 10.4-14.3‰; diazotrophy produced particulate material $\delta^{15}N$ values measured at ~0‰; and N_2-fixation contributions in sediment particles measured between 4‰ and 18‰ (White et al. 2013). These estimates are consistent with the range of $\delta^{15}N$ values we measured in turtle skin (see the Gulf of California $\delta^{15}N$ probability density function in Figure 4b).

The relative medium niche breadth of foragers in the North Equatorial Current (0.77‰^{2}) is likely reflective of a range of distance-from-shore basal signals, as individuals in this sub-region were effectively sampled along a longitudinal transect of the feature. Oligotrophic pelagic areas are depleted in both ^{13}C and ^{15}N relative to near-shore areas, and so this current is expected to support a wider trophic niche given the baseline isotopic variation within its geographic dimensions.

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[2] Variable N_2 fixation rates: e.g., during summer months 15-70 μmol N m^{-2} d^{-1} with bloom rates as high as 453-795 μmol N m^{-2} d^{-1}, lower in the late summer (White et al. 2013). In contrast, N_2 fixation rates in the oligotrophic North Pacific subtropical gyre is ~estimated 111 ±66 μmol N m^{-2} d^{-1} (Luo et al. 2012).

The East Pacific Warm Pool supports an impressively small isotopic niche area for olive ridleys ($0.57‰^2$) considering it is the largest sub-region by geography, spanning 10 degrees of latitude and 16 degrees of longitude. This speaks to the robustness of denitrification across the feature. Characteristics such as temperature, pycnocline, and mixed layer depth are particularly stable and reliable during the season in which our sampling was conducted (Fiedler & Talley 2006). The Gulf of Tehuantepec and the Gulf of Papagayo are two especially productive gulfs (high chlorophyll and nitrate concentrations) within the East Pacific Warm Pool due to wind-driven upwelling. As a result of our sampling platform (NOAA *R/V David Starr Jordan*) spending concerted time observing those two regions, many of the samples we examine within the East Pacific Warm Pool were in or near those two gulfs.

The Costa Rica Dome supports an isotopic niche area almost equal in size ($0.51‰^2$) to the East Pacific Warm Pool, but is considerably smaller in geographic size (we consider it 800-1000 km in diameter). It is the most seasonally dynamic oceanographic feature making up our study's sub-regions, in its spatial location and characteristics. It has a seasonally predictable strong and shallow thermocline (15 m at the peak of the dome, shoaling off to 50 m to the N and S) and from July through November, during the time period of our study, the Dome is defined by countercurrent thermocline ridging and expansion, primarily westward (Fiedler 2002). The Costa Rica Dome experiences productivity blooms May through December, and regional maximum chlorophyll concentration is present over the Dome from May-September (Fiedler 2002). High chlorophyll combined with nutrients brought to the surface via wind mixing and/or upwelling leads to high primary productivity in this region. Upwelling is uncommon in the eastern tropical Pacific, and thus the Costa Rica Dome is a unique area where cold, nutrient rich water is brought to the surface (Peña et al. 1994, Fiedler 2002). It also is not as iron-limited as other high-nitrate low-chlorophyll regions of the equatorial Pacific (Fung et al. 2000), a factor which is believed to boost productivity. Both the East Pacific Warm Pool and the Costa Rica Dome are within the Tropical Surface Water mass (Fiedler & Talley 2006) and have high concentrations of chlorophyll and nitrate compared to other areas in the eastern Pacific (Pennington et al. 2006). Some might even consider the Costa Rica Dome as a feature within the East Pacific Warm Pool, for example Pennington et al. (2006) who describes the Gulf of Papagayo (within the East Pacific Warm Pool) and the Costa Rica Dome as interacting and merging during certain times of the year. While driven by different physical sources, the Gulfs of Tehuantepec and Papagayo and the Costa Rica Dome share characteristics that determine basal isotopic signals, such as upwelling, nitrate concentrations, and denitrification, which is why we believe they support such a similar isotope niche for olive ridleys.

2. Elevated trophic position in the Costa Rice Dome

Olive ridleys have the ability to travel great distances (>1500 km) (Pandav & Choudhury 1998, Polovina et al. 2003, Whiting et al. 2007, Plotkin 2010) and we have learned much about their movements and diving behavior, but their role(s) in pelagic food webs have lacked robust empirical estimates. In this study, bulk tissue isotopic analyses were useful in describing and inferring biogeochemical roots of $\delta^{13}C$ and $\delta^{15}N$ value ranges and isotope niche area estimates in each sub-region. However, bulk analysis alone was insufficient to identify local drivers of observed variation in values, particularly $\delta^{15}N$, over ~40 degrees of latitude. As expected, our complimentary amino acid compound-specific isotopic analyses (AA-CSIA) produced information at the resolution needed to measure both local basal and trophic $\delta^{15}N$ values. This allowed us to calculate trophic position by accounting for variation in baseline while preserving variation in values due to differences in trophic function. With this two-pronged analytical approach we learned that regardless of the ^{15}N enrichment with latitude trend (see Figure 2), adult olive ridleys occupy virtually the same trophic position (median $TL_{Glu/Phe} = 3.15$) across the eastern Pacific. This suggests that regardless of foraging location, olive ridleys maintain omnivory and the opportune exploitation of resources across unique oceanographic features. While this finding says nothing about foraging site fidelity or lack thereof, it is consistent with Plotkin (2010) and others who have concluded that olive ridleys in the eastern Pacific have evolved to have such flexible migratory behaviors that they are able to be energetically successful over their entire range. Further, our results imply that their functional role as generalist consumers remains consistent across oceanic food webs separated by thousands of kilometers and with varying biogeochemical and physical characteristics.

There was one exception to the generalist paradigm for olive ridleys: in the Costa Rica Dome individuals fed at a 0.36 median level higher than in any other sub-region ($p \approx 0.5$). As detailed previously, the Costa Rica Dome is unique in many ways, and it shifts in space seasonally due to physical forces. Regardless, many high-level consumers are able to consistently locate the Dome to forage and exploit the relatively high productivity it supports. In fact, the Costa Rica Dome supports the highest productivity (e.g., zooplankton mean biomass) of the regional upwelling areas (equatorial upwelling zone, Gulf of Tehuantepec, Gulf of Papagayo, Gulf of Panama) except for the Peru Current. Cetaceans, seabirds and tuna feed on standing stocks of zooplankton and other prey (Rielly and Thayer 1990,(Sissenwine et al. 1998, Ballance et al. 2006).

As designed, our singular-species study provides estimates of trophic position, but does not reveal the mechanism behind elevated trophic status in the Costa Rica Dome. There are multiple conceivable explanations and future studies could be designed to empirically explore mechanistic hypotheses. In the following paragraphs, we discuss three potential ecological pathways: a decrease in individual specialization, community structure, and food chain length. Note that two or more of these may be interacting simultaneously.

It is conceivable that olive ridleys in the Costa Rica Dome are exploiting a narrow subset of higher-nutrient (i.e., higher trophic position) prey items among the full set of prey resources available. For example, Aresco (2015) observed an omnivorous freshwater turtle opportunistically eat dead predatory fish during a mass mortality event. They simultaneously observed an elevation in the turtle's isotopic trophic level and were able to explain the increase with the facultative scavenging of a new, high-value and high $\delta^{15}N$ prey resource. Intra-individual variation, known as individual specialization theory (Bolnick et al. 2002, Bolnick et al. 2003, Araújo et al. 2011), was used by Vander Zanden et al. (2010) to describe observations of a variable diet in a generalist carnivore population of loggerhead sea turtles (*Caretta caretta*) that occupy a broad isotopic niche. In the Costa Rica Dome we observe the opposite—a narrow isotopic niche and an elevated trophic position of a generalist omnivore. Therefore, we suggest that in the Costa Rica Dome where high value prey is abundant we are observing a decrease in intra-individual variation, presumably otherwise prevalent in olive ridleys as obligate omnivores since they are both habitat and diet generalists (Ducatez et al. 2015).

Community structure discrepancies across oceanic food webs could also lead to an elevated olive ridley trophic position. If the relative biomasses of each prey item were consistent between disparate food webs over the sampling time period, it would be feasible that olive ridleys could actively individually specialize in the Costa Rica Dome based on prey preference. However, if the relative biomasses of higher value prey items were larger in the Costa Rica Dome compared to the other sub-regions, then an elevated trophic position could be explained by prey encounter rate alone: individual turtles could simply encounter higher nutrient prey items more frequently within the Costa Rica Dome compared to in other oceanic food webs. A future study could estimate prey isotope values and biomasses across the eastern Pacific to test and contrast these first two pathways. Layman et al. (2012) outlines other ways in which these hypotheses could be empirically tested and/or modeled.

δ^{15}N-determined trophic positions of consumers reflect the underlying food web structure and food chain length (Cabana & Rasmussen 1996, Vander Zanden & Rasmussen 1999, Post 2002). Theory suggests that food chain length increases with productivity (Tilman 1999, Loreau et al. 2001), and Post and Takimoto (2007) discuss the potential causes for a predator trophic position shift. Empirical studies both support (e.g., Duffy et al. 2005) and contrast (e.g., Post et al. 2000) this theory. Further, community diversity and/or biomoss can significantly interact with food chain length (Mulder et al. 1999, Naeem et al. 2000, Duffy et al. 2005). Typically, productive areas such as upwelling zones are characterized by large phytoplankton cell sizes and high zooplankton grazing rates. In the Costa Rica Dome, grazing rates are definitively high but phytoplankton cell size reports are variable (Franck et al. 2005, Décima et al. 2015), leaving doubt regarding what, if any, chain length assumption can be made. Olson et al. (2010) found that yellowfin tuna in the eastern tropical Pacific had an elevated tropic position in a longer food chain (oligotrophic) than a shorter food chain (near-shore). If the Costa Rica Dome food chain is shorter than other sub-regions, as seems logical based on productivity, then we would expect the trophic position of a secondary consumer to decrease; however, we found the opposite with olive ridleys.

Without understanding the mechanism(s) behind the elevated trophic position estimate in the Costa Rica Dome we are unable to tell if it is a relatively important oceanic foraging habitat for olive ridleys in the eastern Pacific; however, we are confident that between August and December 2006 it was a unique one.

3. Other Considerations

Further highlighting the Costa Rica Dome as a unique foraging area for adult olive ridleys is a qualitative extension of our $TL_{Glu/Phe}$ estimates based on $\delta^{15}N$ values of olive ridley sea turtle skin compared to $TL_{Glu/Phe}$ estimates based on uncorrected muscle $\delta^{15}N$ values from Hawaii[3]. Average $TL_{Glu/Phe}$ of two samples from Hawaii was just above three, which is analogous to the trophic position estimates of olive ridleys from four EP sub-regions, and likewise ~0.4 lower than estimates in the Costa Rica Dome.

Although we only estimated the trophic positions of adult olive ridleys, we explored whether bulk $\delta^{13}C$ and $\delta^{15}N$ values varied with turtle size (proxy for age), but found no association. Therefore, we do not believe olive ridleys experience trophic ontogeny, as seen in other marine turtle species (Arthur et al. 2008, Ramirez et al. 2015, Tomaszewicz et al. 2015). This is intuitive given their lack of habitat ontogeny. There is still tissue from many of the turtles used in this study archived at the Southwest Fisheries Science Center (La Jolla, CA, U.S.A.), as well as from other eastern Pacific individuals of all sizes not included here.

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[3] We are able to refer to two previously analyzed samples collected from stranded olive ridleys in Hawaii (Arthur et al. 2014). AA-CSIA was completed for these pectoral muscle samples, one from September of 2009 and one from July 2010. The average standard error was 0.18.

This serves as a resource for future investigations of ontogeny, or other questions such as inter- and/or intra-variation of the results we've presented.

Density dependence of foraging strategies has been observed in other aquatic consumers. Svanback and Persson (2004) concluded that when adult perch population was high, individual and population niche breadth and individual specialization was highest, and prey switching was observed. Based on what we currently know about sea turtle life history, no density dependence exists in any of the life stages for turtles. However, there have been some proposed contradictions, or exceptions, such as Bjorndal et al. (2000) providing evidence for density dependent somatic growth in green sea turtles — juvenile turtles on crowded feeding grounds grew slower than other stages. The authors theoretically support this claim by noting that in the absence of exploitation, sea turtle populations would likely be regulated by food availability since individuals outgrow most predators. Therefore density-dependent effects would result from competition for limiting resources, and/or as population levels approach carrying capacity. Some researchers suggest that density dependence should be explored more seriously in sea turtle population models before ruling it out.

4. Persistent, but dynamic: oceanic foraging sites

Other sea turtle species exhibit foraging site fidelity, including fellow pelagic consumers, the leatherback (James et al. 2005). Based on satellite tracking, eastern Pacific olive ridleys are observed to be pelagic nomads (Plotkin 2010). Based on our stable isotope foraging ecology investigations, we conclude that in general adult olive ridley movements are likely not dictated by the need to rely on fixed resources in a specific static location, but rather that while migrating, turtles opportunistically exploit suitable resources when present.

Our findings indicate that persistent but dynamic oceanographic features as unique biogeochemical environments offer suitable resources, such that there was no detectable shift in the food web role of olive ridleys across any of the features we sampled within, indicating their energetic requirements are comparably met across the entire eastern Pacific seascape. The exception of the Costa Rica Dome provides further support to the long list of reports that recognize it as a special habitat. Based on this study, the area appears to be a particularly special foraging habitat for turtles, in addition to other consumers. We thus encourage further study of the food web dynamics in the Costa Rica Dome to enable the exploration of mechanisms driving consumer functional role observations.

In addition, it would be useful to explore if the elevated trophic position of olive ridleys remains over intra- and inter-annual time scales. Such investigations may lead to concrete open-ocean habitat-specific management and conservation recommendations under the umbrella of dynamic ocean management (Howell et al. 2008, Maxwell et al. 2014, Maxwell et al. 2015), especially in light of the high olive ridley bycatch in the central and eastern Pacific Ocean. In the Costa Rica longline fishery alone, 9-14 olive ridley sea turtles are caught per 1,000 hooks (Dapp et al. 2013). Fisheries bycatch is the largest threat to all sea turtle species in the open ocean (Lewison et al. 2014). Comprehensive high-resolution spatial and temporal pelagic habitat use and food web structure information gleaned from isotopic, genetic, endocrine, and telemetry studies in combination is critical to enhance our ability to inform marine consumer protection and ecosystem-based management.

At present, it would be speculative to comment on what is influencing adult olive ridley movement, and/or prey and habitat selection in the open ocean; however, we can look to their physiology, life history and reproductive strategies for clues about how olive ridleys successfully meet energetic requirements over such a large and variable biogeographic scale. Relative to other hard-shelled turtles, olive ridleys have lower standing metabolic rates, undergo actively deeper dives (to forage, not to rest), can travel more variable and greater distances between nesting events, show no trophic or habitat ontogeny, and may have less consistent nesting site fidelity (in the eastern Pacific) (Plotkin et al. 1995, Polovina et al. 2003, McMahon et al. 2007, Hamel et al. 2008, Plotkin 2010). It has been speculated that the long-distance movements between nesting events could be attributed to searching for food and/or alternate nesting sites, and that between-nesting time intervals are determined by environmental factors rather than physiology as in other hard-shelled turtles (Hamel et al. 2008). Studies show that olive ridleys also uniquely have plasticity in the timing of oviposition, being able to delay fertilization and egg-laying until suitable environmental conditions and nesting habitat arise, particularly for the females that nest *en masse* (Plotkin et al. 1995, Hamel et al. 2008). These factors explain how olive ridleys can exploit a wide range of prey resources across variable habitats, both benthic and pelagic, over large dynamic areas and depths. While generalism and flexibility in foraging, nesting, and migration (i.e., lack of precise migrating corridors) suggest that olive ridleys in the eastern Pacific may be resilient to the impacts of climate change (Plotkin 2010), their low metabolism, narrow thermal niche and temperature-dependent sex determination suggest they may still be quite vulnerable to environmental change unless they are able to undergo adaptation.

5. Conclusions

This study illustrates the utility of compound specific analyses of amino acids in identifying local drivers of isotopic variation across a seascape. A small skin biopsy of a marine consumer holds the biochemical information needed to isotopically describe trophic roles at much larger scales than with direct observation or animal tracking alone. It is also less invasive than stomach contents analysis. As such, isotopic investigative approaches are especially useful for cryptic, mobile oceanic foragers with a large distribution and range. Here we have used isotopic approaches to empirically corroborate reports that olive ridleys in the eastern Pacific are opportunistic omnivores, we have developed the hypothesis that their trophic role remains constant with ontogenesis, and have shown that during their 2006 breeding season they occupied the same generalist consumer function across their range. Among the persistent but dynamic oceanic foraging habitats that we sampled, the Costa Rica Dome was unique and we encourage further investigation of its food web dynamics.

The isotopic data generated in this study adds to the growing body of work describing stable isotope baseline data for marine organisms in the eastern Pacific Ocean (Arthur et al. 2008, Olson et al. 2010, Ruiz-Cooley & Gerrodette 2012, Seminoff et al. 2012, Lorrain et al. 2015). A natural future direction would be to estimate marine isoscapes across taxa and trophic levels (Somes et al. 2010, Ceriani et al. 2014, Vander Zanden et al. 2015) that can contextualize stable isotope ecological applications in the region.

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II. A Characterization of the At-Sea mtDNA Composition of Panmictic Olive Ridley Sea Turtles (*Lepidochelys olivacea*) in the East Pacific Ocean

Sea turtles generally have female philopatry, while gene flow is presumed to be largely male-mediated. Olive ridley sea turtles are found throughout the Pacific and Indian Oceans in sub-tropical and tropical waters between ~30˚N and ~20˚S, generally bound by the North Pacific and South Equatorial Currents. Mitochondrial DNA (mtDNA) has revealed at least 27 Indo-Pacific olive ridley sequences (haplotypes), and a strong geographic separation of West and East basin breeding populations. Previous studies have found high genetic connectivity and weak structure across olive ridley rookeries. However, little is known about the at-sea genetic composition, or to what extent there is mixing between rookeries and isolated breeding populations. To determine the nesting stock origin of oceanic olive ridleys, we identified haplotypes (~775 bp) of 156 individuals (juveniles n=67, males n=49, females n=40) in the Gulf of California south to Colombia and from the Americas west to -116°. We compared East Pacific (EP) oceanic haplotypes to all known olive ridley haplotypes from rookeries across the Indo-Pacific and grouped similar haplotypes using maximum likelihood estimation. All but one olive ridley sampled in our study area most closely associated with EP rookery haplotypes. The exception was an adult female found in Costa Rican waters with haplotype Lo74, most closely associated with haplotype Lo44 from Northeast India. To our knowledge, Lo74 has not yet been reported at any rookery. 68% of all oceanic olive ridleys were haplotype Lo46, which has been reported at all EP source rookeries except Peru, and once at an Indian Ocean rookery. The detection of one Indian Ocean haplotype among the EP breeding population suggests at least occasional gene flow between geographically isolated breeding regions. The separation of East and West Pacific and Indian Ocean haplotype groups supports the management of each region's rookeries as separate units. We did not find mtDNA evidence differing solitary and mass nesting (arribada) rookeries, despite the uniqueness of the two nesting strategies. Based on these results and records of at-sea mating, we infer that genetic mixing across EP rookeries occurs not just offshore nesting beaches, but also throughout open ocean regions where olive ridleys migrate and forage. With this study we have compiled all known Indo-Pacific olive ridley haplotypes and provided an important first characterization of the mtDNA makeup of oceanic olive ridleys in the EP. Despite the apparent stability and resilience of olive ridleys in the EP, low population structure and connectivity across sources rookeries suggests that disease and catastrophes have the potential to quickly reverberate through the breeding population on ecological time scales.

A. Introduction

Sea turtles face threats to their survival in all oceans around the globe, and the majority of populations are threatened or endangered. One or more rookery can make up a subpopulation, a geographically or otherwise distinct breeding group with little demographic or genetic exchange with other breeding groups distinguishing them evolutionarily and/or ecologically. Understanding the genetic structure of each breeding population is critical to effective conservation and management of rookeries and foraging areas as subpopulations can uniquely be resilient or susceptible to extinction. Examining at-sea genetic compositions and mixing can improve our understanding of how disparate extinction risks impact individuals in various habitats (nesting, foraging, migrating) across large land- and sea-scapes (Wallace et al. 2011), and how those risks reverberate through the population. As such, it is important to understand how genes flow between rookeries of the same subpopulation, and between breeding populations (Shamblin et al. 2014).

Turtle population structure is most commonly determined by studying genes of nesting females and/or hatchlings available on nesting beaches. Such studies have been completed for every extant sea turtle species around the globe, covering many of the known rookeries. Understanding how genes are or are not mixed between rookeries can be accomplished with a description of the genetic makeup of individuals within a breeding population (juveniles, adult males and females) across at-sea foraging and migration habitats, and the subsequent assignment of individuals to their origin (e.g., mixed stock analysis: (Bolker et al. 2007)). Such complimentary oceanic genetic composition studies are few due to logistical and resource constraints, and so relevant evolutionarily and/or ecologically meaningful groupings (e.g., 'Distinct Population Segments' for vertebrates as defined by the 1973 U.S. Endangered Species Act (see Pennock & Dimmick 1997); "Management Units" (MU) (Moritz 1994)) that are valuable to resource managers are weakly or un-defined.

Wallace et al. (2010) addressed this need for sea turtles by dividing species around the globe into "regional management units" (RMUs), using mark-recapture and telemetry data in addition to, or in place of, available genetic information. Other studies with greater genetic resolution have shown that low-resolution approaches may mislead our understanding of breeding population structure, the connectivity between source rookeries within and between distinct populations, and our ability to inform appropriate conservation strategies. For example, a re-examination of the population structure of the critically endangered Caribbean hawksbill sea turtle using longer mitochondrial DNA (mtDNA) sequences revealed new population distinctions and colonization and dispersal pathways

(Leroux et al. 2012). With their results, the authors of the study were able to offer suggestions for updated hawksbill MUs for effective conservation of genetic diversity. The same was true for a re-analysis of mtDNA population structure and phylogeography completed by Shamblin et al. (2014) for Pacific green sea turtles.

1. Male-Mediated Gene Flow

Conventional understanding is that both males and females migrate to nesting beaches during the nesting season, while only females come on to the beach for egg-laying (Dizon & Balazs 1982, Limpus 1993, Plotkin et al. 1996, FitzSimmons et al. 1997a). As such, it is well understood that sea turtle genetic exchange happens during mating adjacent to nesting beaches (Ehrhart 1982, Alvarado & Figueroa 1989). Kopitsky et al. (2000) and Kopitsky (2002) described olive ridley sea turtle mating behavior (e.g., copulating pairs) a mean distance of 138 km from land and determined the reproductive status of females at-sea in the eastern Pacific via ultrasound between 1990 and 2003. In 1999, upon ultrasonic confirmation of enlarged follicles (an indication of preparing for fertilization), post-capture movements of four females were tracked via satellite transmitters. One female that was tagged 180 km from land was observed nesting in Ostional, Costa Rica, and the others emitted transmissions from nesting beaches shortly after release (20-54 days), often indicating several nesting events during the season. In her thesis, Kopitsky (2002) concluded that ~25% of the pelagic olive ridleys she sampled in the eastern tropical Pacific were mating. Myself and other sea-going researchers in the eastern Pacific Ocean note at-sea mating as common for olive ridleys (R. Pitman, pers. comm.). Such records of at-sea mating events broaden conventional understanding of olive ridley mating locations and timeframes, suggesting that genetic exchange among rookeries and isolated breeding populations is likely male-facilitated via movement across the open ocean, in addition to known exchange during the mating season adjacent to nesting beaches (Figure 1, Appendix).

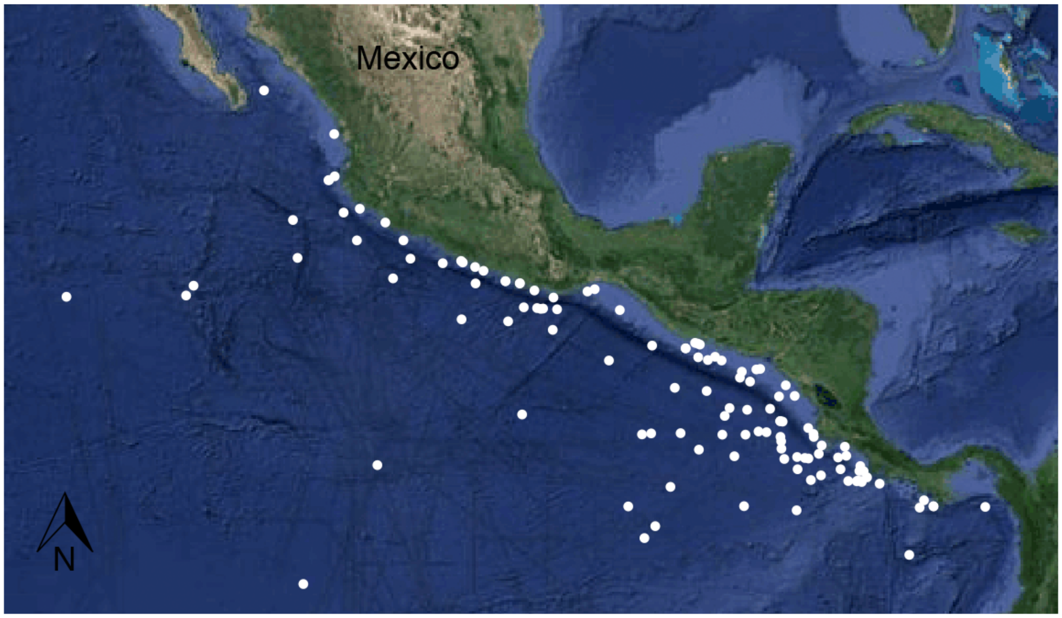

Figure 1. Anecdotal observations (n=116) of at-sea mating olive ridley pairs throughout the eastern Pacific are displayed as white circles. These observations were recorded between 1990 and 2006 by the Southwest Fisheries Science Center, La Jolla, CA. Google Imagery © 2016 TerraMetrics.

mtDNA approaches reveal relationships between lineages, and they do not shed light on male dynamics or male-mediated genetic exchange as nuclear DNA (nDNA) studies can. Further, low sample sizes and under-represented rookeries and oceanic sampling areas can restrain mtDNA studies. But because the relationships between gene flow and genetic differentiation is nonlinear, even small sample sizes can help resolve phylogeographic patterns. Even just a few individuals successfully moving between rookeries per generation can prevent genetic drift (Oyler-McCance & Leberg 2005), and these relationships are detectable with mtDNA approaches. Such studies, along with nDNA studies, estimates of natal homing rates, dispersal probabilities, density-dependence and/or other influences can help resolve metapopulation dynamics, including gene flow.

2. Indo-Pacific Olive Ridley Sea Turtle Phylogeography

Olive ridley sea turtles (*Lepidochelys olivacea*) are widespread throughout the subtropical and tropical Pacific and Indian Oceans (Bowen et al. 1997). They are the second smallest of the seven extant sea turtles in size and are generalist omnivores (NMFS & USFWS 1998, Abreu-Grobois & Plotkin 2008, Peavey Chap 1 of this dissertation). Along with their congener found in the Gulf of Mexico, the Kemp's ridley (*Lepidochelys kempii*) (Shaver & Wibbels 2007), *Lepidochelys spp.* nest both solitarily and *en masse* (100s to 10,000s), known as an "arribada." Olive ridleys are also unique in their lack of, or muted, ontonogenic habitat shifts that other sea turtle species typically undergo (e.g., loggerheads: Tomaszewicz et al. 2015). Instead, olive ridleys spend all life stages in the open ocean and so their behaviors and habitat use are challenging to directly observe.

Although over 1.7 million individual olive ridleys in the eastern Pacific Ocean makes up the most abundant sea turtle breeding population in the world (Eguchi et al. 2007, Seminoff & Wallace 2012), most turtle research and resources have been concentrated on more critically endangered species, and so there are glaring gaps in our understanding of their basic biology and ecology. Shanker et al. (2004) proposed olive ridley colonization from west to east during the late Pleistocene. mtDNA studies have identified 27 Indo-Pacific olive ridley sequences (haplotypes) and a strong geographic separation of western and eastern breeding populations. Bowen et al. (1997), Shanker et al. (2004), and others have shown low mtDNA differentiation across multiple olive ridley subpopulations. Rodríguez-Zárte et al. (2013) showed high connectivity and weak nDNA structure across nesting colonies in

Mexico. From these and other foundational studies, we assume that the Indian and West Pacific breeding populations are genetically separate from the East Pacific breeding population, and that genetic structure within each basin is low. There are not distinct population segment designations to reflect this (NMFS & USFWS 2014), however RMUs designations do (Wallace et al. 2011).

In the East Pacific, there is potentially over 5,000 kms of olive ridley nesting habitat along Mexico, Central and South America. Only six source rookeries have published mtDNA information (Baja California Sur (n=46), Sinaloa (n=14), Guerrero (n=12), Oaxaca (n=17) (Mexico); Costa Rica (n=32); Peru (n=1)); all have low sample sizes. Throughout their range, higher-resolution population structure and comprehensive mixed-stock analyses of olive ridleys are needed. For example, Roberts et al. (2004) found that Indian and Atlantic Ocean green sea turtles (*Chelonia mydas*) are connected via recent or ongoing migration between ocean basins, suggesting the occurrence of more substantial geographic overlap of demographically independent sea turtle populations during foraging or migration than previously presumed. In contrast, although Amorocho et al. (2012) reported juvenile western Pacific green turtles in eastern Pacific waters, no Hawaiian or East Pacific green turtle haplotype has been recorded at West Pacific rookeries (Shamblin et al. 2014). One explanation is that a phylogeographic barrier restricted dispersal between breeding populations around 0.336 mya (Shamblin et al. 2014); which implies colonization occurred at different time scales or mechanisms for Pacific green and olive ridley sea turtles. These studies highlight the evolutionary and ecological insights resulting from an enhanced understanding of the magnitude of geographic overlap and gametic exchange among breeding populations.

The RMU approach assessed conservation status and overlap separately for solitary and *arribada* (mass nesting) olive ridley nesting rookeries. Wallace et al. (2011) concluded that of the eight global olive ridley RMUs, the three Indian Ocean (Northeast (solitary); Northeast (arribada); West (solitary, putative)) are among the top four most endangered in the world due to the presence of high threats (e.g., fisheries bycatch) and low population abundances. In contrast, the two East Pacific (soliatary; arribada) and one West Pacific (solitary) RMUs are currently at a low risk of extinction, despite the presence of threats, because their abundances are relatively high (Wallace et al. 2011). Overlap between the six Indo-Pacific olive ridley RMUs, three with high risk of extinction and three with low risk of extinction, is most probable in the Central Pacific where West and East RMUs meet. Given their disparate extinction risks, it is imperative that we better understand the connectivity between and resilience of each breeding region.

Mysterious inter-nesting behavior presents an added challenge for studying olive ridleys at-sea. Other turtle species show both nesting and foraging site fidelity (e.g., Atlantic green turtles: Makowski et al. 2006), while there is no evidence to suggest olive ridleys have fidelity to specific oceanic feeding locations (Plotkin 2010, Peavey Chap 1 of this dissertation). In this study we use a unique set of tissue samples from a large-scale at-sea sampling effort to describe the haplotype composition of 156 olive ridleys found in the East Pacific basin, and assign them to nesting origins based on haplotype databases. We found a mixed composition of 19 different haplotypes with no apparent spatial pattern across the study area. All but one of the oceanic haplotypes most closely associated with East Pacific rookeries, and Lo46 was overwhelmingly the most abundant (~68%) and widespread haplotype. The apparent connectivity of source rookeries combined with the lack of mtDNA structure among individuals across the eastern Pacific seascape is a classic example of panmixia. We discuss implications for genetic exchange between breeding populations in terms of stability, resilience, and management units.

B. Methods

1. Sample Collection

350 olive ridleys were opportunistically hand-captured and released at-sea from the National Oceanographic and Atmospheric Administration R/V David Starr Jordan during the *Stenella* Abundance Research cruise in 2006 between the months of August and December. Location, sex (generally for turtles with straight carapace length >56 cm: long tail = male, short tail = female), and morphometric information were recorded, and tissue (blood and skin) samples were collected from the dorsal surface of the neck. Red blood cells and plasma were immediately separated using centrifuge, and blood and skin samples were stored at -80°C until transported to archive at -20°C at the Southwest Fisheries Science Center (SWFSC) Marine Mammal and Turtle Molecular Research and Stable Isotope Sample Collections (La Jolla, CA, USA).

2. Laboratory Analysis

Genomic DNA was extracted, amplified, and purified from 156 samples of blood and skin as described by LaCasella et al. (2013). Negative controls were used to detect contamination. Sequences were trimmed to a ~775 base-pair (bp) high quality fragment of the control region and assigned mitochondrial haplotypes based on SWFSC Marine Turtle Molecular Research and GenBank (http://www.ncbi.nlm.nih.gov) databases.

3. Nomenclature

We identified nomenclature for Indian and Pacific Ocean haplotypes, Wallace et al. (2011)-defined Regional Management Units (RMUs)), and associated GenBank IDs. We standardized nomenclature for all 27 Indo-Pacific haplotypes with a “Lo” prefix and numerically sequential names based on Southwest Fisheries Science Center standards, and submitted to GenBank as needed.

4. Statistical Analysis

All 27 known Indo-Pacific haplotypes were assessed for relatedness using maximum likelihood in MEGA6.06 (Tamura et al. 2013). A phylogenetic tree was estimated with *Lepidochelys kempii* as the out-group. Pearson’s Chi-square (χ^2) was used to test the null hypothesis that oceanic haplotype frequency proportions were equal to hypothesized oceanic foraging regions: Gulf of Mexico, North Equatorial Current, East Pacific Warm Pool, Costa Rica Dome (Peavey Chap 1 of this dissertation). Statistical analyses were completed in R (R Core Team 2013).

5. Documentation of Mating Pairs

Between the years of 1990 and 2006, the locations of 123 pairs of mating hard-shell sea turtles (116 olive ridleys) were recording by visual observers during ship-based line transect surveys conducted in the eastern Pacific Ocean by four National Oceanographic and Atmospheric Administration research vessels: David Starr Jordan, McArthur, McArthur II, and Endeavor.

C. Results

We sequenced 19 unique haplotypes across 156 olive ridley sea turtles in the eastern Pacific Ocean (Table 1). Table 2 lists all 27 known haplotypes sequenced across Pacific and Indian Ocean rookeries, previously and in this study. All East Pacific, West Pacific, and Indian Ocean RMUs are represented in this study, except for the putative West Indian RMU. Peru is the most southeast known Pacific olive ridley nesting area, but Peruvian waters were not sampled in this study, and thus Peruvian source haplotype information is not included in Figure 3.

All turtles in this study associate with East Pacific rookery haplotypes, with the exception of one Lo74 adult female (straight carapace length = 60.2 cm) found in Costa Rican waters (Figure 1). Lo74 most closely associates with Lo44, described at the solitary east coast India rookery, Madras.

We observe Lo27 three times in disparate East Pacific locations spanning >15 degrees of latitude and >25 degrees of longitude (see Table 1). Previous studies have only recorded Lo27 three times at nesting beaches in both West and East Pacific breeding populations: twice in Costa Rica; once in Australia (Table 2).

Lo46 accounts for 68% (n=106) of observed haplotypes (Figure 2) and is ubiquitously observed at solitary and arribada East Pacific rookeries except Peru. Prior to this study, Lo46 has been observed once outside of the East Pacific, at Madras (Shanker et al. 2004).

Although Peavey (Chap 1 of this dissertation) hypothesized distinct oceanic foraging sub-regions in the eastern Pacific based on persistent oceanographic features (e.g., Costa Rica Dome), chi-squared analysis of a 4x18 contingency table suggests no significant association between at-sea haplotype frequency and sub-region ($X^2(51) = 46.82$, p-value =0.6402, alpha = 0.05).

Table 1. Olive ridley capture date, location, gender (M = male, F = female, I = immature), straight carapace length (cm; no data is represented as —), and mtDNA haplotype (n=156) based on ~775 bp.

2006 Capture Date		Capture Location		Morphometrics		mtDNA
Month	Day	Longitude	Latitude	Gender	Straight Carapace Length (cm)	Haplotype
8	5	-113.33	23.10	M	56.8	Lo46
8	8	-111.95	20.67	M	61.1	Lo54
8	9	-110.07	21.12	M	67.2	Lo54
8	10	-108.62	23.55	M	63	Lo46
8	10	-108.68	23.60	M	56.4	Lo46
8	10	-108.69	23.60	I	55.6	Lo46
8	10	-108.65	23.62	I	58.9	Lo46
8	10	-108.78	23.70	M	60.2	Lo54
8	10	-108.95	23.88	M	60.6	Lo52
8	10	-108.95	23.89	I	21.3	Lo28
8	10	-109.03	23.94	F	60	Lo46
8	10	-109.02	23.93	F	63.7	Lo46
8	10	-109.07	24.10	F	61.2	Lo46
8	10	-109.17	24.17	I	25.1	Lo54
8	10	-109.20	24.27	M	66.5	Lo46
8	10	-109.20	24.32	F	57.7	Lo71
8	10	-109.30	24.50	F	54	Lo52

8	10	-109.28	24.50	F	60	Lo46
8	11	-111.00	26.48	F	63.2	Lo27
8	13	-107.92	23.78	I	51	Lo46
8	13	-107.65	23.86	I	53	Lo46
8	13	-107.90	23.79	I	48.6	Lo46
8	13	-107.88	23.75	I	59.1	Lo46
8	13	-107.85	23.73	M	58.4	Lo46
8	13	-107.83	23.70	F	59.2	Lo46
8	13	-107.83	23.66	F	60.9	Lo46
8	13	-107.82	23.65	F	59.8	Lo46
8	13	-107.73	23.55	M	—	Lo46
8	17	-106.31	22.76	F	61.9	Lo46
8	17	-106.38	22.67	I	22.9	Lo28
8	20	-111.93	15.60	F	62.1	Lo46
8	30	-92.48	6.27	F	62.9	Lo54
8	30	-92.48	6.27	I	58.4	Lo46
9	2	-87.08	7.13	M	61.4	Lo46
9	3	-86.58	8.60	M	59.7	Lo46
9	3	-86.58	8.60	F	60.2	Lo74
9	9	-84.60	9.29	M	58.8	Lo27
9	3	-86.48	8.57	M	57.8	Lo46
9	12	-78.24	6.65	I	57.4	Lo46
9	14	-78.25	6.66	I	49.3	Lo46
9	18	-84.82	6.82	F	58.4	Lo46
9	18	-84.83	6.93	M	68.8	Lo46

9	18	-84.83	6.97	M	59.6	Lo46
9	18	-84.88	7.18	F	63.3	Lo46
9	19	-85.62	8.85	M	63.3	Lo52
9	19	-85.65	9.10	M	64.5	Lo52
9	19	-85.71	9.20	M	60.1	Lo72
9	19	-85.71	9.26	M	67.7	Lo46
9	19	-85.86	9.86	M	—	Lo46
9	19	-85.86	9.86	F	59.1	Lo46
9	19	-85.72	9.40	F	56.7	Lo46
9	19	-85.72	9.40	I	56.8	Lo54
9	19	-85.85	9.82	F	57.8	Lo55
9	19	-85.92	9.95	M	59.5	Lo46
9	20	-86.80	10.75	F	62.7	Lo46
9	20	-86.79	10.76	M	57.6	Lo46
9	20	-86.79	10.09	M	62.1	Lo46
9	20	-86.96	10.71	F	53.8	Lo46
9	20	-87.15	10.58	I	45.2	Lo33
9	20	-87.15	10.58	M	64.3	Lo46
9	20	-87.22	10.53	F	60.2	Lo46
9	20	-87.22	10.53	M	66.5	Lo57
9	20	-87.49	10.48	M	65.4	Lo46
9	23	-91.62	7.33	I	22.8	Lo46
9	23	-91.66	7.55	M	65.7	Lo46
9	23	-91.85	7.88	F	59	Lo46
9	24	-91.72	7.96	I	28.6	Lo46

9	24	-91.61	7.94	I	29.5	Lo46
9	24	-91.75	7.95	I	32	Lo79
9	24	-91.77	7.99	M	64.6	Lo57
9	24	-90.77	8.98	I	17.4	Lo46
9	24	-90.74	9.18	M	68.2	Lo52
9	24	-90.67	9.40	F	60.3	Lo46
9	24	-90.67	9.38	M	61.3	Lo46
9	25	-89.64	10.70	I	46.5	Lo46
9	26	-90.11	12.51	M	65.2	Lo52
10	3	-90.83	13.72	M	63.1	Lo46
10	3	-90.83	13.72	M	61.8	Lo52
10	3	-90.88	13.64	F	59.4	Lo46
10	3	-90.89	13.61	M	64.3	Lo46
10	3	-90.95	13.60	I	55.8	Lo31
10	3	-90.98	13.53	F	—	Lo27
10	4	-91.68	11.92	M	66.2	Lo46
10	4	-91.68	11.87	M	62.3	Lo46
10	4	-91.72	11.82	I	46.7	Lo62
10	4	-91.73	11.80	M	63.3	Lo46
10	4	-91.80	11.68	F	61	Lo60
10	4	-91.93	11.33	F	58.9	Lo46
10	5	-92.90	9.33	I	43.2	Lo46
10	7	-94.30	10.28	I	31	Lo46
10	7	-94.26	10.38	M	64	Lo59
10	7	-94.26	10.39	F	63.5	Lo46

10	7	-94.25	10.60	M	62.1	Lo46
10	7	-94.25	10.55	M	59.3	Lo46
10	7	-94.23	10.58	M	61.6	Lo72
10	7	-94.23	10.58	F	65	Lo46
10	7	-94.25	10.53	I	54.2	Lo71
10	7	-94.25	10.55	I	57.4	Lo46
10	7	-94.25	10.53	I	42.1	Lo46
10	7	-94.25	10.87	I	30.2	Lo46
10	8	-94.06	13.67	I	28.3	Lo46
10	9	-94.02	15.47	F	58.7	Lo31
10	9	-94.02	15.47	F	62.8	Lo46
10	11	-98.20	10.67	F	63.2	Lo46
10	21	-101.23	15.18	I	24.5	Lo46
10	26	-100.42	15.80	I	28.2	Lo46
10	26	-100.42	15.80	I	27.4	Lo46
10	26	-100.52	15.88	M	60.6	Lo34
10	27	-99.90	15.35	I	24	Lo52
10	27	-99.88	15.38	M	57.1	Lo54
10	27	-99.86	15.40	M	61.7	Lo46
10	27	-99.80	15.50	F	58.9	Lo46
10	28	-99.17	15.02	I	27.4	Lo46
10	28	-99.52	15.05	I	24.7	Lo54
10	29	-98.45	14.75	I	26.3	Lo46
10	29	-98.62	14.82	I	6.6	Lo46
10	29	-98.53	14.57	F	56.3	Lo46

10	30	-98.95	14.98	F	56.9	Lo83
11	1	-100.93	15.50	I	52.8	Lo46
11	1	-100.90	15.53	F	59.7	Lo61
11	2	-100.05	15.58	I	33.5	Lo52
11	2	-99.95	15.37	I	25.7	Lo46
11	3	-99.50	15.47	M	62.5	Lo46
11	4	-98.70	14.98	I	29.5	Lo46
11	4	-98.45	14.95	I	22.1	Lo54
11	5	-97.05	15.17	I	4.18	Lo46
11	5	-97.08	15.17	I	17.6	Lo59
11	5	-97.10	15.13	F	53.5	Lo46
11	6	-99.40	12.63	M	63.5	Lo46
11	12	-106.35	13.97	I	22.2	Lo46
11	25	-104.57	19.00	I	58.4	Lo46
11	26	-105.90	19.00	I	29.2	Lo46
11	26	-105.95	18.98	I	19.7	Lo46
11	26	-105.95	18.95	I	25.1	Lo34
11	26	-105.97	18.95	I	33.6	Lo54
11	26	-106.07	18.92	I	46.8	Lo28
11	26	-106.10	18.80	I	22.5	Lo46
11	26	-106.10	18.80	I	32.8	Lo46
11	26	-106.12	18.78	M	63.6	Lo60
11	26	-106.12	18.78	I	25	Lo46
11	26	-106.17	18.72	I	24.4	Lo46
11	26	-106.18	18.72	I	21.9	Lo57

11	26	-106.17	18.72	I	28.9	Lo46
11	26	-106.20	18.68	I	27.5	Lo46
11	26	-106.22	18.60	I	30.3	Lo46
11	26	-106.25	18.55	F	63	Lo46
11	26	-106.28	18.45	I	22.9	Lo54
11	26	-106.30	18.48	I	30.6	Lo54
11	26	-106.31	18.49	I	20.2	Lo46
11	26	-106.37	18.45	F	61.2	Lo46
11	26	-106.37	18.45	I	28.6	Lo46
11	26	-106.38	18.45	I	24.7	Lo46
11	26	-106.43	18.43	I	25.4	Lo46
11	27	-108.03	16.75	I	54.6	Lo46
11	30	-113.40	16.98	I	27.6	Lo46
12	2	-116.32	19.63	M	59.8	Lo54

Table 2. 27 mtDNA haplotype frequencies across the Indo-Pacific are organized according to GenBank identifiers, Regional Management Units (Wallace et al. 2010), and hypothesized oceanic foraging sub-regions (Peavey Chap. 1). Only one haplotype sequenced in this study exists in GenBank (Lo27, Accession No. KC207830). Although the other 18 have been sequenced, they have not yet been submitted to GenBank but have been cataloged in the Southwest Fisheries Science Center Marine Turtle Molecular Research database (La Jolla, CA, USA). With this paper, the remaining 18 haplotype sequences are submitted to GenBank. Frequencies highlighted in gray represent haplotypes that closely associate with East Pacific rookeries (see Figure 1).

Haplotype			**GenBank**		**Frequencies at Rookeries**													**Frequencies At-Sea**					
					East Pacific (Arribada RMU)		East Pacific (Solitary RMU)				Northeast Indian (Arribada RMU)			Northeast Indian (Solitary RMU)	West Pacific (Solitary RMU)			Hypothesized oceanic foraging region (Peavey Chap. 1)					
					Guerrero, Mexico	Oaxaca, Mexico	Baja California Sur, Mexico	Sinaloa, Mexico	Costa Rica	Peru	Gahirmatha	Devi Mouth	Rushikulya	Madras (India)	Tiwi Islands (Australia)	McCluer Group Islands (Australia)	Flinders Beach (Australia)	Gulf of California	North Equatorial Current	East Pacific Warm Pool	Costa Rica Dome	Other	TOTAL
Current	Previous	No. of base pairs	Accession No.	ID	Lopez-Castro and Rocha-Olivares 2005	Lopez-Castro and Rocha-Olivares 2005	Lopez-Castro and Rocha-Olivares 2005	Lopez-Castro and Rocha-Olivares 2005	Jensen et al. 2013; Lopez-Castro and Rocha-Olivares 2005	Kelez et al. 2009	Shanker et al. 2004	Shanker et al. 2004	Shanker et al. 2004	Shanker et al. 2004	Jensen et al. 2013	Jensen et al. 2013	Jensen et al. 2013	n = 25	n = 31	n = 55	n = 38	n = 4	156
Lo1		782	JN391445	399658676											51	7	9						
Lo15		782	JN391459	399658690							2				7	3	6						
Lo2		781	JN391446	399658677												1	2						
Lo21		782	JN391465	399658696											1								
Lo27		**774 (this study);** 782 (GenBank)	KC207830	478304818					2								1	1		1	1		3
Lo28		**774**																	2	1			3
Lo3		782	JN391447	399658678											1		9						
Lo31		**773**																		2			2
Lo33		**774**																			1		1
Lo34		**774**																	1	1			2
Lo4		782	JN391448	399658679											3								
Lo44		unknown									18	12	12	13									
Lo46		**774**			11	13	44	9	12					1				19	20	31	29	7	106
Lo5		782	JN391449	399658680											1								
Lo52		**776**				1	1	2	7									2		5	2		9
Lo54		**775**																2	6	3	1	1	13
Lo55		**752**																			1		1
Lo57		**776**																	1		1	1	3
Lo59		**774**																		2			2
Lo60		**774**																	1	1			2
Lo61		**775**			1	3	1	3	10	1										1			1
Lo62		**775**							1											1			1
Lo71		**775**																1		1			2
Lo72		**775**																		1	1		2
Lo74		**768**																			1		1
Lo79		**775**																				1	1
Lo83		**775**																		1			1
TOTAL					12	17	46	14	32	1	20	12	12	14	64	11	27						

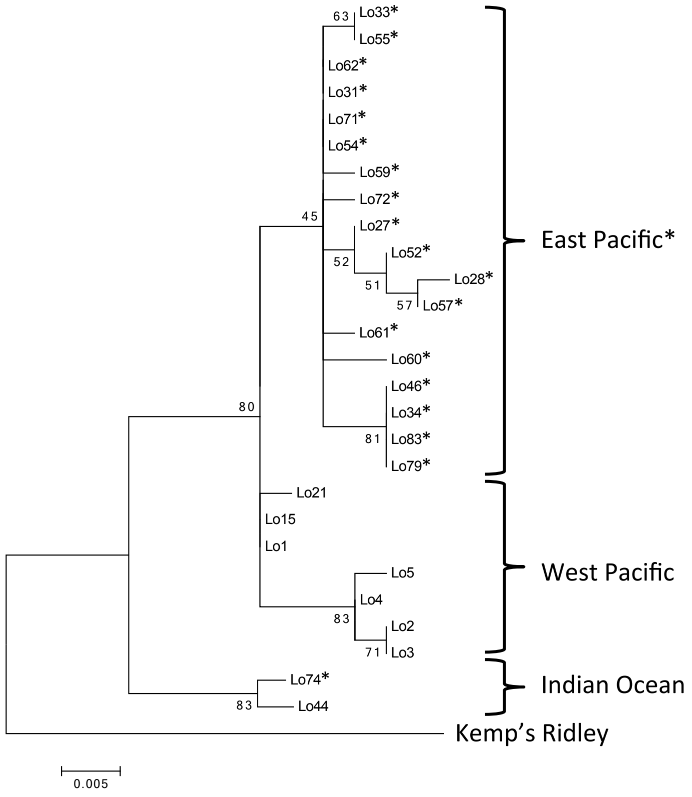

Figure 1. Maximum likelihood phylogenetic tree showing genomic similarities between mtDNA haplotypes, using *Lepidochelys kempii* (Kemp's Ridley) as the out-group. Haplotypes are geographically grouped with brackets by ocean basin: East and West Pacific, Indian Ocean. Haplotypes indicated with an asterisk were sampled in the eastern Pacific Ocean in this study.

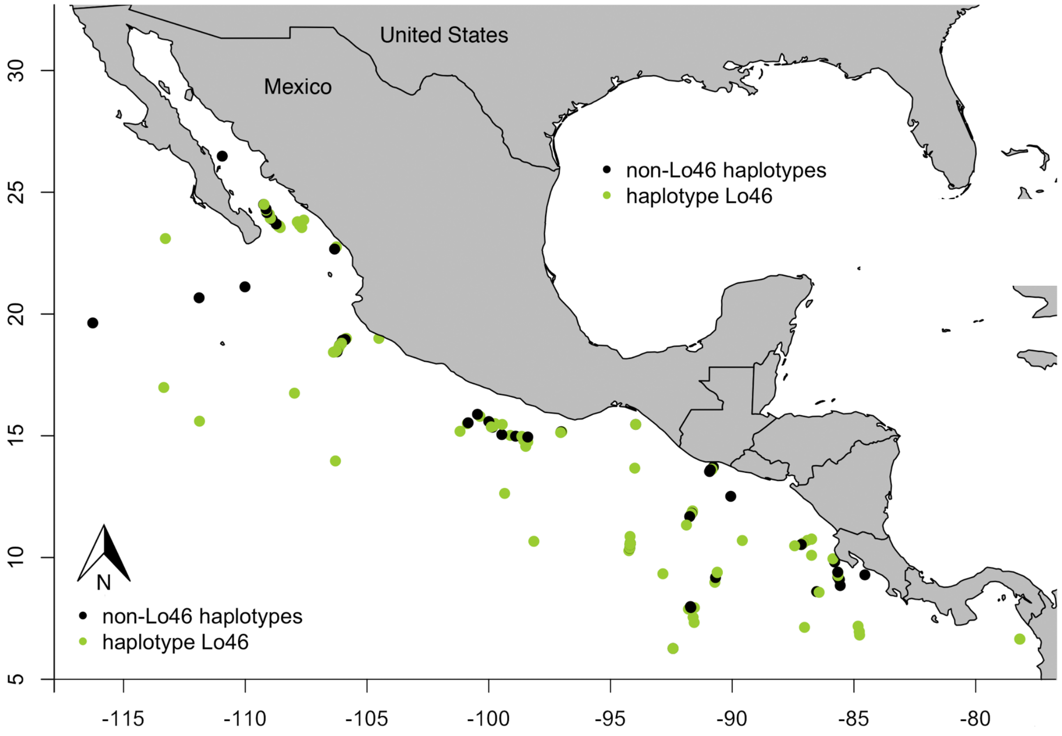

Figure 2. 156 individual oceanic Pacific olive ridleys are displayed as circles across the eastern Pacific seascape study area; mtDNA samples are from 2006 (see Table 1 for sample details). Haplotype Lo46 is displayed in green across 106 individuals, while all other haplotypes (n=18) across the remaining 50 individuals are displayed in black. See Figure 3 to view the haplotypes of the non-Lo46 individuals.

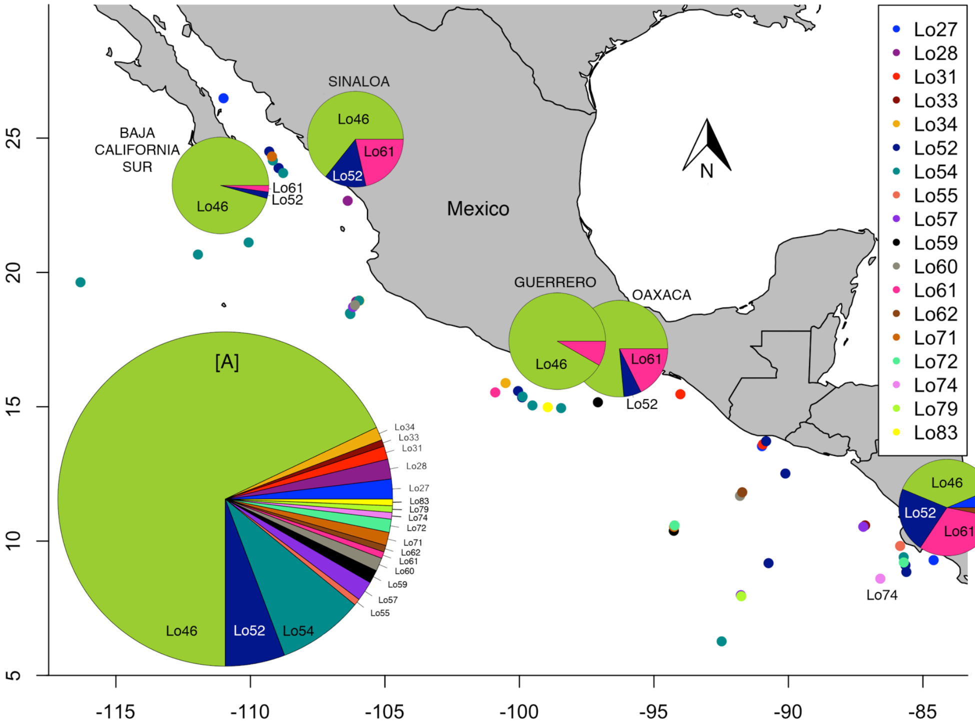

Figure 3. Fifty individual oceanic Pacific olive ridley non-Lo46 haplotypes (n=18) are displayed as colored circles across the eastern Pacific seascape study area; mtDNA samples are from 2006 (see Table 1 for sample details). Haplotype sources (n=5) that were collected over several years and studies (see Table 2) are displayed as pie charts. Inset [A] on the bottom left is a breakdown of all the at-sea haplotypes (n=19), including the most common haplotype, Lo46. See Figure 2 for the location of at-sea Lo46 individuals.

D. Discussion

This study provides the most comprehensive summary of Indo-Pacific olive ridley haplotypes since Bowen et al. (1997). It also represents the only characterization of at-sea mtDNA composition of olive ridley sea turtles in the eastern Pacific. We found that the tropical and sub-tropical eastern Pacific Ocean appears to provide habitat to a mix of turtles from East Pacific source rookeries, with minor contribution from West Pacific and Indian Ocean source rookeries. Lo46 was overwhelming the most common and widespread at-sea haplotype. Aside from Lo46, the distributions of the other 18 haplotypes are heterogeneous across space and demography. These results reflect the weak population structure observed across East Pacific rookeries, as well as across eastern India (Bowen & Karl 2007). These results are not unexpected based on our previous understanding of the separations between olive ridley breeding populations, and mirrors other highly mobile, broadly distributed oceanic megavertebrates with mixed stock composition (e.g., sharks: Cardenosa et al. 2014; Atlantic bluefin tuna: Carlsson et al. 2006). Analogous with other migratory species characterized by both philopatry of females and opportunistic mating by males, the East Pacific olive ridley breeding population structure is complex (Bowen et al. 1997, Kassahn et al. 2003, Bowen et al. 2005).

Lo46 has previously been recorded at all East Pacific rookeries (solitary and arribada) (Figure 3) except in Peru, and once at Madras, India (Shanker et al. 2004). Only one haplotype has been described in Peru to date, Lo61 (Kelez et al. 2009). Lo61 has been described at all six source East Pacific rookeries (Baja California Sur, Sinaloa, Guerrero, Oaxaca, Costa Rica, Peru), yet in this study we only observed one Lo61 at-sea individual. Similarly, while Lo54 was the distant second most common at-sea haplotype (n=13) observed in our dataset, it has yet to be formally described at any rookery in the East Pacific. These observations illustrate that even with modest sample sizes, gene flow between rookeries and between breeding populations is detectable.

One observation of an Indian Ocean haplotype (Lo74) among eastern Pacific oceanic olive ridleys is interesting, but not extraordinary. Recall that Lo46, the ubiquitous East Pacific haplotype, has been recorded in India; and that Lo27 has been recorded in Australia. Although one in 156—less than 1%—may seem small, we do no believe that this is a rare event. Instead, we believe that because detection from one ocean basin to another occurred in just over 150 samples out of a breeding population of >1.7 million, it reveals that connectivity between distinct breeding populations exists. In other words, we believe that if we were to sequence additional eastern Pacific oceanic olive ridleys, we would find additional haplotypes associated with non-EP rookeries. This could be tested, for example by sampling individuals bycaught by fishing vessels e.g., (Stewart et al. 2016).

We conclude that it is reasonable to assume that any olive ridley sea turtle encountered in the eastern Pacific Ocean is most likely native to an East Pacific rookery; but drawing conclusions about fidelity to a specific rookery is premature. Likewise, it is unreasonable to presume any association between olive ridleys observed in a persistent and distinct oceanographic region (e.g., the Gulf of California) and specific East Pacific rookery. Bowen and Karl (2007) previously suggested that shallow population structure may indicate low site specificity in nesting females, as compared to other sea turtles (e.g., Florida loggerheads: Tucker 2010). While our results cannot test their hypothesis, they do support the consideration of olive ridleys across the eastern Pacific seascape as one panmictic population, a theory pervasive in the literature to date. Strong site fidelity is understood to restrict gene dispersal in sea turtles (Allard et al. 1994), and so future research that describes the degree and variation of fidelity across East Pacific rookeries could refine our understanding of olive ridley population structure and connectivity.

Although Wallace et al. (2011), the IUCN Red List (Abreu-Grobois & Plotkin 2008), and the U.S. Endangered Species Act distinguishes between East Pacific rookeries by nesting strategy (solitary vs. arribada), and East Pacific rookery decline estimates vary dramatically between arribada and non-arribada strategies (as low as 31% vs. upwards of 83%, respectively (Abreu-Grobois & Plotkin 2008)), our mtDNA analyses are not able to distinguish between the contributions of the two rookery types to at-sea stock composition. Literature suggests that isolated management units will thrive or expire without significant input from other populations, thus supporting the need for unit-specific management regimes (Bowen et al. 2005). In this case, encapsulating all East Pacific rookeries into one RMU as does Wallace et al. (2011) appears sensible. Our results provide less support for separate RMUs for disparate nesting strategies, however follow-on studies testing olive ridley site fidelity specific to the two contrasting nesting strategies, as well as more robust mtDNA descriptions of source rookeries, would help to validate solitary- and arribada-specific RMUs.

While useful to understand population structure of nesting beaches, describing the genetic composition of oceanic sea turtles based on haplotype frequencies alone has its limitations, some outlined in Bowen and Karl (2007). One important limitation is that nesting colonies are not always differentiated in haplotype frequencies, in which case feeding aggregations would not be expected to be, either. Mixed-stock analysis is useful in answering specific questions, here revealing that while not often, olive ridley haplotypes from distant breeding populations can turn up on eastern Pacific feeding grounds (e.g., Lo74, this study), and likewise a haplotype from the East Pacific breeding population can be detected on a distant nesting beach (e.g., Lo27, Shanker et al. 2004). Although mtDNA information does not give indication about male dynamics, Lo46 being so common and widespread in the East Pacific olive ridley breeding population across a substantial sample size provides evidence that fine-scale structure is lacking. This is true in other species with greater population structure, such as haplotype CmP4 in foraging eastern Pacific green turtles (Dutton et al. 2008, Amorocho et al. 2012), making precise stock assignment difficult or, as in this study, nearly impossible.

Mechanism for mixing and connectivity is not apparent with maternally-inherited mtDNA data alone, and gene flow between rookeries of the same and/or distinct breeding populations is not quantifiable with our approach. However, we recognize that exchange cannot be insignificantly low since it was detected in this study. Dispersal in highly migratory marine vertebrates is influenced by behavior and ecology (e.g., Antarctic fur seals: Okuyama & Bolker 2005, Hoffman & Forcada 2012), and as such, genetic mixing can occur on ecological timescales much faster than evolutionary timescales (Bowen et al. 1993, Bowen and Karl 2007). Since it is believed that male sea turtles are the conduit for gene flow within and between breeding populations (Karl et al. 1992, FitzSimmons et al. 1997a, FitzSimmons et al. 1997b, Roberts et al. 2004), we can conclude that at least some males are traveling between rookeries and breeding populations. Our observation of one Indian Ocean rookery-associated adult female among the East Pacific breeding population suggested that perhaps it is not only male olive ridleys that occasionally make trans-oceanic migrations. However such a conclusion regarding social female-facilitated gene flow between nesting populations with overlapping oceanic feeding grounds (Bowen et al. 1989, Bass et al. 1996, Bowen and Karl 2007) requires telemetry and/or biochemical tracking validation.

At present, low population structure, high at-sea mixing of olive ridley mtDNA, and large relative population size in the eastern Pacific suggests resilience against genetic bottlenecking, and high haplotype richness of the at-sea composition is indicative of long-term population stability. More robust sampling would help to confirm low haplotype diversity across East Pacific sources rookeries, and validate analyses dependent on categorizing haplotypes frequencies and distributions.

Although we are unable to assign at-sea individuals at a resolution finer than an ocean basin (i.e., to a specific rookery), we are able to gain useful insights regarding how threats specific to regions and/or habitats will affect the population (see Jensen et al. 2013), and regarding scale of management (e.g., RMUs). If olive ridleys showed strong sub-structuring, like salmon, local or regional impacts could potentially wipe out an entire rookery without affecting the entire breeding population. However, because the East Pacific breeding population is well-mixed, local threats could potentially aggregate up and reverberate to other rookeries quite quickly, affecting overall mortality rates at much larger scales than the threat itself. These dynamics could be particularly concerning for catastrophes (e.g., red tides) and disease outbreaks (Ene et al. 2005, Cross et al. 2009).

The study raises a number of questions that encourage additional data collection and analyses of both mtDNA and nDNA. First, are there certain rookeries that are unique sources for any of the low-frequency haplotypes identified in the at-sea composition? To date, Lo62 has only been observed in Costa Rica rookeries. Only a few Lo62 individuals were observed at-sea across the breeding population, but more robust sampling could validate the distribution of Lo62 and other uncommon haplotypes. From the results of this study, probabilities could be calculated to develop testable hypotheses for future studies. Second, is low richness at source rookeries representative of founder events? Theoretically: establishment (e.g., Ixtapilla, Michoacán, Mexico) and re-establishment after genetic bottlenecking (relatedness of individuals increase with associated loss of heterozygosity (Nei et al. 1975)) occurred when this population was greatly reduced due to overharvest between 1960-1990 (Rodríguez-Zárate et al. 2013). Detecting a bottleneck is difficult without pre-bottleneck samples, an illustration of why baseline genetic characteristics are so important for population biology, monitoring, and conservation. Even with a large reduction in population size, if recovery happens quickly genetic variation may not be lost (Queney et al. 2000, Spencer et al. 2000). Due to cessation of commercial fishing and decades of conservation efforts, olive ridleys in the eastern Pacific have recovered to over 1.7 million at-sea individuals (Eguchi et al. 2007) and over 75,000 nesting females (Abreu-Grobois & Plotkin 2008), conservatively around 8% of its assumed pre-harvest nesting population of well over 10 million. While some rookeries are thought to be stabilizing, others are still low and/or in decline, and yet others have not been re-colonized (e.g., arribadas in Jalisco and Guerrero, Mexico: Abreu-Grobois & Plotkin 2008; solitary rookery Mismaloya, Mexico: Rodríguez-Zárate et al. 2013). Lastly, it is unclear if any haplotypes (lineages) are unique to nesting strategy. Addressing these questions on the heels of this study and Rodríguez-Zárate et al. (2013), we can better understand conservation implications of location-based threats and thus prioritize the protection of rookeries and oceanic habitats based on explicit objectives to preserve the stability and resilience of this population as changes such as climate and habitat loss loom.

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Appendix

Anecdotal observations (n=123) of at-sea mating hardshell sea turtles throughout the eastern Pacific, recorded between 1990 and 2006 by the Southwest Fisheries Science Center, La Jolla, CA. Species codes: LO = *Lepidochelys olivacea*, CM = *Chelonia mydas*, CC = *Caretta caretta*, unid = unidentified hardshell sea turtle. LO mating pairs are mapped in Figure 1.

Month	Day	Year	Longitude	Latitude	Species
9	26	1990	-81.95	5.09	LO
9	28	1990	-83.92	8.08	LO
9	28	1990	-83.93	8.12	LO
8	5	1992	-97.23	15.08	LO
8	5	1992	-96.55	15.06	LO
8	6	1992	-97.93	15.14	LO
8	6	1992	-97.38	15.11	LO
8	7	1992	-90.30	13.03	LO
8	8	1992	-87.07	12.01	LO
8	8	1992	-87.72	11.06	LO
8	9	1992	-88.20	10.14	LO
8	10	1992	-89.20	9.13	LO
8	18	1992	-93.03	10.02	LO
8	19	1992	-92.65	10.06	LO

8	20	1992	-93.60	7.08	LO
8	21	1992	-91.43	10.07	LO
8	25	1992	-89.73	13.01	LO
8	31	1992	-90.70	13.14	LO
9	1	1992	-89.40	11.09	LO
9	4	1992	-86.60	9.10	LO
9	4	1992	-86.28	9.06	LO
9	4	1992	-86.13	9.04	LO
9	5	1992	-84.48	8.12	LO
9	10	1992	-86.03	8.16	LO
9	23	1992	-83.18	8.01	LO
9	23	1992	-84.13	8.11	LO
9	24	1992	-84.90	9.08	LO
10	21	1992	-81.52	7.03	LO
10	8	1993	-106.00	20.15	LO
8	19	1998	-105.79	21.92	unid
8	22	1998	-103.67	18.49	LO
8	23	1998	-102.92	17.79	LO
8	25	1998	-97.13	15.09	LO
8	26	1998	-94.99	15.86	LO
8	29	1998	-90.35	11.78	unid
8	30	1998	-90.63	13.65	LO
8	31	1998	-88.97	12.32	LO

9	1	1998	-87.35	11.56	unid
9	3	1998	-84.56	9.15	LO
9	4	1998	-84.02	8.53	LO
9	6	1998	-78.80	7.06	LO
9	6	1998	-80.95	7.09	LO
9	6	1998	-80.94	7.08	LO
9	24	1998	-105.40	18.88	LO
10	4	1998	-100.51	14.66	LO
10	21	1998	-89.70	10.01	LO
10	30	1998	-86.62	6.92	LO
8	10	1999	-108.70	23.58	LO
8	20	1999	-104.73	19.03	LO
9	7	1999	-103.35	16.29	LO
9	13	1999	-99.95	16.74	LO
9	13	1999	-99.93	16.09	LO
9	20	1999	-96.73	14.24	LO
9	28	1999	-85.61	8.35	LO
9	29	1999	-87.30	10.56	LO
9	29	1999	-87.30	10.56	LO
9	30	1999	-85.58	9.57	LO
10	7	1999	-87.25	9.44	LO
10	7	1999	-87.26	9.74	LO
10	7	1999	-87.29	9.92	LO

10	7	1999	-87.13	9.02	LO
10	8	1999	-83.98	8.73	LO
10	8	1999	-83.70	8.27	LO
10	8	1999	-84.08	8.14	LO
10	9	1999	-88.74	10.01	LO
11	22	1999	-88.80	7.09	LO
11	25	1999	-116.89	15.56	LO
8	8	2000	-108.87	21.87	CC
8	12	2000	-106.03	20.14	LO
8	15	2000	-105.77	20.29	LO
8	20	2000	-107.31	17.10	LO
9	7	2000	-98.69	16.18	LO
9	7	2000	-98.69	16.18	LO
9	12	2000	-100.53	16.98	LO
9	26	2000	-94.40	13.01	LO
9	27	2000	-91.22	13.50	LO
9	27	2000	-91.22	13.50	LO
9	28	2000	-88.55	12.16	LO
9	30	2000	-86.14	10.28	LO
10	5	2000	-84.62	9.52	unid
10	10	2000	-87.87	10.11	LO
10	20	2000	-97.49	15.81	unid
11	24	2000	-92.93	5.78	LO

11	28	2000	-104.00	8.77	LO
8	21	2003	-104.85	17.79	LO
9	5	2003	-102.63	17.07	LO
9	6	2003	-100.46	16.92	LO
9	17	2003	-99.60	16.59	LO
9	18	2003	-98.10	16.09	LO
9	18	2003	-98.09	16.10	LO
9	22	2003	-107.07	3.89	LO
9	23	2003	-92.61	13.61	LO
9	26	2003	-88.67	11.02	LO
9	27	2003	-88.89	12.56	LO
9	27	2003	-88.29	12.65	LO
9	27	2003	-88.13	12.67	LO
9	28	2003	-86.70	11.58	LO
10	7	2003	-84.80	8.60	LO
10	11	2003	-91.00	-0.22	CM
10	29	2003	-93.95	15.03	LO
10	30	2003	-95.29	15.77	LO
11	16	2003	-101.29	16.89	LO
11	20	2003	-81.34	7.33	LO
11	21	2003	-83.84	8.52	LO
11	23	2003	-107.50	18.59	LO
8	20	2006	-111.62	16.00	LO

8	20	2006	-111.93	15.61	LO
8	30	2006	-92.48	6.27	LO
9	3	2006	-86.58	8.60	LO
9	19	2006	-85.70	9.23	LO
9	19	2006	-85.91	9.92	LO
9	19	2006	-85.92	10.04	LO
9	20	2006	-87.20	10.54	LO
9	23	2006	-91.85	7.88	LO
9	24	2006	-90.67	9.40	LO
9	26	2006	-89.60	10.77	LO
9	26	2006	-90.00	13.16	LO
9	27	2006	-90.72	13.69	LO
10	3	2006	-90.83	13.72	LO
10	4	2006	-91.67	11.91	LO
10	11	2006	-98.00	10.83	LO
10	29	2006	-98.58	14.58	LO
11	5	2006	-96.70	15.53	LO

III. Formally Integrating Empirical Information and Expert Opinion to Assess Cumulative Impacts on Marine Mammals

Endangered species are threatened by the cumulative impacts of multiple pressures, but management controls of human-caused mortality can be biased towards threats that are easiest to quantify. As such, recovery in some marine mammal populations has been limited despite single-threat reduction efforts such as the cessation of commercial whaling and fisheries bycatch quotas. Certain pressures exert disproportionate effects, but empirical studies measuring effects to marine mammals, especially highly mobile and pelagic species, are sparse. Here, we present an approach that formally quantifies individual, interacting, and cumulative impacts of multiple human activities by integrating empirical data and expert knowledge. Using the endangered Southern Resident Killer Whale (SRKW) population as a case study, we use structured expert surveys to estimate the relative influences of multiple pressures on vital rates. With this approach, we estimate per capita annual mortality rates for five threats contributing to lethal injury and find that anthropogenic SRKW mortality exceeds the limit of 10% Potential Biological Removal (PBR) set by the U.S. Marine Mammal Protection Act. Our conclusion contrasts the findings of the most recent U.S. stock assessment for SRKW that measures PBR against two threats, fisheries bycatch and vessel interactions, suggesting human-caused mortality is insignificant and approaching zero. Accounting for the discrepancy are indirect effects of acute chemical pollution, acute noise, and marine debris interactions previously unquantified. This natural and social science integrated approach innovates the concept and use of 'best available science' mandated by environmental laws and has wide applicability to marine mammals and other taxa worldwide.

A. Introduction

Recovery in many marine mammal populations has remained limited (1-5) despite single-threat reduction efforts such as the cessation of whaling and the reduction of fisheries bycatch (6-9). It is generally accepted that it is the cumulative effects of multiple pressures that precludes recovery (5, 10-13). Unfortunately, empirical data for both marine mammal populations and the anthropogenic stresses they are exposed to are poor (5, 14). Therefore the magnitude of individual and combined threats that influence behavior, survival and fitness remain largely unknown (15). Plagued by quantitative data gaps, particularly for indirect and sub-lethal effects, the implementation of top-down management controls of marine mammal mortality is not comprehensive. By mandate, environmental management and policy decisions must be based on quantitative evidence, making up "best available science" (16-18). Since existing empirical studies have not yet quantified how multiple impacts affect marine mammal vital rates, management proceeds with partial information. Here, we present a more comprehensive way to inform management by integrating empirical data with expert opinion to assess how pressures accumulate and interact to impact population vital rates. We relate our findings to a current top-down mortality management tool, Potential Biological Removal (PBR).

In the late 1990s the U.S. Marine Mammal Protection Act (MMPA) defined PBR, an algorithm used to control anthropogenic marine mammal mortality. PBR is transparent, conservative and broadly practical in real-world management, which is why it has also been used in other countries around the world (19, 20). PBR is the number of individuals considered safe to remove from a population, above and beyond natural mortality, without causing depletion[4]. PBR is calculated by the maximum theoretical or estimated intrinsic growth rate at a small population size divided by two ($R_{max}/2$), multiplied by the minimum current population size estimate (N_{min}) and the target population level recovery factor (F_R, a number between 0.1 and 1): $PBR = N_{min}(R_{max}/2)F_R$ (21-23). $R_{max}/2$ is an estimate of the population growth rate when it is at the *Optimal Sustainable Population*, defined as half the current environmental carrying capacity. For endangered populations the MMPA sets $F_R = 0.1$ (24). In general, PBR estimates sustainable anthropogenic mortality scaled to the overall size of the population and takes into account uncertainty in both abundance measurements; bias in our understanding of the population's structure; carrying capacity (K); and ecosystem health. Accurate parameter estimation is crucial for calculating an appropriate PBR. PBR has been criticized for assuming unrealistic population dynamics and the rule's utility and limitations have been discussed in the literature (8, 25, 26). The following provides a list of assumptions of concern: the current population size is known; K is constant (see 26); the population is closed, at a stable age distribution, and experiences logistic growth. These assumptions are often not reasonable for long-lived cetaceans. PBR also applies a blanket population target of 0.5K for all marine mammals, and some have argued that population targets should vary between species and populations (e.g., 25). Regardless of these limitations, PBR has been effective in aiding recovery when fisheries bycatch is the main threat (see 14). However, its effectiveness varies greatly when indirect and sub-lethal threats exceed the threat of bycatch or other direct threats.

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[4] Depletion is defined as abundance below the maximum net productivity level (MNPL). MNPL is assumed to be between 50% and 70% of the historic population size.

PBR is inherently bias towards direct threats because it's original intention was to calculate mortality limits for marine mammal interactions with fishing operations (21). However as soon as the rule was formally included in U.S. policy there was an immediate need to estimate and permit marine mammal "takes" for other anthropogenic mortality sources, as well. With perfect information regarding mortality, a PBR-based control would theoretically work seamlessly. In reality we lack much information regarding indirect and cumulative impacts of threats, and thus new management tools and approaches under the umbrella of ecosystem-based management are needed.

Over the past two decades a body of analytical cumulative impacts marine research has emerged inspiring new ways to tackle objective-based management of the modern ocean (1, 11, 12, 27-33). Human use of marine space, habitats and species are growing each year, and inaction due to data limitations is unsustainable (34, 35). Indeed, decision-support priorities for the Coastal and Marine Spatial Planning strategic action plan of the U.S. National Ocean Council include compiling data, models and other information; and identifying gaps relative to assessing cumulative impacts, interactions among human uses and stressors, non-linear responses of systems to increasing human use and natural forces, and developing user-friendly, open-source transparent management tools (35-37). U.S. Ocean Policy and its Implementation Plan calls for integrated and interdisciplinary research to determine the influences of multiple and interacting stressors to improve the efficacy and adaptability of management rules aimed at reducing the cumulative natural and anthropogenic impacts on marine ecosystems (37, 38). In this paper we present a conceptual model and research framework that quantitatively estimates multiple impacts, even for data-poor populations, using an integrated natural and social science approach. We define a general conceptual model in which 16 human activities create 13 threats that act simultaneously in various combinations of type and magnitude to generate six stressors. Those stressors along with natural factors influence marine mammal population vital rates. With this approach it is possible to prioritize action(s) and research need(s) with respect to specific conservation and/or multi-use objectives within an ecosystem-based management framework for any marine mammal population in any ocean. We test proof-of-concept by applying the approach to a case study on endangered Southern Resident Killer Whales in the northeast Pacific Ocean.

We asked 16 experts to provide best estimates, lower and upper bounds around their estimate, and their confidence in their estimate for each link in the model. Experts ranked nodes and estimated historic and current carrying capacity. When possible, we used empirical data as a quantitative anchor to estimate annual rates for each model link. To conclude, we discuss the general utility, scope and limitations of our cumulative impacts assessment approach for marine mammals and hope it inspires continued advancement of adaptive management.

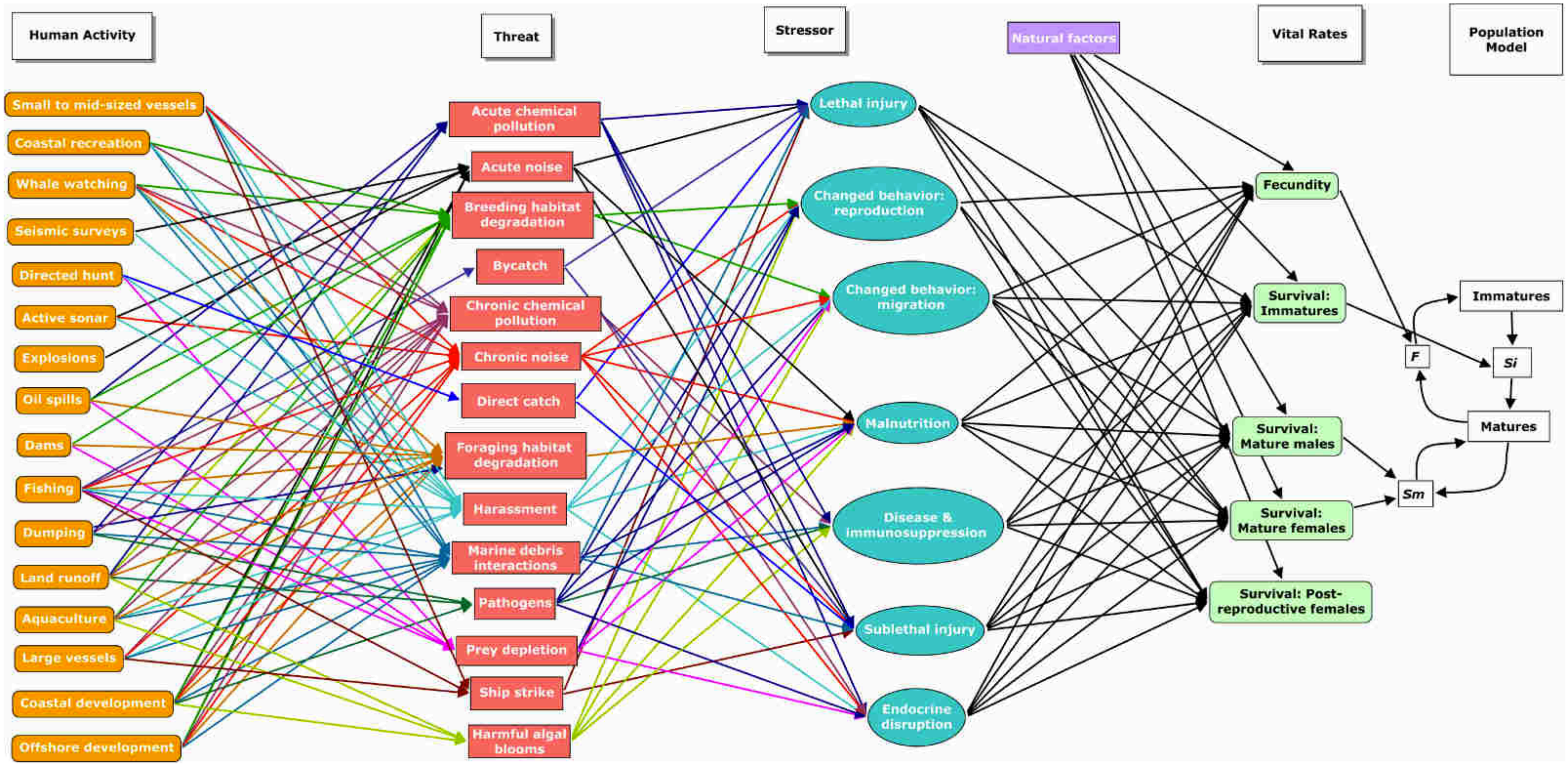
B. Materials and Methods

1. Conceptual Framework to Identify Pathways of Multiple Impacts

We drew from previous works that have conceptually modeled the pathways of multiple pressures, quantified direct impacts, and used expert opinion to rank threats for at-risk species (27, 49, 51). First, we developed working definitions for important terms that underpin our approach. A full list of definitions can be found in Appendix II, and in the survey available online here: https://www.surveymonkey.com/s/CumulativeImpacts_KillerWhales. Definitions for the conceptual model categories are as follows: A “threat” is a possibly deleterious interaction between a human activity and a marine mammal or its surrounding environment. A “stressor” is the biological, behavioral, or physiological consequence of the presence of one or more threat. “Natural factors” are non-anthropogenic influences of individual fitness and survival and population vital rates, including predation, competition, natural pathogens, naturally occurring bio-toxins, intra- and interspecific aggression, natural climate variability, and others.

We constructed a general conceptual model (Figure 1) that identifies how 16 present human activities pose 14 threats, which in turn generate 7 stressors that potentially affect a specific marine mammal in a given area. On a case-by-case basis, threats interactions may be incorporated, however interactions are not inherently included / assumed in the general model. Finally, we map how stressors along with natural factors influence the vital rates of a given marine mammal population. Depending on the life table of the population of interest, the number and type of vital rates can be amended, and in turn the stressor(s) to vital rates links. This general conceptual model serves as a multi-faceted, comprehensive framework transferable to any marine mammal population in any ocean, and it is updatable as relationships and/or data change over time and/or space. To apply this framework to a specific case study, one must turn nodes and links of the network ‘on’ and ‘off’ as applicable for the population of interest.

Figure 5. This schematic represents a general conceptual model where 16 present human activities pose 14 threats, which in turn generate 7 stressors that potentially affect a specific marine mammal in a given area.



2. Case Study: Endangered Southern Resident Killer Whales

To illustrate how our approach can quantify relative effects of natural and anthropogenic threats and stressors we apply it to a case study on endangered Southern Resident Killer Whales. Southern Resident Killer Whales are a genetically distinct population of *Orcinus orca* found in the northeast Pacific Ocean (52) that is protected under the U.S. Endangered Species and Marine Mammal Protection Acts, and the Canadian Species at Risk Act since 2005, 1972, and 2001, respectively. Both countries have legally designated Southern Resident critical habitat, in the U.S. since 2006 and in Canada since 2008. As a species, orcas are apex predators found in every ocean and receive protection under two international agreements, Appendix II[5] of the Convention of International Trade in Endangered Species of Wild Fauna and Flora (53) and Appendix II (Migratory species conserved through Agreements) of the Convention on the Conservation of Migratory Species of Wild Animals (54). The IUCN lists orcas globally as Data Deficient, but the Southern Resident population is recognized as endangered (55).

Southern Residents are specialist consumers, dependent mostly on Chinook salmon (*Oncorhynchus tshawytscha*), particularly in the summer (18, 56, 57). Reports have estimated that Southern Resident Killer Whale carrying capacity was 97-140 individuals prior to when extensive wild whale collections began in 1967 for public displays (18, 58), but the U.S. National Oceanic and Atmospheric Administration (18) has called for improved robust estimates of historic abundance and current carrying capacity (18). Genetic analyses

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[5] CITES Appendix II includes species not necessarily threatened with extinction, but in which trade must be controlled in order to avoid utilization incompatible with their survival.

of museum collections suggest that the historical range of Southern Residents is similar to what it is today: California to Southeast Alaska (53, 59).

When Southern Residents were first censused in 1974 at the end of wild collections they had depleted to 71 individuals (60). That rapid initial population decline of 25-30% was disproportionately made up of calves targeted for public display, which altered the age structure of the small population and, along with other factors, is thought to have played a role in the periodic trends of recovery and decline in the years that followed (58). But by 1995 the population had recovered 74% and was back up to pre-collection numbers (61). Suddenly between 1996 and 2001 the population dropped again by 15-20% down to ~80 (18, 58), which was later found to be correlated with a decline in Chinook salmon due to unfavorable El Niño conditions and continued fishing pressure (43, 61). This five-year decline is what prompted the U.S. to declare Southern Residents an endangered distinct population segment under the ESA in 2005. Although the population has bounced back to 84 individuals (62) it is still believed to be under carrying capacity. Chinook salmon in the Sacramento River winter-run and the Upper Columbia River spring-run have been endangered since 1994 and 1999, respectively, and are threatened in several Oregon, Idaho, and Washington state waterways.

Given this history, prey depletion is a major concern for the viability of this population (18, 43, 46, 61, 63). Also of concern are acute (e.g., oil spill) and chronic (e.g., bio-accumulated DDT, PCBs, and PBDEs) pollutants—it is among the most contaminated marine mammal populations in the world (18, 64). In addition, there is evidence that when vessels (motorized and/or un-motorized) are nearby, Southern Residents reduce their time spent foraging and travel more (65), altering their energetics and stress levels. Further, ships produce elevated background noise at a range of frequencies within orca hearing, potentially interfering with both communication and echolocation. These and other threats act simultaneously over space and time. For example in the Salish Sea, critical habitat for Southern Residents during spring through fall, there is continual threat of oil spill with increasing oil tanker traffic with as many as 20 ships transiting each day (32, 66), underwater noise from ship traffic (67), interaction with whale watching vessels (18, 68), and an inadequacy of Chinook salmon abundance. Multiple population viability analyses show the same general conclusion: if the status quo continues, Southern Residents are in danger of quasi extinction; if any major threats (e.g., oil spill) increase, extinction is almost inevitable in as few as 100 years (63, 69).

Even though this population is one of the most well studied marine mammal populations in the world, and it is well understood that the population is exposed to multiple pressures, managers are still unsure which threats are responsible for limiting the population’s recovery (18, 46). It is impossible to eliminate all threats, and therefore the over-arching goal of recovery is to reverse population decline; specifically that means raising the population growth rate to ≥1.0 for several generations. We know that older adult males and reproductive females tend to be more successful in reproducing and caring for calves, which suggests that the survival of males and reproductive females are key vital rates to focus on (18, 39, 70). Understanding the relative effects of pressures on those vital rates in particular may shed light on which threats are limiting recovery.

3. Literature Review

We completed a literature review to synthesize existing empirical studies, white papers, government documents, and unpublished reports. From this product, we specified the general multiple impacts model for Southern Resident Killer Whales and compiled empirical estimates for each node and link, as available.

4. Expert Elicitation and Consensus

Using SurveyMonkey® (www.surveymonkey.com), we administered two voluntary online surveys to 16 killer whale experts with >300 years of combined experience to ask a series of questions regarding the SRKW model. Studies have suggested that 8-15 experts are a viable number and that findings do not change significantly with >15 experts (71). We conducted these confidential surveys in 2013 and 2014 with permission and guidance from the Arizona State University Human Subjects Institutional Review Board (ASU IRB #1304009117), through the ASU Office of Research Integrity and Assurance, and the University of California (UCSB Pro Number MSII-HA-BE-011-2N, Submission ID 13-0581). Following the first survey, we held a three-hour "consensus webinar" on 21 August 2013 using WebEx^{TM} (www.webex.com) software and recorded the audio. 10 of the 16 experts participated in the webinar. Experts could identify each other and discuss openly. During the webinar we presented the aggregate results from the first survey to the experts without identifying individual responses. We then moderated discussion regarding definitions, wording of questions, and the results. We used feedback from the consensus to revise the survey. A second, updated survey was administered to all 16 experts and can be viewed here: https://www.surveymonkey.com/s/CumulativeImpacts_KillerWhales.

We asked experts to base their responses on what they know of the SRKW population and environmental conditions over the last 30 years (i.e., baseline reference), and to make assessments of how the identified threats, stressors and natural factors will impact the population over the next three generations (i.e., a ~75-year time horizon). We first asked experts to rank anthropogenic threats and stressors, aside from natural factors, and vital rates in order of their influence on the population, with 1 being the most influential. We gave the option to answer “not applicable” if the expert believes the influence is negligible or zero. We then asked for best estimates (i.e., educated guess for the mean) and lower and upper bound around the estimate for each link in the SRKW model. For each question regarding a rank or best estimate, we asked the expert to indicate their overall confidence (a measure of uncertainty) in their answer between 0% and 100%. Threat, Stressor and Vital Rate ranks and estimates were weighted by expert-stated confidence, using the following algorithm where n = expert, E_n = expert estimate, and C_n = expert confidence:

$$\textbf{Rank or Best Estimate} = \frac{\boldsymbol{\Sigma(E_n * C_n)}}{\boldsymbol{\Sigma C_n}} \qquad \text{Eq. 1}$$

Threat and Stressor percent influences (I) upon a given Stressor or Vital Rate, respectively, were scaled between 0% and 100% by multiplying the Best Estimate defined by Eq. 1 by a scalar defined as:

$$\text{Scalar} = \frac{100}{\Sigma \text{Best Estimate}} \qquad \text{Eq. 2}$$

With these surveys, weighting and scaling approaches, we captured ranks, mean influence estimates, uncertainty for each rank and mean estimate, and upper and lower bounds around each mean estimate.

5. Integrating Expert Opinion and Empirical Data

We validated expert ranks (nodes) with published severity and importance categorizations (45). All expert ranks matched published information, so for this case study we did not need to adjust any ranks. We also used published data to anchor expert opinion.

When data was available as a rate for a particular link (e.g., observed bycatch, vessel strike), we effectively weighted empirical data more than expert opinion and used proportions of expert-estimated percent influence to empirical rates to solve for unknown rates. For example, for the five threats (acute chemical pollution, ship strike, acute noise, bycatch, marine debris interaction) that lead to the "Lethal Injury" stressor, we identified proportional relationships between weighted mean expert influence estimates (I) and the known per capita annual mortality rate (M): $I_1M_2 = I_2M_1$. Since all I variables have values derived from expert opinion, only one proportional relationship (I_1/M_1) is needed in order to solve for all other M values (Table 1):

Table 1. This is an example of the integration of expert opinion and empirical data using one link in the multiple impacts model: threats → Lethal Injury. Knowing that ship strikes cause a 0.0014 annual lethal injury rate, that is set equal to 30.8% contribution based on expert estimates, and then all other annual lethal injury rates can be solved for.

	Mean confidence of expert estimates of percent influence of Threat to Lethal Injury, weighted by their overall confidence of Lethal Injury	Mean percent influence (I) of Threat to Letha Injury (out of 100%), weighted by expert confidence (C)	Empirical estimate of annual anthropogenic Lethal Injury rate is starred, estimated annual rates based on expert estimates are not starred
Threat	C	I	M
Acute Chemical Pollution	0.70	34.4%	**0.0016**

Ship Strike	0.71	30.8%	**0.0014***
Acute Noise	0.65	17.6%	**0.0008**
Bycatch	0.70	9.3%	**0.00042**
Marine Debris	0.65	7.9%	**0.00036**

C. Results

Expert ranks of Southern Resident threats, stressors, and vital rates are listed in Table 2, including range of rank responses and average expert-stated confidence. Final expert threat ranks are as follows: (1) prey depletion, (2) chronic chemical pollution, (3) chronic noise, (4) harassment, (5) acute chemical pollution, (6) acute noise (tie), (6) pathogens (tie), (8) breeding habitat degradation, (9) foraging habitat degradation, (10) ship strike, (11) fisheries bycatch, (12) marine debris interactions (tie), (12) harmful algal blooms (tie). Stressor ranks are as follows: (1) malnutrition, (2) endocrine disruption, (3) disease & immunosuppression, (4) lethal injury, (5) changed behavior: reproduction, and (6) sub-lethal injury. Vital rates are as follows: (1) fecundity and survival of mature females, (3 & 4) survival of mature immatures and mature males, and (5) post-reproductive females. Expert ranks of threats, stressors and vital rates align with categorizations available in government reports (see Table 3).

Table 2. Threats, stressors, and vital rates ranked according to their likely influence on the Southern Resident Killer Whale population dynamics over the next three generations (~75 years). Influences were ranked via expert opinion. Final ranks were calculated by weighting each expert rank by their stated confidence in their estimate. Ranks were validated with published categorizations as available. The ranges of expert ranks and mean expert confidence of ranks (out of 100%) appear in brackets, respectively.

Rank	Threat	Stressor	Vital Rate
1	prey depletion [1-3, 80%]	malnutrition [1-5, 80%]	fecundity [1-4, 80%]
2	chronic chemical pollution [1-7, 80%]	endocrine disruption [1-4, 70%]	survival: mature females [1-4, 80%]
3	chronic noise [1-3, 80%]	disease & immunosupression [2-6, 70%]	survival: immatures [1-5, 80%]
4	harassment [2-8, 80%]	lethal injury [1-6, 80%]	survival: mature males [1-5, 70%]
5	acute chemical pollution [1-12, 70%]	changed behavior: reproduction [2-6, 70%]	survival: post-reproductive females [4-5, 80%]
6	acute noise [3-9, 70%] (40)	sub-lethal injury [3-6, 70%]	
6	pathogens [3-11, 70%] (40)		
8	breeding habitat degradation [8-13, 70%]		
9	foraging habitat degradation [3-12, 70%]		
10	ship strike [4-13, 70%]		
11	fisheries bycatch [9-13, 70%]		
12	marine debris interactions [9-13, 70%] (40)		
12	harmful algal blooms [7-12, 70%] (40)		

Table 3. This is an example of the integration of expert opinion and empirical data for one link in the multiple impacts model: Threats → Lethal Injury. PBR stands for Potential Biological Removal and is calculated as the minimum population size (N_{MIN} = 85) times one-half the default maximum growth rate for cetaceans (0.5 of 3.2%) multiplied by an endangered stock recovery factor of 0.1 (24, 40).

	Expert rank of Threats influencing Lethal Injury, weighted by expert confidence	Published categorization of threat	Mean confidence of expert estimates of percent contribution of Threat to Lethal Injury, weighted by their overall confidence of subject of interest	Normalized mean percent contribution of threat to Stressor (out of 100%), weighted by expert confidence	Empirical estimates of annual anthropogenic Lethal Injury rate between 2004 and 2011 is starred, estimated annual rates based on expert estimates are not starred.	Per capita number of Lethal Injury whale deaths per year, based on N_{MIN} = 85.	Exceeds 10%(PBR)?	References	Notes
Threat	**Stressor: Lethal Injury**								
Acute Chemical Pollution	1	Medium to High with low to high probability	0.70	34.4%	**0.0016**	**0.13**	**YES**	Ross et al. 2000; Krahn et al. 2004; Ylitalo unpubl. data; NOAA 2008 & 2011	
Ship Strike	2	Medium to High & increasing	0.71	30.8%	**0.0014***	**0.14**	**YES**	Baird 2001; Carretta et al. 2010; Williams & O'Hara 2010; NOAA 2011	One lethal ship strike was observed in 2006. NMFS 5-Year Status Review concludes zero non-fishery related human-caused deaths from 2007-2011.

Acute Noise	3	Medium to High	0.65	17.6%	**0.0008**	**0.07**	**YES**	NOAA 2011	
Bycatch	4	None to Low	0.70	9.3%	**0.00042**	**0.04**	**YES**	NMFS 2008; Caretta et al. 2010; NMFS 2011	Annual observed bycatch rate is zero, however that is considered an underestimate.
Marine Debris	5	Low & increasing	0.65	7.9%	**0.00036**	**0.03**	**YES**	Williams et al. 2011	
Overall		PBR = 0.14 whales/yr; 10%(PBR) = 0.014 whales/yr	0.68	100%	**0.0047**	**0.40**	**YES**	Carretta et al. 2014	NMFS measures PBR against zero human-caused mortalities between 2007 and 2011. This study's estimates lethal injury annual mortality between 2004-2011.

For Southern Resident Killer Whales NOAA assumes $N_{min} = 85$, $R_{max} = 3.2\%$ and $F_R = 0.1$, resulting in a PBR of 0.14 whales per year (40-43). This is equivalent to the removal of one whale every 7 years. There has been no recorded direct mortality or serious injury caused to this population by fisheries (Table 4). In 2006 one whale was killed during a vessel interaction, and no other deaths from vessel interactions or any other non-fisheries sources have been recorded since. The most recent Southern Resident Killer Whale stock assessment estimates that human-caused mortality between 2007 and 2011 is zero and therefore does exceed 10% of PBR and is thus considered insignificant and approaching zero under the MMPA (40). However, in reality Southern Resident whale deaths are observed almost every year (44) (see “Observed Deaths” column of Table 4), and so we understand that commercial fisheries bycatch and vessel interactions are not the only threats that impact Southern Resident Killer Whale survival. While the NOAA Southern Resident stock assessment, population recovery plan and status review acknowledge indirect affects on survival, they are not considered when measuring recorded mortalities and serious injuries against PBR (40, 41, 45, 46). We consider all effects in this study and compare our results to the NOAA Southern Resident Killer Whale stock assessment’s conclusion regarding PBR. Empirical and expert integrated relative contribution estimates for Southern Resident lethal injury are summarized in Table 3. Knowing that one whale died from a vessel strike in 2006, we estimate the annual mortality rates for each of the five threats that contribute to lethal injury between 2004 and 2011 using expert estimates of relative influences to lethal injury (see Table 1). Although NOAA only considered five years of data (2007-2011) in the latest Southern Resident Killer Whale stock assessment (40), we chose to include eight years of data based on the National Marine Fisheries Service’s 2011

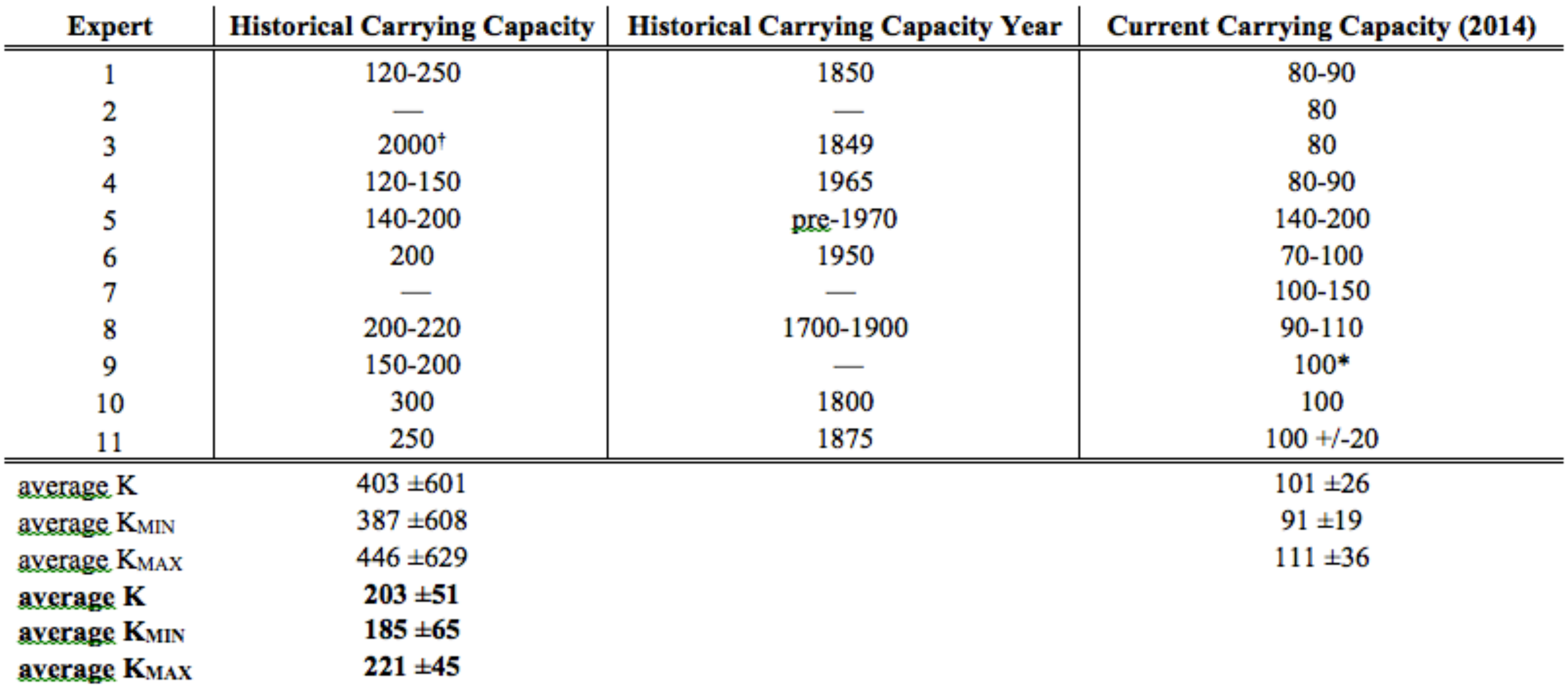
Guidelines for Assessing Marine Mammal Stocks to attain approximately unbiased (median absolute bias <25%) estimates of average annual fisheries-related mortality and serious injury, relative to PBR (see Table 4). The recommendation is to pool eight years of data when observer coverage is 10% and PBR is ≥1. The average per capita annual death rate is virtually the same when averaged across 5 and 8 years (Table 4). In doing so we find a 0.0047 cumulative mortality lethal injury rate across five threats, equivalent to 0.4 whale deaths per year (Table 3). The mortality rate from this one stressor alone exceeds 10% of PBR, as do each of the five threats that contribute to lethal injury, which under the MMPA means that human-caused lethal injury cannot be considered insignificant and approaching zero, and the stock is not meeting the MMPA's zero mortality rate objective.

11/16 experts provided estimates of historic carrying capacity and year, and current (2014) carrying capacity in Table 5. Average historic carrying capacity was estimated to be 203 ±51. The range of years given for the historical carrying capacity estimate was 1700-1950. Average carrying capacity in 2014 was estimated to be 101 ±26.

Table 4. Southern Resident Killer Whale abundance, births and deaths have been recorded by the Center for Whale Research (whaleresearch.com and orcanetwork.org) since 1998. Those estimates are compiled here, as well as observed vessel strikes and bycatch rates. The observer coverage, when known, in these fisheries is 10%. The annual per capita mortality and lethal injury rates are calculated, and the average (μ) rates are calculated for the five-year span (2007-2011) used in the 2013 Southern Resident Killer Whale stock assessment (40), highlighted in gray, and the 8-year span (2004-2011) used in this study, outlined in black.

	Southern Resident Killer Whale Population							Fisheries Bycatch	
Year	Population	Observed Births	Per Capita Birth Rate	Observed Deaths	Per Capita Mortality Rate	Empirical Lethal Injury Rate	Vessel Strikes	WA Salmon Drift Gillnet Fishery	WA tribal coastal drift gillnet salmon fishery
t	N	B	B/N	D_{obs}	D_{obs}/N	F+S/N	S	F	F
1998	89	2	0.022	6	0.067	0	0	0	0
1999	85	2	0.024	4	0.047	0	0	0	0
2000	82	0	0.000	7	0.085	0	0	0	0
2001	79	4	0.051	0	0.000	0	0	0	0
2002	80	1	0.013	2	0.025	0	0	0	0
2003	82	4	0.049	3	0.037	0	0	0	0
2004	83	4	0.048	0	0.000	0	0	0	0
2005	91	2	0.022	3	0.033	0	0	0	0
2006	89	1	0.011	6	0.067	0.011	1	0	0
2007	86	3	0.035	1	0.012	0	0	0	0
2008	85	1	0.012	8	0.094	0	0	0	0
2009	85	4	0.047	1	0.012	0	0	0	0
2010	86	5	0.058	6	0.070	0	0	0	0
2011	87	2	0.023	0	0.000	0	0	0	0
2012	85	2	0.024	7	0.082	0	0	0	0
2013	82	0	0.000	1	0.012	0	0	0	0
2014	85	1	0.012	4	0.047	0	0	0	0
Total		38		59	μ = 0.036	μ = 0.0014	1	0	0
					μ = 0.037	μ = 0			

Table 5. 11/16 experts provided estimates of historical carrying capacity (K), historical K year, and current K for the year the survey was completed (2014). * indicates that the expert expressed great uncertainty about the estimate. Average K, K_{MIN}, and K_{MAX} ± standard deviation are calculated at the bottom of the table. For historical K, the first three averages include all expert estimates, while the second set of averages in bold omit expert 3's estimate, indicated with †, as an outlier because it greatly reduces standard deviation.



Expert	Historical Carrying Capacity	Historical Carrying Capacity Year	Current Carrying Capacity (2014)
1	120-250	1850	80-90
2	—	—	80
3	2000†	1849	80
4	120-150	1965	80-90
5	140-200	pre-1970	140-200
6	200	1950	70-100
7	—	—	100-150
8	200-220	1700-1900	90-110
9	150-200	—	100*
10	300	1800	100
11	250	1875	100 +/-20
average K	403 ±601		101 ±26
average K_{MIN}	387 ±608		91 ±19
average K_{MAX}	446 ±629		111 ±36
average K	**203 ±51**		
average K_{MIN}	**185 ±65**		
average K_{MAX}	**221 ±45**		

D. Discussion

In 2009 NOAA identified the need for a conceptual framework that can account for the cumulative impacts of multiple stressors acting on marine mammal populations and has prioritized developing innovative assessment approaches (18). Governments have invested resources in understanding indirect and sub-lethal effects, for example underwater noise (47), but no tangible updates have been made to the way human-caused mortalities are managed since the 1990s. With this study we propose one way to assess cumulative impacts by integrating empirical data and expert opinion. We apply the approach to endangered Southern Resident Killer Whales to rank threats, stressors, and vital rates, and to estimate historic and current carrying capacity. While all of the current threats directly and indirectly affecting Southern Resident vital rates have been identified, the *relative contributions* of each threat have not yet been quantified and so only recorded mortalities from fisheries bycatch and ship strike threats are measured against Potential Biological Removal (PBR). We have identified 11 *additional* threats and two interactions (see Appendix I G) that were unaccounted for but are cumulatively responsible, along with natural factors, for the 28 deaths between 2005 and 2011 not considered against PBR in the 2013 Southern Resident Killer Whale stock assessment.

With our cumulative impacts assessment we find that when all five direct and indirect threats producing the one of six stressors are considered against PBR, lethal injury, the U.S. MMPA zero mortality rule is not met, which contrasts the conclusion of NOAA's Southern Resident Killer Whale stock assessment that mortalities and serious injuries[6] from

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[6] NOAA defines "serious injury" as an injury that is more likely than not to result in mortality (61).

interactions with fisheries and vessels are insignificant. This discrepancy is because this study and NOAA's stock assessment are comparing PBR to cumulative and partial quantifications of mortality, respectively. We do not believe the authors of the stock assessment or killer whale experts will find this result surprising, as the implementation of PBR is limited to observations of direct threats and therefore does not account for multiple and interacting threats acting simultaneously and accumulate over time, particularly indirect effects (e.g., contaminants) (26, 47).

This mismatch of management rule and reality suggests that PBR alone is inadequate to manage cumulative impacts, and illustrates is why the use of PBR for Southern Residents and other marine mammals may not be as conservative as it should be. For this case study, cumulative estimates of specific-threat mortality are very small and actual threats could be much smaller or much larger depending on uncertainty and stochasticity. We have largely ignored this variability for now to show proof-of-concept, however if this comprehensive integrative approach were to be used for management deviations should be quantified and considered.

1. Summary of cumulative impacts assessment for Southern Resident Killer Whales

Experts identified prey depletion, chronic chemical pollution, chronic noise, and harassment as the top four most detrimental threats to Southern Resident Killer Whales. This is in-line with threat concerns discussed in the population's recovery plan and status review in the U.S. and recovery strategy in Canada. Also important to note are the three threats identified as least threatening, fisheries bycatch, marine debris interactions, and harmful algal blooms. In light of this, Southern Residents are particularly useful in illustrating that when fisheries bycatch is not a major threat to a particular marine mammal population, PBR as currently used may be ineffective for assessing mortality limits. The only known Southern Resident Whale direct mortality came from a collision with a tugboat in 2006, and in this assessment the threat of ship strike ranks 10th out of 13. There is speculation that that death may have resulted from prolonged harassment and/or habituation to vessels (40). Nonetheless, 44 Southern Residents have died since 2005, 43 due to natural factors or threats other than fisheries bycatch and ship strikes and thus not measured against PBR.

While harmful algal blooms tied with marine debris interactions as least important threats to Southern Residents, it was also the threat that experts were consistently least confident in estimating how it influences specific stressors. For example, while experts were ~63% confident in estimating how both chronic chemical pollution and chronic noise influence change in reproductive behavior (~19% and ~16%, respectively), they were only ~37% confident that harmful algal blooms have less influence (~11%), uncertainty also captured with the large bounds around the weighted estimate. Harmful algal blooms are a threat that is increasing across the globe and may require additional research before ignoring.

Acute chemical pollution was ranked 5th, followed by acute noise and pathogens. This is interesting considering some population viability analyses show that an acute chemical pollution event, such as an oil spoil, would have the largest negative effect on Southern Resident Killer Whales and could induce extinction. Acute noise seems to be a consistent worry in regards to military and construction operations and has received much research attention to date. Their relative middle-of-the pack rank could be because while perhaps both threats are great, the probability of occurrence is low. Likewise, breeding and foraging habitat degradation ranked 8th and 9th out of 13, respectively. This may be reflective of years of critical habitat protection by both the U.S. (2006) and Canada (2008), and/or that this population does not migrate long distances and therefore threats and protections work rather uniformly across space and time.

Malnutrition, endocrine disruption, and disease & immunosuppression are the top three stressors that experts believe are impacting Southern Resident Killer Whale vital rates, followed by lethal injury, changed reproductive behavior, and sub-lethal injury. This explains why a fisheries- and/or serious injury-focused mortality reference point such as PBR is not as effective or conservative as it should be for Southern Resident Killer Whales. The implementation of the rule essentially ignores four of the six stressors, including the top three.

Adult males and reproductive females have been shown to be important for reproduction and calf rearing, which implies that their survival along with fecundity might be most important to the success of the population. Confidence-weighted expert ranks agree, ranking fecundity and survival of mature females most important. Interestingly the survival of mature males and immatures are tied for second-most important. There may be several reasons for this nuance, and while not explored in this study could be a fruitful area of further research. Possibilities include recovering a stable age distribution since it was altered when 76% of the calves produced between 1959 and 1970 were captured for display, reproductive potential concerns, or others.

Our findings for Southern Resident Killer Whales may be of interest on the heel of one of the strongest El Niño of record, given that prey depletion and competition with other salmon predators for resources are of major concern for the sustainability of Southern Residents. It remains to be seen how threatened and endangered populations of Chinook will respond to the impending combined pressures of salmon fishing and 2015-2016 El Niño conditions.

2. Utility and applications

In the five-year review of the 2008 Recovery Plan for Southern Resident Killer Whales, authors state that, “Although the population has been studied for more than 30 years, we are not certain which threat is the most important to address in order to ensure recovery. The Recovery Plan therefore addresses each of the threats based on the best available science. NMFS has linked the management actions in the Recovery Plan to research and monitoring actions to gather information, to inform prioritization, refine recovery actions, and identify new actions as needed.” These types of formal reviews are important to assess progress that management rules and actions are making towards species recovery objectives. In this situation, we argue that while the Southern Resident Killer Whale five-year review was thorough, it highlights that collectively and collaboratively as scientists and managers, our view of the ‘best available science’ needs to be more comprehensive, especially in data-poor situations. The multiple impacts framework and data synthesis approach presented here show how expert knowledge can be incorporated, both qualitatively and quantitatively, to augment empirical information and generate quantitative priors for data gaps. We believe that the Southern Resident Killer Whale case study illustrates how empirical data and expert opinion combined can make up the best available science. We have presented one set of analyses to complete the cumulative impacts assessment, however within the framework we have developed there are perhaps several other viable analytical solutions a user might choose based on case study-specific objectives and available data. Similar but variant approaches that integrate expert and empirical information for cumulative impact assessments have been undertaken for the Mojave Desert Tortoise (48, 49) and the Pacific walrus (30, 50), as well as others.

Developing comprehensive management tools for marine mammal management is urgent as the uses of the ocean increase, and new threats such as ocean acidification and warming seas loom. The need is even more apparent for endangered and threatened species, and for populations whose main threats are not the easiest or most direct to quantify. A natural and social science integrated approach innovates the concept and use of 'best available science' mandated by environmental laws and has wide applicability to marine mammals and other taxa. The good news is that combined, we have the empirical, institutional, and expert knowledge to move forward with such necessary management innovations. The U.S. Endangered Species and Marine Mammal Protection Acts can be powerful ways to evoke action to reduce threats, and this case study shows that if our evaluation tools are not able to show violations of those legislations, action may be slow or inadequate. An amendment to current law and policy to update marine mammal mortality reference levels, the implementation of PBR, and/or cumulative impact assessment approaches would catalyst a new wave of comprehensive management.

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Appendix

I. Considerations for a cumulative impacts model that integrates multiple sources of information

The assessment approach we have described offers a new way to consider the cumulative effects of lethal and sub-lethal pressures to marine mammal populations against objective-based reference points, like PBR. There are other feasible approaches to assess cumulative impacts, including other ways to use expert judgment. To advance the use of such integrative approaches in management we discuss the justification, function and limitations of the elements we have incorporated into our approach.

A. Expert Opinion

Subjective probabilities or artificial intelligence is more commonly termed expert knowledge/opinion/judgment in conservation science (74). It has conceivably been used by decision makers throughout human history, but it's structured use in the aerospace industry, military intelligence, the commercial nuclear industry and other probabilistic risk analysis, and policy analysis has been documented back to the 1940s (75). In the 1970s it began to be systematically used to model scientific reasoning under uncertainty. With this study we assert that integrating empirical data with expert opinion is a viable way to inform management decisions and research directions for marine mammals now, in the face of data gaps and uncertainty. Drawing from both natural and social sciences (35) innovates the notion of "best available science," especially in data-poor cases.

B. Definitions

Definitions of terms (Appendix II) were critical for standardizing the way in which we framed our research approach and communicated with and across experts (28) who individually and anonymously provided their opinion. The research team initially defined terms before administering the first survey and then edited, omitted, and added terms as-needed based on the feedback received from experts during the consensus webinar, prior to the administration of the second survey. Defining terms is an important first step in building a multiple impacts model, and definitions can and should evolve with the research process. Lexicons are living products of biological conservation research and management efforts (e.g., IUCN Red List of Threatened Species' Classification Schemes (76)).

C. Reference Point(s) and Time Horizon

Equally as important as definitions is ensuring all estimates in a multiple impacts model are made based on the same baseline reference point(s) and over the same time horizon (77). Here, we asked experts to consider what they know about the Southern Resident Killer Whale population over the last 30 years and provide estimates regarding how multiple threats and stressors will influence population dynamics over the next three generations (~75 years). We chose the baseline of the last 30 years because the population has been monitored closely since the mid-1970s[7] and so we assume that experts are familiar with population dynamics, threats, and stressors across at least the last three decades. 30 years has also been used by NOAA (46).

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[7] The Center for Whale Research (Friday Harbor, Washington, U.S.A.) has been counting Southern Resident Killer Whale births and deaths since 1974.

We asked experts to provide estimates over the next three generations because that is typically the time horizon the IUCN Red List uses when making assessments regarding reduction in population sizes for long-lived mammals (78). This is in part because uncertainties and data gaps make predictions over periods longer than three generations unreliable (79). The IUCN defines generation length as follows: “the average age of parents of the current cohort (i.e., newborn individual in the population). Generation length therefore reflects the turnover rate of breeding individuals in a population. Generation length is greater than the age at first breeding and less than the age of the oldest breeding individual, except in taxa that breed only once. Where generation length varies under threat, the more natural, i.e., pre-disturbance, generation length should be used.” Taylor and others (80) estimated generation length for orcas for conditions present in 2007 as 24.0 years, and for pre-disturbance conditions as 25.7 years. Therefore, we assume that 75 years represents roughly three generations for orcas. A population viability analysis (PVA) for Southern Residents by Taylor and Plater in 2001 predicted a median time to extinction of 74 years with a 95% confidence interval of 33-121 years (69), other PVAs have predicted a bit longer. Akcakaya and others (79) caution against using time horizons that are not long enough to reflect the affects of climate change, noted to be 50+ years. Given all of this information and advice, we believe three generations is an acceptable time horizon to quantify cumulative impacts for Southern Resident Killer Whales.

D. Natural Factors

Marine mammals are influenced by natural factors such as stochastic environmental change and pulsed resources. These natural stresses combine with multiple human-induced stresses to influence population dynamics across space and time. There are many justifiable ways to parse and account for natural and anthropogenic factors in a multiple impacts model. Perhaps the most recognizable is from fisheries science where total mortality of a fish population is the sum of fishing and natural mortality. Of course for marine mammals there are more anthropogenic sources of lethal and sub-lethal stress than fishing/direct take. In this study we focus on threats and stressors that can be modified by managing human activities—by definition an ecosystem-based management approach (34). We expanded sources of stress caused by humans to include all direct and indirect sources, but kept natural factors collapsed into one summative stressor. We asked experts to provide estimates for anthropogenic influences on Southern Resident Killer Whale population dynamics above and beyond natural factors, and treated natural factors as a direct influence on vital rates (see Figure 1). In the final model, experts were asked to estimate the influences of natural factors (e.g., what proportion of mortality will be due to natural factors over the next three generations) with respect to all anthropogenic stressors combined on vital rates. This is because during the consensus webinar experts collectively expressed that they did not feel confident to estimate influences of natural factors at the threats → stressor level, but more confident in estimating how natural factors influence vital rates relative to how anthropogenic stressors influence vital rates. Additionally, treating natural factors as a direct input to vital rates allows us to quantify the influence of anthropogenic stressors but leave natural factors as an unquantified input, if desired. Once the expert opinion and empirical data are integrated, the influence of natural factors can then be solved for, rather than estimated: survival = 1 – mortality(anthropogenic, natural). The two methods for estimating the influences of natural factors can be compared and contrasted depending on the objective.

E. Uncertainty & Confidence Intervals

After Cooke (75), Halpern and others (29) argued that failure to assess uncertainty in expert opinion threats assessments diminished their efficacy as a substitute for empirical data. In light of this, we designed explicit ways in which experts could state their confidence in their overall knowledge of the subject of interest, as well as their confidence of each estimate. Further, we asked each expert to provide a lower and upper bound for each best estimate, which in effect translates to a confidence interval. These measures of uncertainty and confidence were useful in two ways. First, we were able to weigh each expert's response based on their self-defined certainty of the general subject (e.g., harassment) and specific model links (e.g., harassment → malnutrition). Therefore, when aggregating expert estimate distributions, opinions of experts who are most knowledgeable about a particular response contribute more to the aggregate (final) distribution than opinions of experts who were less knowledgeable. This has been called "unequal expert weights in opinion pooling" (71, 74, 75). Second, we were able to bound each expert's best estimate with an expert-defined confidence interval. Draws were made from each expert's bounded distribution when calculating the aggregate (final) distribution for a given link in the model (71, 74, 75). For example, if an expert thought a given threat, say harassment, contributed 30% to a given stressor, say malnutrition, their estimated lower and upper bounds effectively indicated the functional shape of the distribution around their best estimate. If no confidence intervals had been specified, one might assume every expert estimate is bounded between 0% and 100% and takes on the same functional shape, perhaps a uniform distribution. From our results we can see that would have been an erroneous assumption, as confidence intervals and functional shapes varied. Thus one of the novel contributions of this cumulative impacts assessment approach is the explicit incorporation of uncertainty and confidence intervals around expert judgment (23, 29, 74, 81).

F. Consensus

The Delphi communication method was developed in the 1950s & 1960s and is based on the principle that information amassed from a structured group of experts is more accurate than those from an unstructured group of experts (82). The method allows for facilitated interaction among experts (83-86) and the format is typically as follows: Experts individually complete a questionnaire and are then brought together and provided with an anonymous summary of aggregate expert responses. Then, experts are encouraged to converse and explain reasoning. After the structured interaction, experts are able to, but not required to, revisit and revise the questionnaire. Iteration through this cycle can be completed two or more times until consensus is achieved (83, 86). During the research process presented here, following the first anonymous online survey we facilitated voluntary structured interaction via a webinar and 10 of the 16 experts participated. The tone of the three-hour interaction was pleasant and amenable. While each expert was initially unaware of which other experts would be participating in the webinar, no one was surprised to ‘see’ the other, as they were all familiar with one another from their combined >300 years experience studying killer whales. Following the webinar a second anonymous online survey was administered. Experts had the opportunity to carry over applicable responses from the first survey and/or make revisions. The last question of the second survey asked if the expert felt another structured interaction (webinar) was warranted. 5/10 respondents said no, 3/10 said possibly / if time permits, and 2/10 said yes. While this iterative process could go on and on and continue to be improved, and we did receive indication that at least some experts would be willing to participate in a second webinar, we decided to publish the results after one facilitated interaction and two surveys. Both because we felt it would take much more time (perhaps 1+ years) for unknown added benefit, and because time is short for the critically endangered case study population and we hope our findings might aid recovery efforts.

We found the consensus portion of our research process valuable in a two main ways. Frist, it improved and refined the content of the Southern Resident Killer Whale multiple impacts model and the information we gleaned from the elicitation process. While the research team has expertise in cumulative impacts assessment, conservation biology, and applied ecology, the 10 experts that provided feedback during the webinar have far more experience with both marine mammals in general, and of course killer whales. Having their concerted energy and input for a focused three hours was invaluable. Notably experts were able to tell us what information they were confident in estimating based on their knowledge, and what information they were not. For example, they were uncomfortable providing estimates for natural factors and functional forms of their responses, and so we omitted both of those questions from the survey. In contrast, they were comfortable estimating interactions between threats and the open platform allowed us to discuss how to best include interactions in the model. The ways in which we updated the survey based on their feedback is detailed in the online survey itself (https://www.surveymonkey.com/s/CumulativeImpacts_KillerWhales). Some examples of the suggestions that were incorporated in the second survey are: the consideration of adult male, female and post-reproductive female survival separately, versus a combined ‘adult survival’ vital rate; the change of the “disease” stressor to “disease and immunosuppression;" the addition of stressors "endocrine disruption" and "malnutrition," and the removal of stressors "changed behavior: foraging" and "compromised body condition;" the addition of "marine debris interactions" and "harmful algal blooms" threats; the addition of interactions between chemical pollution (acute and chronic) and pathogens threats. Some of the revisions were important improvements to the general conceptual model design and assessment approach, such as assessing anthropogenic influences separate from natural factors. Many of the content and survey revisions were modifying the Southern Resident Killer Whale-specific model. This would likely be valuable for any case study.

The second major value the consensus webinar added to the process was credibility. Asking experts to volunteer to anonymously complete a time-consuming (upwards of one hour or more) online survey is a hefty request. The webinar took even more time, as did the second online survey. While we did not ask the question, we speculate that being able to deliberate directly with the researchers and other experts during the webinar gave each expert additional incentive to participate. We feel that full participation and buy-in among experts was essential to our ability to complete a robust cumulative impacts assessment, and it is often an under-recognized benefit of structured consensus-building interaction (28, 87). While we don't doubt that there may have been some skepticism of the elicitation process during the first survey, the consensus-building interaction seemed to provide the overall process with credibility and momentum.

G. Interacting Threats

We do not include interacting threats or stressors in the general conceptual model because we believe they are highly population-dependent. For the Southern Resident case study we discussed interactions during the consensus webinar with experts and decided it was important to include two threat interactions: acute chemical pollution and pathogens; chronic chemical pollution and pathogens. In the second survey experts were asked to provide estimates for individual threat → stressor links, as well as interacting threats → stressor links (e.g., acute chemical pollution X pathogens → endocrine disruption).

H. Anchoring Expert Information with Empirical Data

For each segment within the multiple impacts network model (e.g., multiple threats → one stressor) we anchored expert estimates with empirical data when possible. From our literature review we identified data that are either reported as or easily translated into annual rates: number of individuals affected per year. For example, ship strike rate. Second, we used the expert percent contribution estimates and the rate(s) anchors to calculate estimates for other rates that have not yet been estimated in the literature. Table 2 provides an example of this approach for five threats that influence Lethal Injury. Essentially, this approach integrates expert opinion with empirical data to generate quantitative priors for all impacts, even those that have not yet been empirically assessed. All priors are updateable as new information becomes available.

II. Glossary

ACUTE NOISE: Short term (seconds to days in duration), high magnitude underwater noise resulting from human activities
ACUTE CHEMICAL POLLUTION: Short-term exposure to a sudden release of dissolved chemicals, nutrients, oil or other minute human-caused pollution that impairs water quality and animal health
BREEDING HABITAT DEGRADATION: Either total or partial damage to biological and/or physical aspects of the environment that reduces or impedes successful reproduction
BYCATCH: Unintentional or incidental capture in active or passive fishing gear targeting a different species
CHANGED BEHAVIOR: REPRODUCTION: Alteration of typical reproductive behavior, such as fewer breeding attempts, changed mating system, shorter nursing periods, etc.
CHRONIC CHEMICAL POLLUTION: Long-term exposure to a sustained release of

dissolved chemicals, nutrients, oil or other minute human-caused pollution that impairs water quality and animal health

CHRONIC NOISE: Long-lasting or repetitive underwater noise resulting from human activities, generally lower in magnitude than acute noise

DISEASE & IMMUNOSUPPRESSION: Changes in cellular and humoral immunity, including, but not limited to impairment of natural killer (NK) cell activity, T-lymphocyte function, antigen-specific lymphocyte proliferative responses, delayed-type hypersensitivity, and antibody responses that impair normal cellular and humoral response to infectious agents (natural or foreign) such as viruses, bacteria, fungi and parasites.

ENDOCRINE DISRUPTION: Mimicry or interference with bodily hormone function by naturally occurring or man-made compounds; disruption can occur by turning on, shutting off, or modifying signals that hormones carry and can affect the normal functions of tissues and organs. Consequences can include developmental, reproductive, neural, or other problems.

FECUNDITY: Number of female offspring produced per female in the population per year

FORAGING HABITAT DEGRADATION: Either total or partial damage to biological and/or physical aspects of the environment that reduces or impedes successful foraging

HARASSMENT: Intentional or unintentional disturbance from their natural behavior, including tourism disturbance, aggression from fishers or fish farmers, etc.

HARMFUL ALGAL BLOOMS: A rapid increase in the abundance of algae that are either toxic to marine mammals (causing illness or death), or adversely affect marine mammal prey (e.g. clog gills of fish and invertebrates)

LETHAL INJURY: Physical trauma resulting in death

MALNUTRITION: The inadequate intake of any of an animal's required nutrients; note that malnutrition can occur in an animal receiving large amounts of food when they are not able to ingest, digest, absorb, or use the food, or if the food is inadequate in one or more of the required nutrients. When an animal is not able to obtain food for an extended period of time either for the previously listed reasons or due to an unavailability of food or insufficient energy intake, this is defined as starvation.

MARINE DEBRIS INTERACTIONS: Exposure to relatively large pieces of human-produced products possibly resulting in entanglement, plastic ingestion, etc.

NATURAL FACTORS: non-anthropogenic factors that influence individual fitness and survival and population vital rates. Natural factors include predation, competition, natural pathogens, naturally occurring biotoxins, intra- and interspecific aggression, natural climate variability, and other factors. Note that some threats may have both natural and anthropogenic components (e.g., prey depletion).

PATHOGENS: Microorganisms, including bacteria, viruses, and fungi, which cause disease

PREY DEPLETION: Reduction of favorable or important prey species populations

SHIP STRIKE: Physical impacts or interactions with a vessel or its propeller

SUBLETHAL INJURY: Physical trauma resulting in impaired health or function but not leading to immediate death

SURVIVAL: IMMATURES: Proportion of immature individuals in the population surviving and growing to sexual maturity per year

SURVIVAL: MATURES FEMALES: Proportion of sexually mature female individuals in the population surviving to the next year

SURVIVAL: MATURES MALES: Proportion of sexually mature male individuals in the population surviving to the next year
SURVIVAL: POST-REPRODUCTIVE FEMALES: Proportion of reproductively senescent (or post-menopausal) female individuals in the population surviving to the next year