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# Sex ratios of olive ridley sea turtles in the North Pacific high seas: implications for climate change research

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ABSTRACT: Size-class distributions and sex ratio data provide critical information to assess the demography and reproductive potential of animal populations, such as sea turtles. Sea turtle sex is determined by incubation temperature, whereby warmer temperatures during a certain period of embryonic development produce more female hatchlings. Whereas hatchling sex ratios have been well-studied, sex ratios of sea turtle foraging aggregations are less known for most populations. Here we report on sex ratios of immature and mature olive ridley sea turtles *Lepidochelys olivacea* in the Eastern Tropical Pacific (ETP) and Central North Pacific (CNP) based on blood plasma hormone analysis, refined with Bayesian modeling, or gonad examination. Our findings established that (1) the commercial enzyme-linked immunosorbent assay used in the present study was appropriate to analyze testosterone concentration in olive ridley blood plasma to determine foraging ground sex ratios (via a Bayesian model); (2) size-at-maturity is generally larger in males than females in the ETP; (3) the overall sex ratio among all turtles was 1.2F:1.0M; and (4) the sex ratio of smaller-sized immature turtles from both study regions was female-biased (ETP, 1.6F:1.0M and CNP, 2.1F:1.0M). These are the first sex ratio estimates for olive ridleys foraging in the high seas of the North Pacific Ocean. The data can inform population models for species conservation, particularly those that contribute to the development of conservation plans that consider climate change projections.

KEY WORDS: Female bias · Environmental impacts · Marine turtle · Incidental mortality · *Lepidochelys olivacea* · ELISA · Endocrinology · Necropsy

## 1. INTRODUCTION

Sex ratio is a key demographic parameter used to predict reproductive potential, which in turn influences population growth or decline (Tarsi & Tuff 2012). If there is a moderate female bias, population growth may boom (i.e. more females can produce more offspring), whereas an extreme bias towards females may limit mating opportunities and potentially hinder population growth (Tarsi & Tuff 2012).

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Understanding population sex ratio is beneficial for threatened and endangered species management, as these data can guide management efforts and shape how conservation plans are developed (Santidrián Tomillo et al. 2021). Furthermore, effects of sex ratio on population trajectories can provide insight into potential impacts of climate change on wildlife taxa that are acutely sensitive to environmental variability, such as sea turtles (Patrício et al. 2021).

### 1.1. Temperature-dependent sex determination in sea turtles

As with many reptiles, sea turtle sex is not determined genetically (Bull 1980), and instead is dependent on incubation temperature of developing embryos (Standora & Spotila 1985), with warmer temperatures producing more females (Mrosovsky & Yntema 1980). Consequently, sea turtles are significantly impacted by climate change and elevated temperatures because embryonic sex is directly linked to environmental temperature (e.g. Patrício et al. 2021).

### 1.2. Immature and mature sea turtle sex ratios

Sea turtle populations do not usually exhibit a 1:1 sex ratio and are often female-biased (e.g. Work & Balazs 2010, Allen et al. 2015, Shertzer et al. 2018), which can foster recovery of depleted populations (e.g. more females produce more hatchlings, Wibbels 2007). Limited recent studies suggest a male bias for populations of hawksbills *Eretmochelys imbricata* (Pilcher et al. 2015), loggerheads *Caretta caretta* (Casale et al. 2002, Limpus 2003), and leatherbacks *Dermochelys coriacea* (James et al. 2007), while studies conducted several decades ago show no sex bias (e.g. green turtle *Chelonia mydas*, Wibbels et al. 1993; Kemp's ridley *Lepidochelys kempii*, Gregory & Schmid 2001). To date, no sex ratios have been reported for immature olive ridley sea turtles *L. olivacea* (hereafter referred to as olive ridley) foraging groups.

### 1.3. Determining sea turtle sex

External sexual dimorphisms in immature turtles are not apparent (Eckert et al. 1999), although adult males present a substantially longer tail than females (Pritchard et al. 1983). However, determining the sex of turtles can be difficult because the tail length of adult females can be similar to that of large immature males (Limpus 1985), so an adult-sized immature male turtle may be misidentified as an adult female. These challenges for determining sex of immature sea turtles indicate a need for a reliable and efficient sex identification technique. Using testosterone concentration ([T]) assays of blood plasma—a reliable and minimally invasive technique—the sex of immature turtles can be determined (e.g. Owens et al. 1978). However, because ELISA methods were not created specifically for analysis of hormone concentration in sea turtle tissue samples, each assay must undergo quality assurance tests to validate its applicability for the species of interest (e.g. the current study on olive ridleys) before broad application of the assay technology. Generally, [T] is significantly greater in males than in females (a bimodal distribution), but there can be some overlap or a gap in [T] between males and females, rendering sex assignment challenging for individuals with [T] in these ranges. To confirm the sex of such individuals, previous studies have used mark–recapture (blood sampling at immature life stage and re-capture in adult life stage to confirm external sex) and/or visualization of gonads via laparoscopy, then created statistical models to assist in predicting the sex of individuals within the same population that fall in the indeterminate [T] range (e.g. Allen et al. 2015, Shertzer et al. 2018). Relevant to sex prediction statistical models, the [T] that indicates the sex of a sea turtle is dependent upon the species, the maturity state, and water temperature at time of capture (Braun-McNeill et al. 2007, Blanvillain et al. 2011, Hawkes et al. 2013). Although predicting the sex of turtles using [T] is only reliable for immature turtles, [T] analyses in adult turtles can be informative about an individual's reproductive state (e.g. Allen et al. 2015).

### 1.4. North Pacific olive ridley biology

The olive ridley is 1 of 6 'hardshell' (family Cheloniidae) sea turtle species found globally. It is the most abundant species, has a circumtropical distribution, and lives large portions of its life in the oceanic realm (Plotkin 2007). In the North Pacific, olive ridleys mate, nest, and forage within either the Eastern Tropical Pacific (ETP) or western Pacific, and individuals from both regions forage in the middle, i.e. the Central North Pacific (CNP) (Dutton et al. 1998, Polovina et al. 2004, Peavey 2016). In the ETP, olive ridleys are described as nomadic with migratory flexibility, making them adaptable to environmental change relative to other sea turtle populations (Plotkin 2010). Like other

species, they nest on beaches as solitary individuals, with copulation (i.e. egg fertilization) occurring adjacent to nesting beaches. However, olive ridleys also nest en masse, known as an 'arribada', during which thousands of female olive ridleys nest on the same beach during a 3–5 d period (Fig. 1). Moreover, the species is also known to copulate in high-seas regions distant from nesting beaches (Kopitsky et al. 2000, Kopitsky 2002, Peavey 2016). Overall, the population size of olive ridleys in the North Pacific, as well as globally, is depleted relative to historic levels, although several breeding populations are increasing (Abreu-Grobois & Plotkin 2008, NMFS & USFWS 2014). As a result, olive ridleys are listed as Vulnerable on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Abreu-Grobois & Plotkin 2008) and as 'globally threatened' under the US Endangered Species Act (NMFS & USFWS 2014).

Size at sexual maturation is unknown for most populations of sea turtle species, including olive ridleys. In the CNP, female and male olive ridleys reach sexual maturity at approximately 60.0 cm straight carapace length (SCL; Zug et al. 2006). For olive ridleys in the ETP, individuals with SCL ⩾56.0 cm are considered putative adults (NMFS & USFWS 1998, Peavey et al. 2017), while turtles of this size with long tails (generally >20 cm, Peavey et al. 2017) or plastron softness (L. E. Peavey Reeves pers. obs.) are typically considered to be putative adult male turtles (Wibbels et al. 1991), as SCL alone is not indicative of sex. Understanding size at sexual maturation is important for developing accurate population viability models and informing species conservation efforts.

### 1.5. Objective and outcomes

The present study is the first to assess sex of free-swimming immature olive ridleys via [T] analyses. The objectives of this study were to (1) confirm the applicability of an ELISA for assessment of [T] in olive ridley blood plasma using samples collected in the ETP, (2) determine the sex ratio for ETP olive ridleys using the blood plasma [T] analysis results, (3) determine the sex ratio for CNP olive ridleys using gonad visualization data from necropsied olive ridleys incidentally caught in the Hawai'i-based longline fishery, and (4) provide insight into olive ridley maturation and reproductive state. The results of these efforts will serve as a baseline with which to compare results of future studies of olive ridleys, which is especially important in the context of ongoing climate change.

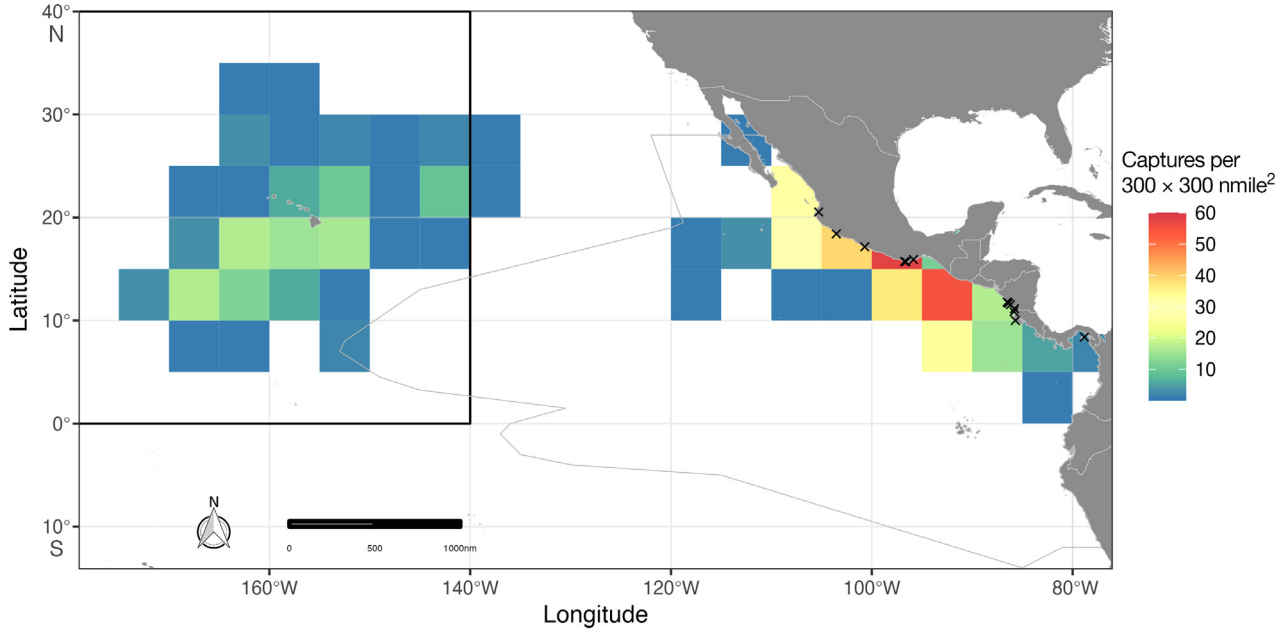


Fig. 1. Sampling locations of olive ridley sea turtles *Lepidochelys olivacea*, where turtles were free-swimming in the Eastern Tropical Pacific (ETP, n = 332) during a research survey conducted in 2006 or incidentally captured by the Hawai'i longline fishery in the Central North Pacific (CNP, n = 137) from 2009 to 2019. Catch rates ranged from 1 to 60 turtles per 300 × 300 nmile$^2$ (~555 × 555 km$^2$) between the locations (ETP and CNP); warmer (red) colored areas have higher catch rates than cooler (blue) colored areas, and no turtles were sampled in cells with no color (white) that fall within the fishery (black outline) or survey (grey outline) areas. Arribada nesting beaches in the eastern Pacific are marked by an ×. Created using the 'ggplot2' package (Wickham 2016) in R version 4.2.2 (R Core Team 2023)

## 2. MATERIALS AND METHODS

### 2.1. Turtle sampling

#### 2.1.1. Capture of free-swimming olive ridleys in the ETP

Between August and December 2006, the National Oceanographic and Atmospheric Administration (NOAA) undertook a ship-based research survey (*Stenella* Abundance Research Cruise) in the tropical and subtropical regions of the ETP (Fig. 1). Free-swimming olive ridleys (n = 332; Table S1 in Supplement 1 at www.int-res.com/articles/suppl/m748p149_supp1.xlsx) were hand-captured from a small, rigid-hull inflatable boat (RHIB) operated from NOAA's RV 'David Starr Jordan'. After capture, turtles were transferred via net from the RHIB to the research vessel. Once aboard the larger ship, olive ridleys were tagged with a uniquely coded Inconel® flipper tag, body size (SCL) and tail length were measured, tissues (e.g. blood) were sampled, and behavior at time of capture (e.g. mating/mounting observed) was recorded (Eguchi et al. 2007, Peavey et al. 2017). Upon completion of sampling, turtles were released in the general area where they were caught.

#### 2.1.2. Incidental mortality in the CNP

Incidental interactions of olive ridleys (often leading to mortality) occur within the Hawai'i-based longline (HLL) fishery in the CNP (Fig. 1). Between 26 April 2009 and 8 July 2019, olive ridley carcasses (n = 137; Table S2 in Supplement 1) were brought to NOAA's Pacific Islands Fisheries Science Center for necropsy and sampling. During the necropsy, sex and maturity state (i.e. mature or immature) were determined by visual assessment of gonad development (by veterinary pathologist Dr. T. Work) and SCL according to previously published methods (Work & Balazs 2010). Incidental mortality locations are subject to confidentiality constraints and, therefore, we depict capture locations as the sum of turtles per 300 × 300 nmile$^2$ (~555 × 555 km$^2$) for both populations (Fig. 1).

Animals were captured under approved permits: US Endangered Species Act permit #10(a)(1)A-#22991 and NOAA PRD Research Permit #774 1714. All research and sampling were conducted under National Marine Fisheries Service (NMFS) Institutional Animal Care and Use Committee (IACUC) Protocols #00-037-2 and #SWPI-2021-01M in accordance with the protocols and handling guidelines set forth by the NMFS IACUC.

### 2.2. ETP blood collection and handling

Blood was collected from ETP turtles via the dorsal cervical sinus using a 3.8 cm × 21-gauge needle connected to a 10 ml sodium heparin vacutainer blood collection tube (Becton, Dickinson) per Allen et al. (2015). Blood samples were stored in a cooler, on ice, until centrifugation. Plasma was aliquoted into 2 ml cryovials (Corning), and subsequently stored at −80°C for the remainder of the cruise. Once the cruise was completed, the samples were archived and stored at −80°C until hormone analysis, which was conducted between November 2015 and June 2016.

### 2.3. ETP blood plasma steroid hormone extraction

Chemical solvents (ether and acetone) were used to extract steroid hormones from ETP blood plasma samples per Allen et al. (2015). The following day, the extracted samples were then reconstituted in 250 µl of 0.01 M phosphate-buffered saline (PBS; Sigma) with 0.1% bovine serum albumin (BSA; Amresco). The samples were subsequently vortexed for 15 min and then placed in a water bath for 30 min at 37°C.

Prior to analyzing any ETP samples, we pooled previously collected (via stranded and temporarily captive olive ridleys) olive ridley blood serum/plasma samples of known low [T]. To determine extraction efficiency, we used this 'low [T]' sample to determine extraction efficiency by spiking the sample with 10 ng of T standard provided in the ELISA T assay kit. This was paired with extraction of an aliquot of the pooled sample, which was not spiked with T to determine the baseline [T] of the pooled sample. We extracted and quantified the amount of T in the spiked sample (added T), which was diluted 1:100, and the non-spiked T sample (no T added), which was not diluted. The extraction efficiency (%) was obtained by the following equation:

$$\frac{final\ T\ concentration - concentration\ from\ the\ non\text{-}spiked\ sample}{concentration\ of\ T\ added} \times 100 \quad (1)$$

Mean extraction efficiency was 104.6%, indicating that all hormones were extracted.

### 2.4. ELISA testosterone assay

We used an ELISA T kit (Catalog # ADI–900–065, ENZO Life Sciences) to quantify [T] in each of our extracted plasma samples collected from ETP olive ridleys. As outlined previously by Allen et al. (2015), we made a total of 7 standards ranging from 1.9525 to 2000 pg $ml^{-1}$ using PBS with BSA; therefore, the PBS with BSA was considered the zero ($B_0$) standard. The manufacturer's sensitivity of the assay was 2.0 pg $ml^{-1}$, whereas the effective sensitivity of the assay with the added standards was calculated to be 1.2 pg $ml^{-1}$. The manufacturer characterized the cross reactivity with other hormones, including 100% reactivity with testosterone, 14.64% reactivity with 19-hydroxytestosterone, 7.20% reactivity with androstenedione, 0.72% reactivity with dehydroepiandrosterone, and 0.40% reactivity with estradiol. There was also less than 0.001% cross-reactivity with estriol, corticosterone, cortisol, cortisone, estrone, progesterone, and pregnenolone. Assay drift was evaluated using the same low (15.3 pg $ml^{-1}$), medium (150.2 pg $ml^{-1}$), and high (369.7 pg $ml^{-1}$) control olive ridley plasma/serum samples throughout all assays and using standards in each assay at the beginning and the end of each plate. Overall, the control samples did not exhibit any detectable trend throughout the assay, signifying that there was no substantial assay drift.

Reconstituted extracted samples were pipetted into the assay plate undiluted, randomly distributed throughout the assays, and quantified in duplicate in each assay to ensure data accuracy. When samples contained a [T] that exceeded the threshold of the assay detection limit, we diluted a subsample of the plasma sample extract in PBS (with BSA) until we obtained a [T] within the detection limit on a subsequent assay.

The optical density within each well of the ELISA plate was determined via a Tecan Sunrise spectrophotometer (Phenix Research Products). The results were reached by employing a 5-parameter logistic curve fitting program (Magellan 3.11, Tecan Group), and the resulting [T] values (pg $ml^{-1}$) were exported for further data analysis.

The same low, medium, and high control samples (pooled olive ridley plasma/serum) that were used to determine assay drift were also used for quality control to confirm both within- and between-assay variation. The mean intra-assay coefficients of variation for the low (15.3 pg $ml^{-1}$), medium (150.2 pg $ml^{-1}$), and high (369.7 pg $ml^{-1}$) controls were 30.2, 9.9, and 7.6%, respectively (n = 21 assays). The inter-assay variation for the 3 controls was 13.0, 9.4, and 12.2%, respectively (n = 21 assays).

To demonstrate that the commercial ELISA could be used to analyze [T] in olive ridley plasma, assay precision tests were performed. First, a linearity assessment was performed, to determine if the T ELISA measured the same antigen in hormone standard provided in the ELISA kit and serial dilutions of pooled olive ridley plasma extracts (n = 4) of known [T] (1689.2 pg $ml^{-1}$). Serial dilutions of the hormone standard and pooled olive ridley plasma extracts were assayed in duplicate and triplicate, respectively. Linearity was determined by comparing the slopes of the binding curve of the serially diluted plasma extract pool (9 dilutions spanning 1:1 to 1:256) to the slope of the hormone standards. When conducting a linear regression and *F*-test, we confirmed that the T ELISA measured the same antigen in the standard controls and plasma extracts because the slopes of curves from the known standard controls and serial dilutions of pooled plasma extracts demonstrated parallelism and were not significantly different ($r^2$ = 0.968, p = 0.2638; Fig. S1 in Supplement 2 at www.int-res.com/articles/suppl/m748p149_supp2.pdf). Second, a matrix-interference test was conducted to examine if there was any potential interference caused by substances within our plasma samples, which are independent of specific antigen–antibody binding. We made a sample pool (n = 3) with low [T] and spiked aliquots (120 µl) of the pooled sample with an equal volume (120 µl) from each of the assay standards. The concentration of T contributed from the pooled sample was subtracted from each sample-spiked measurement so its contribution would be factored out of the assessment. A simple linear regression was used to determine the degree to which the measured [T] corresponds to the true concentration of the spiked sample. When assessing for matrix interference, we found no significant difference (p = 0.43) in expected and observed concentrations when pooled plasma extracts were spiked with standard solutions; this finding is consistent with little or no evidence of matrix interference.

Within each assay run, if the associated standards were not monotonically increasing, we discarded the inaccurate duplicate of the standard from the curve (i.e. the value causing the deviation from monotonic progression); the resulting curve was deemed allowable if the curve fell within 2 SDs from the mean of remaining control values.

A sample was re-assayed if the variation between duplicate analysis of the same sample was >10% (n = 14). We also re-extracted and re-assayed samples when their [T] values were anomalous (n = 16; e.g. a

sample had unusually high or low [T] for that size/sex of turtle).

### 2.5. Data analysis

Raw [T] values that were obtained from the ELISA analysis software were corrected for plasma volume, acetone volume, extraction efficiency, reconstitution volume, and dilution, using the equation:

$$Testosterone\left(\frac{pg}{ml}\right) = \frac{[Raw] \times [Reconstitution\ Volume\ (ml)]}{[Plasma\ Volume\ (ml)] \times ([Acetone\ Volume\ (ml)] \times [Extraction\ Efficiency] \times [Dilution])} \quad (2)$$

Testosterone concentration data are presented as the mean ± SD of the duplicate values for each sample.

#### 2.5.1. Body size difference between two sampling locations in the North Pacific

To compare size distributions of olive ridleys between CNP and ETP, we used Bayesian hierarchical cluster analysis using the R package 'mclust' (v 6.1.1; Scrucca et al. 2023). We restricted the maximum number of clusters to 3 in order to avoid biological nonsensical results.

#### 2.5.2. Estimating probability of sex assignment: capture of free-swimming olive ridleys (ETP)

We used the approach of Allen et al. (2015) to estimate the probability of sex for each individual of unknown sex (including some putative adult females) caught in the ETP (Supplement 2). Briefly, measured [T] was modeled as a linear function of sex, total tail length (tail), length between cloaca and tail tip (tail2), SCL, the ratio between tail and SCL (tail/SCL) and between tail2 and SCL (tail2/SCL), and water temperature (Temp). Models were fit to the observed data via a Bayesian approach using Markov chain Monte Carlo (MCMC).

More specifically, data from putative mature adult turtles were used to define the relationship between [T] and the covariates (Sex, SCL, tail, tail2, and Temp), and that relationship was used to determine the sex of unknown-sex turtles; the putative mature turtles included 19 mature-sized females mounted by males at the time of capture and 119 putative mature male turtles with total tail length generally >20 cm and/or with soft plastrons. For mature-sized males, 1 male had a tail length <20 cm and 3 males had unreliable tail lengths (e.g. broken tails). For individuals with no *in situ* water temperature records (n = 26, captured between 25 and 28 October 2006), we used the average temperature from available records within the same geographic range (i.e. individuals caught on the same date in the same latitude/longitude box as the missing-data individuals); the computed mean ± SST was 29.8 ± 0.677°C. The temperature range for the study period was 23.1–31.5°C with a mean of 29.5°C. Because the variability among the computed mean water temperatures was small (SD = 0.667°C) and similar to the average temperature for the whole study, we used the computed mean for individuals without *in situ* water temperature records. For individuals with unknown sex (n = 194), sex was estimated as a missing variable; note that there were 6 males greater than the mature-size threshold (56.3–64.3 cm SCL, 13.0–16.1 cm tail length) included in this 'unknown' category. For turtles (n = 7) with no tail measurements (tail and/or tail2), we treated these tail lengths as unknown. During the MCMC sampling, the number of times the samples were drawn from each sex for those individuals was counted. The proportions to the total MCMC samples were then treated as the probability for each sex.

We considered the following linear models to determine the relationship between [T] and the covariates (1) Sex, (2) Sex + tail, (3) Sex + tail2, (4) Sex + Temp, (5) Sex + tail + SCL, (6) Sex + tail2 + SCL, (7) Sex + tail + Temp, (8) Sex + tail2 + Temp, (9) Sex + tail + SCL + Temp, (10) Sex + tail2 + SCL + Temp, (11) Sex + tail/SCL, (12) Sex + tail2/SCL, (13) Sex + tail/SCL + SCL, (14) Sex + tail2/SCL + SCL, (15) Sex + tail/SCL + Temp, (16) Sex + tail2/SCL + Temp, (17) Sex + tail/SCL + SCL + Temp, (18) Sex + tail2/SCL + SCL + Temp, (19) Sex + SCL + Temp. The appropriateness of the models to the observed data was determined via the Pareto *k*-statistic (Vehtari et al. 2017). Performance of the models was compared using deviance information criteria (DIC) and leave-one-out information criteria (LOOIC) (Vehtari et al. 2017).

MCMC sampling was conducted using JAGS language (v. 4.3.1; Plummer 2022) and executed in the R statistical environment (v. 4.3.2; R Core Team 2023) via the 'rjags' (v. 4.15; Plummer 2023) and 'loo' (v. 2.7.0; Vehtari et al. 2024) packages. We ran 5 independent chains of 50 000 burn-in steps, followed by 100 000 steps to sample from the joint posterior distribution. The samples were thinned by taking every tenth sample to reduce the autocorrelation

among samples and size of output. The Gelman–Rubin diagnostics statistic (Gelman et al. 2014) was used to determine the convergence of the chains. JAGS and R code used in this analysis are provided in Supplement 2.

## 3. RESULTS

### 3.1. ETP and CNP turtle body sizes

For the ETP SCL data set, the Gaussian mixture model with 2 means (33.5 and 60.8 cm) and 2 variances (99.5 and 12.7 $cm^2$) was determined to be most appropriate according to the Bayesian information criterion. For the CNP SCL data set, the best model included 2 means (50.6 and 58.5 cm) and 2 variances (54.4 and 6.7 $cm^2$). The ETP data set contained a greater number of smaller individuals than the CNP data set.

The turtles captured in this study consisted of a range of size classes and life stages. The overall mean ± SD (range) of SCLs for all turtles (n = 469) from both the ETP and CNP was 53.2 ± 12.5 cm (17.4–69.3 cm). The SCL was 52.0 ± 14.3 cm (17.4–69.3 cm) for the ETP (n = 332; Table S1) and 56.1 ± 5.9 cm (29.9–66.9 cm) for the CNP (n = 137, Table S2). The body size (SCL) structure of each region is presented in Fig. 2; both the smallest and the largest turtles in this study were captured in the ETP, and more small turtles (<40 cm SCL) were captured in the ETP overall than in the CNP for both sexes (Fig. 2). The number of male olive ridleys captured within the ETP was greater than the number of females, with the greatest number falling within 60–65 cm SCL (Fig. 2a). Six turtles of unknown sex were larger than the ETP minimum nesting female size, but with short tails (56.3–64.3 cm SCL and 13.0–16.1 cm total tail length); nonetheless, the model predicted them to be males and they were included in the immature male category. As expected, putative mature male tail length was greater than putative mature female tail length for olive ridleys captured in the ETP (Table 1). Similarly, mature male tail length was greater than mature female tail length in the CNP foraging aggregation, where sex and maturity state were determined by gonad visualization during necropsy.

### 3.2. ETP testosterone concentration

The range of [T] for olive ridleys foraging in the high seas of the ETP was 13.1–82 804.1 pg $ml^{-1}$. Mean [T] was greater in males compared to females regardless of maturity state (Table 1, Fig. 3). However, there was no clear delineation in [T] range between imma-

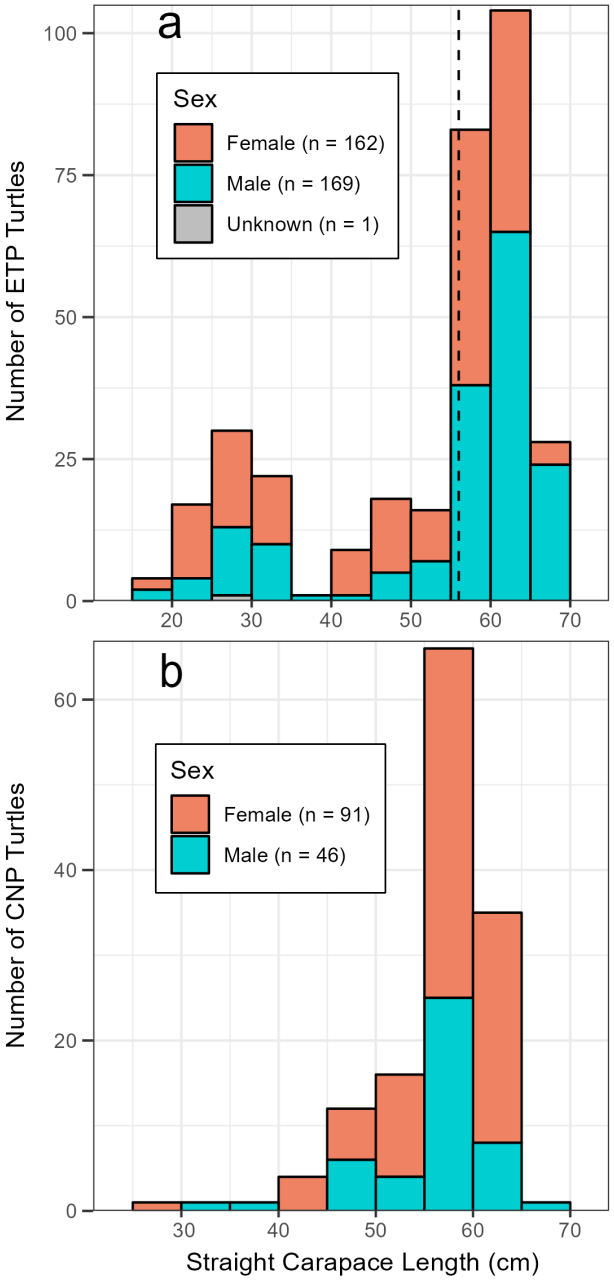


Fig. 2. Number of female olive ridley sea turtles *Lepidochelys olivacea* grouped by 5 cm size-class bins (straight carapace length, SCL) that were sampled in (a) the Eastern Tropical Pacific (ETP, n = 332) and (b) the Central North Pacific (CNP, n = 137). The vertical dashed line represents the size-threshold used to designate maturity for the ETP population (56 cm SCL). Testosterone concentration was analyzed in blood collected from free-swimming turtles captured in the ETP and was used to predict the sex via a Bayesian model. The sex and maturity state of CNP turtles were determined upon visualization of the gonads during necropsy

Table 1. Morphological measurements (straight carapace length [SCL] and tail length) and testosterone concentration [T] of olive ridley sea turtles *Lepidochelys olivacea* sampled in the Eastern Tropical Pacific (n = 331) or Central North Pacific (n = 137) (mean ± SD, range, n = sample size). [T] was analyzed in blood collected from free-swimming turtles captured in the Eastern Tropical Pacific and was used to predict the sex via a Bayesian model. The sex and maturity state of Central North Pacific turtles was determined upon visualization of the gonads during necropsy

| Life stage | Sex | SCL (cm) | | | Tail length (cm) | | | [T] (pg ml$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Range | n | Mean | Range | n | Mean | Range | n |
| **Eastern Tropical Pacific** | | | | | | | | | | |
| Putative mature | F | 60.4 ± 2.5 | 56.3–69.3 | 84 | 12.9 ± 1.9 | 9.6–17.9 | 80 | 280.3 ± 508.4 | 13.1–2686.8 | 84 |
| | M | 62.1 ± 3.2 | 55.3–68.8 | 119 | 29.2 ± 4.1 | 17.3–39.5 | 116 | 18608.9 ± 15288.7 | 350.2–82804.1 | 119 |
| Immature | F | 36.5 ± 11.7 | 17.4–55.8 | 78 | 7.1 ± 2.9 | 2.8–15.8 | 78 | 82.3 ± 53.0 | 32.8–391.3 | 78 |
| | M | 38.7 ± 13.4 | 17.6–64.3 | 50 | 8.2 ± 4.2 | 2.3–18.2 | 50 | 3060.8 ± 4444.7 | 179.3–18972.6 | 50 |
| **Central North Pacific** | | | | | | | | | | |
| Mature | F | 60.6 ± 1.9 | 55.5–63.9 | 34 | 12.3 ± 1.4 | 9.2–15.5 | 34 | – | – | – |
| | M | 59.9 ± 2.5 | 55.0–66.9 | 19 | 23.6 ± 4.1 | 15.0–30.0 | 18 | – | – | – |
| Immature | F | 54.0 ± 5.7 | 29.9–63.7 | 57 | 9.9 ± 2.0 | 5.5–14.0 | 57 | – | – | – |
| | M | 52.5 ± 6.5 | 33.7–59.2 | 27 | 13.6 ± 4.8 | 4.5–20.5 | 27 | – | – | – |

ture females and males (Table 1, Fig. 3), as [T] overlapped between 179.3 and 391.3 pg ml$^{-1}$.

### 3.3. ETP and CNP sex ratios

All MCMC runs converged (Gelman–Rubin statistics: R-hat < 1.1). According to LOOIC, sex + tail/SCL + SCL was the best model and sex + tail/SCL + SCL + temp was the second best. DIC selected sex + SCL + temp as the best and sex only was the second best model, although the difference in DIC values between the first and second was large (Table S3 in Supplement 1). For the 2 top models according to LOOIC, Pareto *k*-statistics indicated that the first model (sex + tail/SCL + SCL) did not fit well to all data points. The second model (sex + tail/SCL + SCL + temp) fit better to the data so we used it to estimate the probability of sex. The linear regression of estimated probability of being male (pMale) between the 2 models indicated that the 2 models were virtually identical ($pMale_{Model[2]} = -0.00004 + 0.998 \times pMale_{Model[1]}$, $r^2 = 1.0$), when using pMale = 0.5 as the cutoff value. The sex of one ETP turtle remained as unknown and was not included in sex ratio analyses because pMale was 0.50058; although this value was greater than 0.5, it was too close to the cutoff value to be included as a male.

For all olive ridleys (n = 468) included in this study, the sex ratio was 1.2F:1.0M. While overall we found an unbiased sex ratio (0.96F:1.0M) for the ETP olive ridleys, we found a female bias in the immature cohort and a male bias in mature turtles (Table 2). For the CNP olive ridleys whose sex and maturity state were determined during necropsy, we found female-biased sex ratios overall (2.0F:1.0M) and regardless of maturity state (Table 2), despite an increase in the number of males (mature and immature) captured in

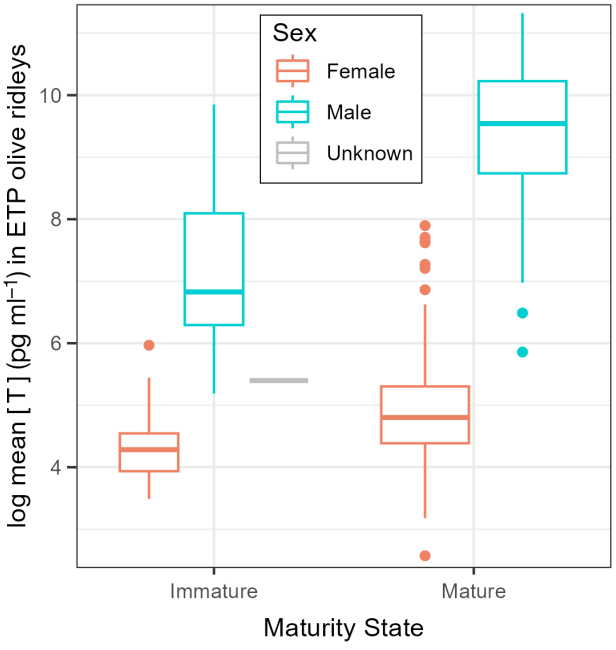


Fig. 3. Testosterone concentrations [T] (pg ml$^{-1}$) of plasma samples collected from immature and putative adult olive ridley sea turtles *Lepidochelys olivacea* (n = 332) captured in the Eastern Tropical Pacific (ETP). Putative maturity for males was based on straight carapace length (>56 cm) and tail length (>20 cm) or soft plastron. Sex was predicted via a Bayesian model that incorporated testosterone concentration, water temperature, tail length, and turtle size. Bar: median; box: interquartile range (IQR); whiskers: max./min. values ≤1.5 × IQR above/below box; dots: outliers

later years (47.8% of males were captured between 2017 and 2019; Table S2).

## 4. DISCUSSION

Here, via assay precision tests, we established that the commercial ELISA is appropriate to analyze [T] in olive ridley plasma. Additionally, we established size at maturation for Pacific olive ridleys (via gonad visualization during necropsy of turtles incidentally captured in the CNP HLL fishery). Finally, we determined sex ratios at 2 different olive ridley foraging regions in the high seas of the North Pacific Ocean. This information could be useful for investigating potential impacts of climate change on reproductive success, sex ratio, and population feminization, especially when compared to hatchling sex ratios at olive ridley nesting beaches in the Pacific (Table 2). In combination, these new demographic estimates can be incorporated into future population viability models and conservation status assessments to bolster population trends and stability, as well as sex-based survivorship of this species for more effective management.

### 4.1. CNP and ETP olive ridley body sizes: potential sampling biases

The distributions of SCLs were different between the 2 sampling locations. The difference came from (1) the presence of small individuals (<29.9 cm) in the ETP and (2) proportionately more larger individuals (>60 cm) among ETP captures (130/332 = 39.2%) than for CNP captures (34/137 = 24.8%). Similar to other sea turtles captured in the HLL fishery (loggerheads and leatherbacks; Wallace et al. 2008, Swimmer et al. 2017), few (n = 3) small turtles <40 cm SCL were captured incidentally in the CNP, perhaps due to hook size/type, bait size/type, or depth of gear that prevented smaller turtles from depredating longlines (Gilman et al. 2012, Swimmer et al. 2017). The HLL fishery also incidentally captured more female than male CNP olive ridleys over the 10 yr study period (Table 1); this finding suggests that there may be a catchability bias for female turtles. Consequently, with the HLL fishery capturing more female than male olive ridleys, the fishery may be interacting with turtles of greater reproductive value (Wallace et al. 2008), as female fecundity is most important for population growth in sea turtles (e.g. Santidrián

Table 2. Olive ridley sea turtle *Lepidochelys olivacea* sex ratios for different life stages (hatchling, immature, and mature) at various locations within the North Pacific Ocean. Hatchling sex ratios are from nesting beaches, whereas immature and mature sex ratios are from foraging grounds

| Life stage | Sexing method | Location | Location type | Year(s) | Sex ratio (F:M or %F) | Source |
|---|---|---|---|---|---|---|
| Hatchling | Nest temperature & gonad histology | Eastern Pacific: Oaxaca, Mexico | Arribada nesting beach | 2010–2011 | 1.2:1.0 | Hernández-Echeagaray et al. (2012) |
| Hatchling | Nest temperature & gonad histology | Eastern Pacific: Baja California Sur, Sinaloa Nayarit, & Guerrero, México | Solitary nesting beach | 2008–2010 | 2.1:1.0–124.0:1.0 | Sandoval-Espinoza (2012) |
| Hatchling | Nest temperature & gonad histology | Eastern Pacific: Jalisco, Mexico | Solitary nesting beach | 1993<br>1994<br>2010 | 7.0:3.0<br>1.3:1.0<br>77% | Valadez-González et al. (2000), García et al. (2003), Sandoval-Espinoza (2012) |
| Hatchling | Nest temperature | Eastern Pacific: Ostional, Costa Rica | Arribada nesting beach | 2017 | ⩾80% | Wen (2018) |
| Hatchling | Nest temperature | Eastern Pacific: Guanacaste, Costa Rica | Solitary nesting beach | 2013–2017 | 78.6% | Binhammer et al. (2019) |
| Hatchling | Nest temperature (hatchery) | Indo-western Pacific: East Java, Indonesia | Solitary nesting beach | 2009–2010 | 31.5–45.9% | Maulany et al. (2012) |
| Immature | Endocrinology & modeling | Eastern Tropical Pacific | Foraging ground | 2006 | 1.6:1.0 | This study |
| Immature | Necropsy | Central North Pacific | Foraging ground | 2009–2019 | 2.1:1.0 | This study |
| Putative mature | Endocrinology & modeling | Eastern Tropical Pacific | Foraging ground | 2006 | 0.7:1.0 | This study |
| Mature | Necropsy | Central North Pacific | Foraging ground | 2009–2019 | 1.8:1.0 | This study |

Tomillo et al. 2015). Thus, our results provide information that may be valuable for conservation actions and successful management of olive ridleys in the North Pacific. Nevertheless, studies should use caution when fishery bycaught (dead) animals are used to make inferences about live populations because it is possible that there is an inherent interaction bias (e.g. Casale et al. 2002). Ongoing data collection in the CNP is likely to shed light on the persistence of this finding.

There may be differences in spatial distributions of size and sex classes. For example, in the ETP, the proximity of the eastern-most turtle capture locations to nesting beaches might have provided greater access to smaller/younger turtles and reproductively active/mating turtles, although many mating pairs were observed hundreds of kilometers from shore (Kopitsky et al. 2000, Peavey 2016). Mating pairs are relatively easy to spot from a vessel because they bob on the surface for long periods of time; however, many hand-captured males were swimming independently at the time of encounter offshore. Sex-based behavioral differences may also have played a factor in catchability, but this would be speculative at this time. To better understand potential catchability biases between sexes and across maturity classes, we encourage additional study of CNP and ETP sex ratios across all female and male life stages.

### 4.2. ETP [T]

Because of the great number of archived ETP plasma samples, the present study greatly expands knowledge about the reproductive biology of olive ridleys, especially adult males. Similar to other studies examining hormone concentration in sexually mature turtles (Plotkin et al. 1996, Blanvillain et al. 2011), we found higher mean [T] in both putative mature females and males compared to immatures of either sex (Table 1, Fig. 3). Mature females with high [T] (>700 pg $ml^{-1}$; see outliers in Fig. 3) were likely going to reproduce that season as they were all mounted by males prior to capture. Some putative mature males in this study had very high [T] (up to 82804.1 pg $ml^{-1}$) in comparison to another endocrine study in the southern ETP where the maximum [T] of a copulating male was 12210 pg $mL^{-1}$ (Plotkin et al. 1996). Additionally, putative adult males mounted on females in the ETP had a [T] range of 2002.1–50124.2 pg $ml^{-1}$, which is a greater range than previously found in reproductively active males in the Eastern Pacific (Plotkin et al. 1996); perhaps some mounted males from this study were not reproductively active or were prepubescent males. It is evident that there is large variation in [T] across individual male olive ridleys in the ETP regardless of reproductive state at the time of sampling. Our results revealed that male olive ridleys have much greater range of [T] than previously known, and putative sexually mature male olive ridleys have a relatively very high maximum [T]. Information about adult male sea turtle reproductive biology is severely lacking (especially for olive ridleys), and the estimates presented here boost the available knowledge in the general literature, which may allow inferences about reproductive state of male turtles in future studies that assess [T].

### 4.3. ETP male maturity

Male sea turtle size at sexual maturity is known for only a few sea turtle species and populations (e.g. loggerhead, Casale et al. 2005, Ishihara et al. 2011, Avens et al. 2015; hawksbill, Limpus 2009b; green, Limpus 2009a) because males spend the majority of their lifetime at sea, making them difficult to access (Morreale et al. 2007, Girard et al. 2021), unlike females that crawl on land to lay eggs when they are sexually mature. In our study, we considered ETP olive ridleys >56.0 cm SCL and with a tail ⩾20 cm or with a soft plastron as putative mature males. Putative adult male turtles mounted on females ranged from 52.8–68.8 cm SCL. Only 1 mounted (model-predicted) male smaller (52.8 cm SCL) than the mature-size threshold (56.0 cm SCL) had a short tail (12.4 cm) and a high [T] (4094.4 pg $ml^{-1}$); this turtle was considered immature due to size and tail length (despite displaying mounting behavior) and was likely a prepubescent male approaching maturation and perhaps not successfully copulating. Interestingly, 6 turtles classified as immature due to short tail lengths (13.7–16.1 cm) were larger (56.3–64.3 cm SCL) than the mature-size threshold (56.0 cm) used in this study; 2 had high [T] (10204.4 and 10393.6 pg $ml^{-1}$), suggesting that these males were likely in the process of maturing. These findings suggest that most male olive ridleys in the ETP may not mature until >56 cm SCL, and further that males may mature at a larger size than females (56 cm SCL), considering that all but 1 putative adult male mounted to females were >56 cm SCL and the majority of males considered putatively mature had high [T]. Similarly, a previous study, albeit with a small sample size (n = 8), found that mature male olive ridleys off Costa Rica ranged from 63.3–67.0 cm SCL (Plotkin et al. 1996). Conversely, 1 turtle in the present study

was categorized as a putative mature male based on size (59.6 cm SCL) and tail length (23.6 cm); however, the low [T] (350.2 pg $ml^{-1}$) indicates the male was likely not yet reproductively active.

### 4.4. CNP male maturity

In the CNP, the previously described size threshold for maturity is 60 cm SCL (Work & Balazs 2010). However, when classifying males as mature based solely on size, it is useful to incorporate the assessment of tail length to avoid misclassifying mature males as immature. Based on gonad visualization during necropsy, 52.6% (10/19) of mature male CNP olive ridleys were <60 cm SCL and all immature males were <60 cm SCL. For male olive ridleys in the CNP <60 cm SCL (n = 37), tail length was a good indicator of maturity state, as 77.8% (7/9) of male turtles <60 cm SCL with tails ⩾20 cm in length were found to be mature upon necropsy; male olive ridleys ⩾60 cm SCL and with tail lengths ⩾19 cm (n = 9) were also all determined to be mature upon necropsy. All females (immature and mature, n = 91) had tail lengths ⩽15.5 cm. Therefore, the 60 cm SCL maturity size threshold originally described for both sexes of olive ridleys in the CNP (Work & Balazs 2010) may require refinement to include tail length ⩾20 cm as an indicator for mature males. Thus, turtles <60 cm SCL, but with a long tail, are likely males.

### 4.5. ETP and CNP sex ratios

We found no bias in the overall sex ratio (0.96F:1.0M) for free-swimming olive ridleys captured in the ETP in 2006 (n = 331), but a female bias (2.0F:1.0M) for those incidentally captured by longline fisheries in the CNP from 2009 to 2019 (n = 137). In the ETP, we found a male-biased (0.7F:1.0M) sex ratio for putative mature olive ridleys (n = 203) and a female-biased (1.6F:1.0M) sex ratio in the immature cohort (n = 128). In the CNP, we found a female-bias in both maturity stages (mature 1.8F:1.0M, n = 53; immature 2.1F:1.0M, n = 84).

Sea turtle foraging ground sex ratio data are informative as they reflect an amalgamation of decades of hatchling cohorts, often produced from many nesting beaches. Sex identification of turtles captured in foraging grounds can fill data gaps when sex ratio data are limited for immature or adult life stages (NMFS & USFWS 2014). While foraging ground sex ratio data cannot provide complete information on the (operational) sex ratio of the adult mating/breeding cohort (e.g. Stewart & Dutton 2014), sex-based survivorship after hatching could be gleaned from current sex ratios at foraging grounds. Foraging ground sex ratio data can provide insight into significant population impacts that may arise in the future (e.g. Jensen et al. 2018), such as increased nesting beach temperatures producing more female hatchlings.

There could be myriad reasons for the female-biased immature olive ridley sex ratios found at foraging grounds in the present study (e.g. males and females having different distributions, mortality rates, and movement patterns related to breeding); however, the increased female bias in immature (compared to putative mature) sex ratios at both foraging locations (Table 2) may be indicative of the demographic effects of changes in environmental conditions, such as rising global temperatures. Ultimately, linking sex ratios of sea turtle foraging aggregations to the nesting beach of origin for cohorts of later life stages would be ideal for constructing reliable population models (Maurer et al. 2021), which are integral to species conservation.

The impacts of climate change (e.g. warmer temperatures, sea level rise) are occurring more rapidly than sea turtles have experienced in the distant past. At nesting beaches, olive ridleys lay their eggs in nests deposited in open, sun-exposed sand rather than in shaded sand near beach-back vegetation (Plotkin 2007). Such nest site selection may increase nest temperatures if nests are constantly exposed to full sun; this is exacerbated by that fact that many olive ridley beaches are composed of dark, highly organic sand with high solar heat absorption (Seminoff & Wallace 2012). Therefore, olive ridley nests may be more prone to warmer nest temperatures that ultimately produce more female hatchlings (Valverde et al. 2010). Recent estimates (subject to a high degree of error) of hatchling sex ratios at many olive ridley nesting beaches across the Eastern Pacific found a female bias based on nest temperature analysis and inferred sex ratios (Table 2). The majority of turtles examined in this study originated from Eastern Pacific rookeries based on genetic analysis (P. H. Dutton & E. L. LaCasella pers. comm.), and female-biased immature sex ratios found here may suggest population feminization, consistent with increased temperatures (due to climate change) at nesting beaches.

Conservation planning for ETP olive ridleys can now incorporate foraging ground sex ratio data into population viability models to inform management decisions. While sex ratio for the North Pacific olive ridley foraging aggregations was 1.2F:1.0M, the sex ratio of

smaller-sized immature turtles from both sampling locations was female-biased (ETP 1.6F:1.0M and CNP 2.1F:1.0M). Poloczanska et al. (2009) suggested a 4F:1M sex ratio to be of grave concern for hatchlings for any given regional population, but so far this threshold does not appear to have been reached at ETP nesting beaches (Table 2). It would be informative to build upon this study to evaluate long-term trends in sex ratio for these 2 genetically mixed foraging groups and link individuals to source nesting beaches where environmental conditions may be different, thus enabling a broader view regarding feminization.

While sex ratio data obtained from a single sampling year (i.e. 2006 for the ETP) or limited number of turtles over many years (i.e. 2009–2019 for the CNP) may be characterized as snapshots, the data presented here reflect decades of reproductive output. These findings provide initial evidence of foraging population feminization and baseline information from which to further examine feminization of North Pacific olive ridley populations. Continued monitoring and sampling of olive ridleys in the ETP and CNP foraging regions should occur to obtain more contemporary data to compare to the sex ratios found here.

### 4.6. Climate impacts

Climate change impacts, such as increased air and water temperatures and sea level rise, may affect sex ratios of olive ridley populations through population feminization, changes in prey availability, and nesting beach habitat loss, thereby affecting the species' ability to thrive. However, their life history plasticity, including their diverse mating strategies and nomadic behavior, could potentially mitigate the impacts of climate change on olive ridleys more so than for sea turtle species that have less variable habitat use and behavior patterns (Bernado & Plotkin 2007, Plotkin 2010, Seminoff & Wallace 2012, Peavey et al. 2017). Additionally, because olive ridleys mature faster and have higher fecundity rates than other sea turtle species (Plotkin 2007), the species has a record of sustaining overharvest better, and recovering faster from the effects of overharvest (Eguchi et al. 2007); these life history traits may also provide benefits as environmental conditions change. Still, in the future, feminization may increase to the point where populations may collapse because there are not enough males to maintain genetic diversity and necessary female fertilization rates. This study underscores the need to continue research on sex ratios of olive ridleys around the world to aid conservation and management efforts, especially in locations where the species experiences multiple threats (e.g. climate change, fishery incidental mortality, and illegal harvest). The present study also provides valuable, difficult-to-obtain empirical data and results on olive ridleys within the Pacific to compare with future studies on the effects of anthropogenic impacts.

*Dedication*. This paper is dedicated to the late Dr. Wallace J. Nichols, our dear friend and colleague who pioneered sea turtle research in the eastern Pacific Ocean. 'J.' was exceptional at building networks of all kinds, for scientific monitoring, creative innovation, and conservation movements. Across the entire Pacific and many man-made boundaries, J. carried the lesson that sea turtles are ocean connectors and conservation ambassadors. We, authors and readers, all continue his important work to monitor sea turtle populations as part of J.'s "Blue Mind" network, and the ocean ecosystem.

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