

Morphology of the limb, shell and head explain the variation in performance and ecology across 14 turtle taxa (12 species)

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Given that morphology directly influences the ability of an organism to utilize its habitat and dietary resources, it also influences fitness. Comparing the relationship between morphology, performance and ecology is fundamental to understand how organisms evolve to occupy a wide range of habitats and diets. In turtles, studies have documented important relationships between morphology, performance and ecology, but none was field based or considered limb, shell and head morphology simultaneously. We compared the morphology, performance and ecology of 14 turtle taxa (12 species) in Mexico that range in their affinity to water and in their diet. We took linear measurements of limb, shell and head variables. We measured maximum swimming speed, maximum bite force and how often turtles were encountered on land, and we used stable isotopes to assess trophic position. We used these data to test the following three hypotheses: (1) morphology, performance and ecology covary; (2) limb and shell variables, like hand length, are correlated with swimming speed and the percentage of time spent on land; and (3) head variables, such as head width, are correlated with bite force and stable isotopes. We find support for these hypotheses and provide the first evidence that morphology influences performance and ecology in turtles in the field.

ADDITIONAL KEYWORDS: bite force – evolution – habitat use – morphology – stable isotopes – swimming – turtles.

INTRODUCTION

Natural selection favours morphologies that maximize the ability of an individual to utilize a specific range of resources (Irschick *et al.*, 2008). For this reason, studies that test directly how morphology impacts the ability of an organism to utilize a resource provide key insights into how different morphologies arise (Arnold, 1983). Fulton *et al.* (2005), for example, demonstrated that pectoral fin aspect ratio in labriform fishes

is positively correlated with swimming speed and water velocity. This finding, and the fact that coral reef habitats are characterized by a mosaic of different water velocities, suggests that pectoral fin aspect ratios in coral reef fish evolve in response to variation in water velocity (Fulton *et al.*, 2005). Similar relationships have been observed in nearly all major vertebrate groups, including primates (Fabre *et al.*, 2019), bats (Aguirre *et al.*, 2002), lizards (Losos, 1990), frogs (Moen, 2019) and fishes (Wainwright, 1991; but see Schulte *et al.*, 2004), crystallizing the assertion that ecology drives the evolution of morphology through its impact on performance (Arnold, 1983).

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The ways in which ecology can impact morphology are complex, and measuring organismal performance in ecologically relevant tasks can help to strengthen the understanding of morphology by providing context on how morphology can affect the ability of an organism to utilize resources (Losos, 1990; Wainwright, 1991, 2007; Moen, 2019). Many studies have investigated morphology, performance and ecology in a diverse range of organisms, but few have investigated this in freshwater and terrestrial turtles in the wild.

Turtles are an ideal model organism to address questions that might not be feasible in other taxa. For example, there are fewer turtle species than most major vertebrate groups (365 turtle species vs. 10 000+ bird species; Turtle Taxonomy Working Group, 2017; Gill *et al.*, 2020), they exhibit surprisingly diverse ecologies (Pritchard, 1979; Ernst & Barbour, 1989), and there are communities of interacting species in nearly all regions of the world (Rodrigues *et al.*, 2019). Thus, if biologists aim to understand how ecology influences morphological evolution, turtles are ideal because they are ecologically diverse, there is abundant information that already exists on their ecology, and most species could be measured physically in natural history museums.

Morphology, performance and ecology have been investigated a number of times in freshwater and terrestrial turtles. Studies that use morphological measurements of museum specimens have shown that aspects of limb, shell and head morphology are associated with habitat use (Claude *et al.*, 2003, 2004; Joyce & Gauthier, 2004; Rivera & Claude, 2008; Stayton, 2019) and diet (Foth *et al.*, 2017). Laboratory studies have shown that there are fundamental differences in how terrestrial and aquatic turtles swim (Pace *et al.*, 2001; Stephens & Wiens, 2008; Young & Blob, 2015; Young *et al.*, 2017; Mayerl *et al.*, 2019), and in how head morphology is associated with bite force and diet (Herrel *et al.*, 2002, 2018; Pfaller *et al.*, 2011; Marshall *et al.*, 2012). These studies have paved the way for our understanding of how limb, shell and head morphology interact to influence performance and ecology, yet few studies are field based or have considered measurements of limb, shell and head in the same study or assessed these measurements with field-based measures of ecology.

In this study, we compare the morphology, performance and ecology of turtles from four different field sites in Mexico. The dataset comprised 14 taxa (12 species) that belong to four major turtle families, with species habitat use ranging from highly aquatic to highly terrestrial and diets ranging from herbivorous to carnivorous. Based on previous studies, we predicted that turtle morphology should be correlated with the continuum of aquatic–terrestrial habitats and herbivorous–carnivorous diets that are observed

across the diversity of turtles (Pace *et al.*, 2001; Joyce & Gauthier, 2004; Rivera & Claude, 2008; Foth *et al.*, 2017; Stayton, 2019). For instance, turtle species that are more frequently found in the water should have longer hands, a more streamlined carapace and faster swimming speeds (Pace *et al.*, 2001; Claude *et al.*, 2004; Joyce & Gauthier, 2004; Rivera, 2008), whereas turtle species with more carnivorous diets should have wider heads and stronger bite forces (Herrel *et al.*, 2001). In this study, we test the predictions of three different hypotheses. The first hypothesis is that morphology, performance and ecology should covary significantly. The second hypothesis is that there are specific limb (e.g. hand length) and shell variables (e.g. shell height) that contribute a disproportionate amount of the variation in maximum swimming speed and the percentage of time spent on land. The third hypothesis predicts that specific head variables (e.g. head width) will contribute more to variation in bite force and diet. We test these hypotheses in a phylogenetic framework using independent contrasts.

MATERIAL AND METHODS

STUDY SITES AND SAMPLING

This work was conducted by permission of the Secretary of the Environment and Natural Resources (SEMARNAT) of the Mexican Government under permits SGPA/DGVS/004756/18 and SGPA/DGVS/004756/19. We sampled at four different field sites in the states of Jalisco, Sonora, Oaxaca and Yucatan, Mexico. Turtle species at these sites consist of individuals that belong to four of the most diverse turtle families, Emydidae, Geoemydidae, Kinosternidae and Testudinidae (Fig. 1). Our field site in Jalisco was located at the Chamela biological station (19°29'55.05"N, 105°2'37.00"W) of the National Autonomous University of Mexico, within the larger Chamela-Cuixmala Biosphere Reserve. Our Sonora field site was located in the southeast corner of the state, within the Monte Mojino reserve (27°0'15.32"N, 108°48'34.05"W), within the larger Sierra de Alamos-Río Cuchujaqui natural protected area. The field site in Oaxaca was a network of different privately owned properties near Pochutla (15°46'43.40"N, 96°30'39.61"W). Our field site in Yucatan was located within the Kaxil Kiuc Biocultural Reserve (19°58'31.61"N, 89°11'38.35"W).

Fieldwork was carried out over the span of 4–6 weeks during the rainy season (June–October) from 2017 to 2019. More data were collected in Jalisco as part of a long-term study that began before the present study (Butterfield *et al.*, 2018). Our main methods of capturing turtles were visual encounter surveys and baited hoop

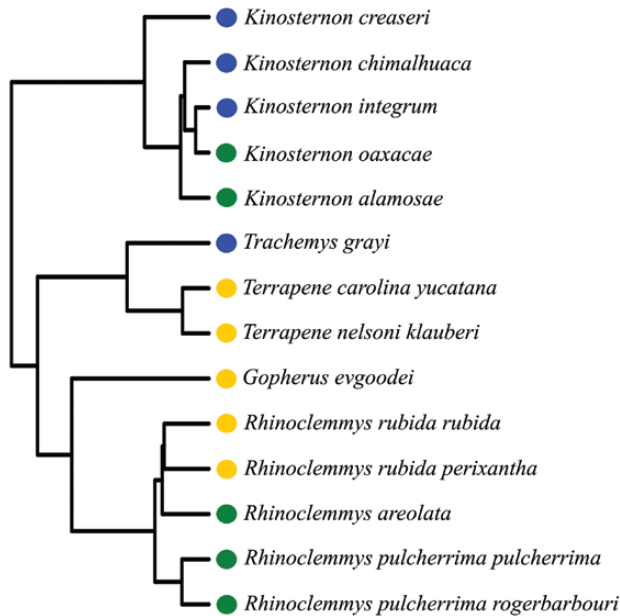


Figure 1. Phylogeny depicting the examined turtle species, based on the study by [Pereira et al. \(2017\)](#). Colours indicate the habitat categorization for each species ([Legler & Vogt, 2013](#); [Berlant & Stayton, 2017](#)), as follows: blue, aquatic; green, semi-aquatic; orange, terrestrial.

traps. Visual encounter surveys consisted of walking through potential turtle habitat with a group of three to ten people in a loose grid fashion to locate turtles on land. Hoop traps were baited with canned sardines and placed in rivers, small creeks and small ponds to trap mainly kinosternid species. In Oaxaca, *Trachemys grayi* was captured using a traditional cast net and by hand. The sampling effort of visual encounter surveys and trapping nights, including the number of people in the survey or number of traps, were recorded in Oaxaca, Sonora and Yucatan, but not in Jalisco.

MORPHOLOGY

We measured shell, limb and head characteristics of adult turtles that were captured in the field throughout the study. The majority of these turtles were used for swimming and/or biting performance trials, in which case turtles were measured after the experiments. The shell characteristics that we measured included straight-line carapace length (CL; cervical scute to cleavage between supracaudal scutes), straight-line plastron length (PL; symmetry axis from epiplastra to xiphiplastra), plastron width (PW; length of the seam that connects abdominal and pectoral plastral scutes), plastral lobe width (Lobe; the length of the seam that connects femoral and abdominal scutes), carapace width (CW; the width between fifth and sixth marginal scutes), shell height (SH; the maximum

vertical height from plastron to carapace) and bridge length (Bridge; the length from axilla to inguinal notch). The limb characteristics that we measured included antebrachium length (Ante; apex of elbow to wrist crease), manus length (Hand; middle of the wrist crease to distal end of third digit where skin meets the nail), surface area of interdigital webbing of manus (FrontWeb; the area of webbing between the second and third digit), crus length (Crus; apex of knee to apex of heel), pes length (Foot; apex of heel to distal end of third digit where skin meets nail) and surface area of interdigital webbing of pes (RearWeb; area of webbing between second and third toe). Interdigital webbing areas were calculated by multiplying the width and length of the interdigital webbing and dividing by two. The head characteristics that we measured included head width (HW; widest part of the skull), head length (HL; premaxilla to posterior edge of supraoccipital) and head height (HH; highest part of the skull at the posterior end of the jaw). All measurements were made to the nearest 0.1 mm using dial callipers.

PERFORMANCE

Maximum swimming speed was measured by filming turtles swimming in a custom-built aquarium. To ensure maximum swimming speed, turtles were motivated to swim by gently tapping on the glass of the aquarium and on the carapace. A black bag was placed around the finishing end of the aquarium to make it appear to the turtle to be a refuge. The fastest of five trials was taken as the ‘maximum swimming speed’ of an individual. In Chamela, the custom aquarium was made of glass and measured 150 cm × 15 cm × 30 cm; in Sonora it was acrylic and measured 200 cm × 30 cm × 30 cm, and in Oaxaca and Yucatán we used the same acrylic tank, which measured 150 cm × 30 cm × 30 cm. Each aquarium was filled with 15 cm of water and allowed to equilibrate to ambient temperature for ≥ 24 h before each trial began. Thin strips of opaque tape placed vertically at 10 cm intervals across the back of each tank were used as reference points when recording swimming speed. Trials were recorded in lateral view using a point-and-shoot camera (Nikon AW120) that was placed perpendicular in front of each tank such that the field of view in the camera encompassed only the entire tank. We made sure that the camera was placed in the same spot for every trial to avoid potential biases owing to parallax.

Swimming experiments took place at ~08.00 h on the day after the initial capture of the turtles that we subjected to the experiment. Turtles were housed overnight in plastic or semi-natural enclosures depending on each field site. During housing and experiments, turtles were exposed to ambient air

temperatures (typically ranging between 25 and 30 °C). All species seemed to respond in a similar manner to being housed overnight, by walking around and scratching the enclosure at intermittent intervals. Thus, the potential effects on energy expenditure that turtles endured before the experiment are assumed to be similar for all individuals subjected to the experiments.

Each turtle was encouraged to swim across the aquarium five times, at 1 h intervals. Using iMovie (Apple, Cupertino, CA, USA; <https://www.apple.com/lae/imovie/>), we analysed the video of each trial and recorded the fastest time that a turtle swam uninhibited between two reference lines. Recording started when the front edge of the carapace touched the first 10 cm reference line that was encountered after beginning to swim uninhibited, i.e. turtles were not scratching or bumping up against the glass. Recording stopped when the front edge of the carapace touched the last reference line that was encountered while swimming uninhibited. The fastest speed across the five trials was taken as the maximum swimming speed of an individual.

We measured bite force (in newtons) in the field using a custom isometric force transducer (type 9203, range ± 500 N; Kistler, Winterthur, Switzerland) that was mounted in a custom-built holder connected to a digital amplifier (type 5058A5; Kistler). Bite force was measured by coaxing turtles into biting thin metal plates that were part of the custom-built mount and noting the maximum force exerted (displayed on the hand-held digital amplifier) while a turtle was biting the plate (for more detail, see [Herrel *et al.*, 2001](#)). These experiments took place within 3 h after capturing an individual, and all individuals were subject to ambient air temperatures. Bite force has been shown to be relatively insensitive to variation in temperature in turtles ([Vervust *et al.*, 2011](#)). For each individual, we conducted three trials that were separated by ≥ 1 h, with the maximum force exerted in the three trials taken as the maximum bite force of an individual. All measurements were taken at the anterior point of the mandible. Bite force values were corrected by 0.67 to correct for the lever arms of the bite plates.

ECOLOGY

Habitat use and diet were the two aspects of ecology that we measured. Habitat use was measured by calculating the percentage of all our observations for each species that was composed of finding a unique individual on land (% land). Trophic position, as a proxy for diet, was assessed using stable isotopes.

During the fieldwork, we used visual encounter surveys and hoop traps to sample turtles, with the exception of *Trachemys grayi*, which we captured using

cast nets and by hand. We did not collect sufficient data to measure the differences in detectability of species during our visual encounter surveys, and it is possible that the % land measure could be biased, in particular for *Kinosternon* spp. A disproportionate number of kinosternids were captured using hoop traps instead of visual encounter surveys, but we choose to use % land because we believe that this measure captures more variation in habitat use than discrete habitat categorizations, such as terrestrial or aquatic.

The ratio between heavier/lighter isotopes of carbon, $^{13}\text{C}/^{12}\text{C}$ (denoted $\delta^{13}\text{C}$), has been used extensively in ecological studies to estimate patterns of resource use ([Ben-David & Flaherty, 2012](#)). This is because $\delta^{13}\text{C}$ values change in semi-predictable ways as they move through one biological system to the next owing to fractionation, which describes the change in stable isotope ratios that results from chemical reactions favouring the most abundant stable isotopes, such as ^{13}C , and ‘wasting’ uncommon isotopes, such as ^{12}C , resulting in a $\delta^{13}\text{C}$ that increases gradually from primary producers (plants or phytoplankton) to carnivores ([McConnaughey & McRoy, 1979](#); [Ben-David & Flaherty, 2012](#)). In this study, our aim was to understand where turtles fall on the herbivore–carnivore spectrum and not to identify specific food sources. For this reason, we sampled the $\delta^{13}\text{C}$ of turtle shell keratin and interpreted lower values as turtle species with more herbivorous diets and higher values as turtle species with more carnivorous diets.

Turtle shells are made up of bone, epidermal tissue and keratin, and the $\delta^{13}\text{C}$ values of keratin reflect the isotope values of the resources that were consumed during its development ([Murray & Wolf, 2012](#)). Keratin grows very slowly, and small (1 mg) pieces taken from adults can represent the isotope values of resources consumed over several years ([Murray & Wolf, 2012, 2013](#)). Previous studies have shown that the stable isotope turnover in shell keratin is very slow ([Murray & Wolf, 2012](#)). For this reason, we are confident that samples taken from individuals are likely to represent a weighted average of the resources consumed by individuals over several seasons and are not susceptible to seasonal variation in diet. In this study, we took 1 mg keratin samples from adults of each turtle species. These samples were cut with a razor saw from the peripheral edge of the second or third costal scutes or the peripheral edge of a pectoral scute, and then removed by lifting off the square with a razor blade. This procedure took only several minutes, and turtles showed minimal discomfort during the process. After removal, keratin samples were washed with 99% isopropyl alcohol and placed into tin capsules. Tin capsules were organized into a 96-well plate and sent to the Center for Stable Isotopes (CSI) at the University of New Mexico for analysis.

At the CSI, $\delta^{13}\text{C}$ values are determined using a ThermoScientific Delta V mass spectrometer (Thermo Scientific, Bremen, Germany) interfaced with a Costech 4010 (Costech, Valencia, CA, USA) elemental analyser (<http://csi.unm.edu/instrumentation>). All their sample runs include regularly spaced laboratory standards to monitor the precision of the readings. In our data measurement, the precision was 0.1‰ SD. Stable isotope results are expressed as δ values, $\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$. The R_{sample} and R_{standard} represent ratios of heavy to light isotopes, $^{13}\text{C}/^{12}\text{C}$, for the sample and standard. Stable isotope units are expressed as parts per thousand, or per mil (‰).

Keratin samples were collected in Jalisco during 2015 and 2017. Sonora, Oaxaca and Yucatán samples were collected in 2018. Given that $\delta^{13}\text{C}$ values are negative, we calculated their absolute value before analysing them so that we could \log_{10} -transform and analyse isotope values with the other morphology and performance variables. Lastly, in isotope studies $\delta^{13}\text{C}$ values are commonly adjusted by a trophic discrimination factor, which represents the percentage by which $\delta^{13}\text{C}$ values change from the resource to the consumer during tissue synthesis (Caut *et al.*, 2009). We did not determine trophic discrimination factors experimentally or adjust our values using previously reported values because trophic discrimination has been studied in only one species (*Gopherus agassizii*; Murray & Wolf, 2012). Adjusting all our data by the discrimination factor of this single species would not affect the interpretation of our data.

PHYLOGENY, PHYLOGENETIC INDEPENDENT CONTRASTS, AND BODY-SIZE CORRECTION

Given that turtle species in our study have a shared evolutionary history, the data cannot be treated as independent and need to be adjusted for phylogeny. Likewise, many data we collected are correlated with body size (e.g. head width, swim speed, bite force) and need to be corrected for body size so that interpretation is not biased by body size. To correct for both phylogeny and body size, we first pruned the phylogeny developed by Pereira *et al.* (2017) using the ‘drop.tip’ function in the ‘stats’ package in R to include the turtle species in this study (R Core Team, 2019). The phylogeny of Pereira *et al.* (2017) is fossil calibrated, and branch lengths are proportional to time. Subspecies were not included in the phylogeny published by Pereira *et al.* (2017). Therefore, we added sister taxa manually to *Rhinoclemmys rubida* and *Rhinoclemmys pulcherrima* to reflect the two subspecies of each that we included in the study. Subspecies were added using the ‘bindtip’ function in the R package ‘phytools’ (Revell, 2012). Using the edited phylogeny (Fig. 1), we calculated

phylogenetic independent contrasts (PICs) for all morphology, performance and ecology variables using the ‘pic’ function in the ‘ape’ package in R (Paradis *et al.*, 2004). We used PIC instead of other methods (e.g. phylogenetic residuals; Revell, 2009) because it was the only way we could correct for phylogeny and analyse variables related to body size (e.g. manus) with variables that are not related to body size (e.g. $\delta^{13}\text{C}$ values). All variables were \log_{10} -transformed before calculating PIC to minimize the differences among values attributable to measuring units.

After calculating PICs, we regressed the PICs of morphology and performance variables on the PIC of CL using a linear model forced through the origin by setting the intercept to zero (‘lm’ function, ‘stats’ package; R Core Team, 2019). The residuals of these linear models are referred to as ‘standardized PICs’ and represent size-free PICs of morphological and performance variables. Standardized PICs (morphology and performance variables corrected for CL) and PICs ($\delta^{13}\text{C}$ and % land) were used for all subsequent analyses.

To ensure robustness of our results using standardized contrasts, we also ran our analyses using phylogenetically independent contrasts of log-shape ratios (Mosimann, 1970; Larouche *et al.*, 2020), and we have included these results in the Supplementary Results section in Supporting Information. Contrasts of the log-shape ratios consist of dividing the variables of interest (e.g. SH) by the geometric mean of all variables that are correlated with body size (all morphological variables in this study), taking the \log_{10} , then calculating the contrast of this value.

STATISTICAL ANALYSIS

All statistical analysis was carried out in the statistical environment R v.3.6.1, and our analyses were scrutinized to a significance level of $\alpha = 0.05$ (R Core Team, 2019). To test hypothesis 1, that morphology, performance and ecology covary, we used a two-block partial least squares analysis (2B-PLS) in the R package ‘geomorph’ (Rohlf & Corti, 2000; Adams & Otárola-Castillo, 2013). The 2B-PLS analysis assesses the degree of covariation between two separate matrices or ‘blocks’ and calculates the correlation coefficient between scores of projected values between the first singular vectors of each block, defined as “ R_{PLS} ”. The 2B-PLS analysis is used primarily with landmarks and Procrustes-fitted coordinates but can be used for any matrix of variables (Adams & Felice, 2014). The 2B-PLS method compares the associations between vectors that contain singular value decompositions of variables in a matrix. In this study, we defined three blocks, with the first containing the standardized PICs of all morphological

variables (morphology), the second containing the standardized PICs of maximum swimming speed and bite force (performance), and the third containing PICs of $\delta^{13}\text{C}$ and % land (ecology). These blocks were used to compare covariation between: (1) morphology and performance; (2) morphology and ecology; and (3) performance and ecology. This was executed using the 'two.b.pls' function in the R package 'geomorph', which uses permutation (999 in this study) to assesses the significance of covariation between two blocks (Adams & Otárola-Castillo, 2013).

We fitted general linear models through the origin and used Akaike information criterion (AIC) selection and likelihood ratios to test hypotheses 2 and 3. For hypothesis 2, we tested for significant relationships between the PICs of maximum swimming speed and % land as dependent variables and standardized PICs of limb (Ante, Hand, FrontWeb, Crus, Foot and RearWeb), shell (SLP, PW, Lobe, Bridge and SH) and head (HW, HL and HH) variables as independent variables, resulting in six different linear models (three per dependent variable). Given that three tests were done for each dependent variable, we adjusted *P*-values for multiple comparisons using the Bonferroni correction. A full model with all limb, shell and head variables was not possible because when all morphological characters were included the number of independent variables exceeded the number of turtle species in this study, which led to model overfitting. We tested hypothesis 3, that head morphology is correlated with bite force and $\delta^{13}\text{C}$, with two different linear models, the first between bite force (dependent variable) and head variables (HW, HL and HH) as independent variables, and the second between $\delta^{13}\text{C}$ (dependent variable) and head variables (HW, HL and HH) as independent variables. We reduced each model by one independent variable at a time using the 'drop1' function in the 'stats' package (R Core Team, 2019). The models with the lowest possible AIC values (reduced models) were used to identify which morphological characteristics best explained the variation in performance and ecology of each hypothesis. A likelihood ratio test was used to test the significance of each model compared with a null model. This was done using the 'anova' function in the R package 'stats' (R Core Team, 2019).

To quantify the strength of variables and directions of relationships in the full models, we calculated beta coefficients for independent variables in each full model. Beta coefficients were calculated using 'Beta Coefficient Calculator' by (Gardener, 2015); this function calculates the beta coefficient of the independent variables in a linear model using the model generated with the lm() function.

We did not assess inter-individual variation in this study. However, the Supporting Information (Figs S1, S2)

illustrates intra-individual variation between swimming speed and shell height, and between bite force and head width.

RESULTS

SAMPLING SUCCESS

We gathered morphological, performance and ecological data on all species except for *Kinosternon alamosae* and *Trachemys grayi* (Table 1). For this reason, we decided to divide the data we had for each species into three subsets. The first dataset contains all species, the second contains all species except for *Trachemys grayi*, and the third contains all species except *Trachemys grayi* and *K. alamosae* (Butterfield *et al.*, 2021). Species means were used for all analysis and \log_{10} -transformed before analysis to minimize the variation resulting from different units of measure (the absolute value of $\delta^{13}\text{C}$ was calculated in order to calculate the \log_{10}). The % land variable was arcsine transformed before analysis because raw variables were not normally distributed.

To obtain our % land measurement, we placed four hoop traps on four nights in Yucatan in 2018 and none in 2019, resulting in 27 *Kinosternon creaseri*. Visual encounter surveys were conducted on 15 days in 2018 and 2019 in Yucatán, totalling 170 person-hours (\bar{x} = 2.07 persons per survey) and 16 *Terrapene carolina yucatanica*, 29 *Rhinoclemmys areolata*, and 17 *K. creaseri*. In Oaxaca, we trapped for three nights in 2018 and 2019, averaging 6.5 traps/night and resulting in 26 *Kinosternon oaxacae*. Visual encounter surveys in Oaxaca were conducted on 26 days in 2018 and 2019, totalling 700.5 person-hours (\bar{x} = 8.9 persons/survey) and 24 *Rhinoclemmys rubida rubida*, six *Rhinoclemmys pulcherrima pulcherrima* and 12 *K. oaxacae*. We spent only 2 days in Oaxaca manually trapping *Trachemys grayi* in 2019 with cast nets, resulting in 11 individuals. In Sonora, we placed hoop traps for six nights in 2018 and 2019, averaging 1.7 traps/night, which resulted in 49 *Kinosternon integrum* and 11 *K. alamosae*. Visual encounter surveys were conducted on 32 days in Sonora in 2018 and 2019, totalling 249.6 person-hours (\bar{x} = 2.2 persons per survey) and five *Gopherus evgoodei*, 41 *Terrapene nelsoni*, 11 *Rhinoclemmys pulcherrima rogerbarbouri* and six *K. integrum*. Sampling effort was not recorded in Jalisco, but hoop traps were used for only three nights to capture individuals for this study in 2019. In Jalisco, we did not record sampling effort, but we found unique turtles without traps on 219 days from 2015 to 2019, resulting in 351 *Rhinoclemmys rubida perixantha*, 13 *R. p. rogerbarbouri* and 42 *Kinosternon chimalhuaca*. Sampling effort estimates for Yucatan,

Oaxaca and Sonora are based only on hours that T.G.B. spent in the field, but additional individuals captured by locals and brought to T.G.B. were also used in the % land measure before being released back into the field; these individuals were not included in the sampling effort estimate.

RELATIONSHIP BETWEEN MORPHOLOGY, PERFORMANCE AND ECOLOGY

Using the 2B-PLS test to compare covariation between matrices or 'blocks' of the standardized PICs of morphology, performance and ecology variables,

we found partial support for hypothesis 1. The morphological block covaried significantly with the performance block ($R_{\text{PLS}} = 0.897$, $P = 0.003$; Fig. 2A) and the ecological block ($R_{\text{PLS}} = 0.62$, $P = 0.035$; Fig. 2B). The performance and ecological blocks did not covary significantly ($R_{\text{PLS}} = 0.63$, $P = 0.06$; Fig. 2C). The loadings from each of these analyses suggest that the majority of the variation between turtles is attributed to the affinity of each species for water (Supporting Information, Table S1), such that traits like FrontWeb, swim speed and % land load very strongly on the first vectors of each of the first two 2B-PLS blocks (Supporting Information, Table S1). Similar results were found when using

Table 1. Sample sizes of morphology, performance and ecology variables that were measured for each species

Species	Shell	Limb	Head	Bite force	Swim speed	% Land	$\delta^{13}\text{C}$
<i>Gopherus evgoodei</i>	7	7	5	3	7	10	7
<i>Kinosternon alamosae</i>	7	7	7	7	6	11	–
<i>Kinosternon chimalhuaca</i>	20	14	12	12	7	42	13
<i>Kinosternon creaseri</i>	18	15	18	9	10	44	12
<i>Kinosternon integrum</i>	12	12	11	6	6	55	11
<i>Kinosternon oaxacae</i>	21	20	15	10	11	47	7
<i>Rhinoclemmys areolata</i>	20	19	18	9	9	34	8
<i>Rhinoclemmys pulcherrima pulcherrima</i>	14	14	14	4	6	25	6
<i>Rhinoclemmys pulcherrima rogerbarbouri</i>	20	18	13	8	13	13	21
<i>Rhinoclemmys rubida perixantha</i>	17	13	5	6	11	351	15
<i>Rhinoclemmys rubida rubida</i>	18	18	18	7	15	39	7
<i>Terrapene carolina yucataca</i>	16	16	16	10	11	23	12
<i>Terrapene nelsoni klauberi</i>	17	17	17	9	5	57	11
<i>Trachemys grayi</i>	7	7	7	7	–	11	–

The shell, limb and head columns indicate the number of times we measured the morphological variables that correspond to these regions of the turtle body plan. These sample sizes vary because it was not possible to measure limb or head variables on some individuals. Bite force and swim speed indicate the number of individuals used in each experiment. The % land column indicates the number of times we observed a unique individual of each species. The majority of *Rhinoclemmys rubida perixantha* observations are from a previous study (Butterfield et al., 2018). The $\delta^{13}\text{C}$ indicates the number of keratin samples for each species that were processed by the Center of Stable Isotopes at the University of New Mexico. Males and females were represented equally in all samples except for *Gopherus evgoodei*, for which the sample is dominated by males ($N = 6$).

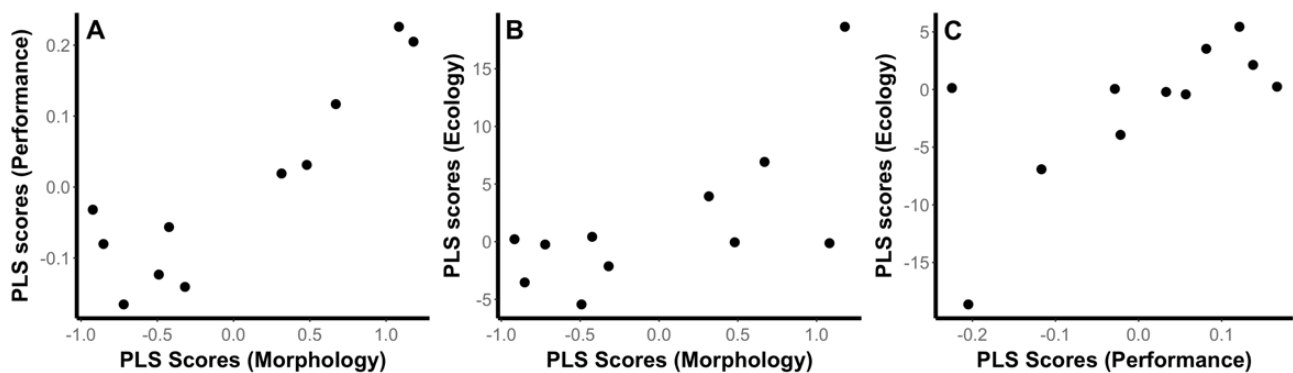


Figure 2. Results from two-block partial least squares (2B-PLS) test between morphology and performance blocks (A), between morphology and ecology blocks (B) and between performance and ecology blocks (C). These scatterplots illustrate the covariation between morphology, performance and ecology. Blocks represent matrices of phylogenetic independent contrasts (and standardized contrasts) of morphology, performance and ecology. *Kinosternon alamosae* and *Trachemys grayi* are not included in this analysis.

contrasts of log-shape ratios (Supporting Information, Supplementary Results; Fig. S3).

Linear models revealed significant support for hypotheses 2 and 3. Most reduced models were significantly different from the null model after using AIC selection, with the exception of the linear models between swimming speed and head variables and between $\delta^{13}\text{C}$ and head variables, which were not significant (Table 2). With respect to hypothesis 2, we found that PW, Lobe, SH and RearWeb were significantly correlated with swimming speed, whereas SLP, Lobe, HW and HL were significantly correlated with % land (Table 2). With respect to hypothesis 3, we found that HW was significantly correlated with bite force and that HL exhibited a marginally significant correlation with $\delta^{13}\text{C}$ (Table 2). The AIC values and R^2 values from these analyses suggest that swimming speed and % land are best explained by shell characteristics overall; most of the variation in bite force is explained by head characteristics, and $\delta^{13}\text{C}$ is explained only in part by HL (Table 2). Beta weights of morphological variables used in the linear models showed similar patterns in the proportion of the variance they explained in each model (Table 3). The results of the full models are presented in the Supporting Information (Table S2). Similar results were obtained when using contrasts of the log-shape ratios (Supporting Information, Supplementary Results; Table S3).

DISCUSSION

Understanding how the ecology of an organism covaries with morphology and performance provides insight

on how variation in ecology could impose selective pressures driving the evolution of morphology. In this study, we compared 14 turtle taxa (12 species) across Mexico to test the hypotheses that morphology, performance and ecology covary (hypothesis 1) and that specific limb, shell and head variables are correlated with our measures of performance and ecology (hypotheses 2 and 3). We found support for all three hypotheses, documenting significant covariation between morphology, performance and ecology and identifying specific morphological variables that correlated performance and ecology. These data are consistent with the notion that the selective pressures associated with habitat use and dietary regimes might be driving the evolution of morphology and performance in a consistent way in turtles.

One of the most valuable findings from this study is that direct measurements of limbs, shell and head reflect consistent and continuous differences in the ecology and diet of different turtle species, even if they belong to very different phylogenetic clades. Previous studies that have investigated the evolution of turtle lifestyles have used habitat or diet categories to depict turtle lifestyles (e.g. aquatic carnivorous). These categorizations have been extremely useful (e.g. Stephens & Wiens, 2003, 2008; McLaughlin & Stayton, 2016; Agha *et al.*, 2018; Stayton *et al.*, 2018; Stayton, 2019), but could conceal the possibility that turtle lifestyles form a continuum, as suggested by the present study. For example, habitat categories would not have detected the significant negative relationship between Hand, Foot, RearWeb and % land identified in the present study (Table 2). Likewise, DonnerWright *et al.*, (1999) demonstrated that continuous differences

Table 2. Summary of reduced linear models that compared performance and ecological variables with morphological variables

Hypothesis	Dependent variable	Independent variables	AIC	R^2	F	P -value
2	Swim speed	PW* + Lobe* + SH*	-88.28	0.93	56.39	< 0.001
	Swim speed	Hand + FrontWeb + RearWeb*	-80.33	0.87	27.62	< 0.001
	Swim speed	HL* + HH*	-62.92	0.41	5.12	0.43
	% Land	SLP* + Lobe*	37.68	0.80	27.45	< 0.001
	% Land	Hand + RearFoot + RearWeb	40.25	0.77	15.78	< 0.001
	% Land	HW* + HL + HH*	46.08	0.65	8.87	0.01
3	Bite force	HW* + HL + HH*	-87.21	0.95	78.36	< 0.001
	$\delta^{13}\text{C}$	HL	-89.96	0.23	4.33	0.06

All variables were corrected for phylogeny using independent contrasts (PIC), and variables that were correlated with body size were corrected by the independent contrast of body size (standardized PICs). The Akaike information criterion (AIC) and R^2 demonstrate the fit of models. The F values and P -values are results from likelihood ratio tests in which we compared the model with a null model. The P -values for hypothesis 2 were corrected for multiple comparisons using the Bonferroni correction.

The following definitions were used to represent standardized PICs or PICs for each variable: Ante, antibrachium length; bite force, maximum bite force; Crus, length of crus; FrontWeb, area of webbing between second and third front digits; Hand, hand length; HH, head height; HL, head length; HW, head width; Lobe, plastral lobe length; MW, width between fifth and sixth marginal scutes; PW, plastron width; RearFoot, rear foot length; RearWeb, surface area of webbing between second and third rear digits; SH, shell height; SLP, straight-line plastron; Swim speed, maximum swimming speed.

*Independent variables that explained a significant amount of the variation in each model.

Table 3. Beta weights, or standardized coefficients, from each of the full linear models before reducing the model via AIC selection

Independent variable	Hypothesis 2		Hypothesis 3	
	Swim	% Land	Bite	$\delta^{13}\text{C}$
SLP	-0.05	-0.11*	-	-
PW	-0.72*	-0.34	-	-
MW	0.04	0.12	-	-
Lobe	0.44*	0.75*	-	-
Bridge	0.07	0.27	-	-
SH	-1.30*	0.46	-	-
Antebrachium	-0.11	0.18	-	-
Hand	-0.18	-0.40	-	-
Front webbing	0.42	-1.3	-	-
Crus	0.07	-0.83	-	-
Foot	-0.10	0.24	-	-
Rear webbing	0.62*	-0.03	-	-
Head width	0.41	-1.10*	0.98*	-0.38
Head length	1.20*	-0.61*	0.006	-0.66
Head height	-1.50*	1.20*	-0.03	0.25

For the definitions of variables, see the footnote to Table 2.

*Independent variables that were significant in the linear models after Akaike information criterion (AIC) reduction.

in the geomorphology and stream velocity of the St. Croix river in Minnesota and Wisconsin, USA were correlated with the occurrence of different species throughout the watershed, suggesting that there are also continuous differences that permit multiple aquatic species to co-exist. Patterns of continuity have been observed in other studies, suggesting that such measures have the potential to provide a better understanding of turtle lifestyles (Lindeman, 2000; Welsh *et al.*, 2017). Future work should aim to use measurements of the limbs, shell and head together when investigating the evolutionary diversification of turtles. We also think that our findings here, and work by others (Pace *et al.*, 2001; Herrel *et al.*, 2002; Stayton, 2011, 2019; Ana *et al.*, 2015; Mayerl *et al.*, 2016; Young *et al.*, 2017), provide enough insight on how turtle morphology is related to ecology to enable the use of measurements of the limb, shell and head of museum specimens to address broader evolutionary questions.

The relationship between morphology, performance and ecology has been measured in many different contexts to address different ecological and evolutionary questions. Many studies focus on a single morphological feature (Collar *et al.*, 2014), a specific measure of performance (Herrel *et al.*, 2001) or a specific aspect of the ecology of an organism (Stephens & Wiens, 2003). In this study, we had a unique opportunity to include measurements of the entire turtle body plan, record two measures of performance and estimate two

aspects of ecology. This approach allowed us to use the 2B-PLS analysis, a multivariate analysis that was developed to test for significant covariation between different matrices of variables (Adams & Felice, 2014). The 2B-PLS analysis allowed us to consider all the variables together and make pairwise comparisons of singular value decompositions that represented the variation in matrices of morphological, performance and ecological variables, respectively. We found significant correlations between morphology and performance and between morphology and ecology variables (or 'blocks'), suggesting that ecology and performance both drive the morphological evolution of turtles. Moreover, the fact that we observed inconsistent relationships between morphology, performance and ecology variables suggests that evolution to habitats and diets could be mosaic in turtles. Mosaic evolution is the concept that evolutionary change can take place in some body parts without causing changes in other body parts (Clarke & Middleton, 2008; Felice & Goswami, 2018). With respect to hypothesis 1, we found significant covariation between morphology, performance and ecology blocks, but for hypotheses 2 and 3 we identified only a handful of traits that correlated significantly with performance and ecology. If the ecological evolution of turtles is mosaic, then limb, shell and head traits could contribute in semi-independent ways to determining the ecology of turtle species, resulting in turtles with similar ecologies but with different morphological traits that covary with performance and ecology. Nonetheless, it is possible that some of these correlations were not significant owing to the low interspecific sample size.

The percentage of time that turtles are found on land (% land) was negatively correlated with how fast turtles swim and with their limb, shell and head morphology (Tables 2 and 3). Turtles that spend more time on land are slower swimmers and are characterized by smaller hands, taller shells, more enclosed plastrons and smaller heads (Tables 2 and 3). This finding builds on previous museum-based studies (Claude *et al.*, 2003; Joyce & Gauthier, 2004; Stayton *et al.*, 2018) by demonstrating that a combination of limb, shell and head characteristics are correlated with swimming performance and habitat use in the wild.

Likewise, we showed that that head morphology (and other variables) directly influence bite force and $\delta^{13}\text{C}$, strengthening findings that head morphology, bite force and diet are correlated (Herrel *et al.*, 2002, 2018). Our study also suggests that increasingly high $\delta^{13}\text{C}$ values (or less negative values) correspond to turtle species that are more carnivorous, have stronger bite forces and wider heads (Tables 2 and 3). This observation is based on the interpretation that increasingly high $\delta^{13}\text{C}$ corresponds to an increasing level of carnivory and that lower $\delta^{13}\text{C}$ values correspond

to more herbivorous species. This relationship between $\delta^{13}\text{C}$ and diet has been observed in other studies, but was not investigated specifically here (Fry *et al.*, 1978; McConnaughey & McRoy, 1979; Ben-David & Flaherty, 2012; Murray & Wolf, 2013). Plants are the only source of carbon in terrestrial ecosystems, and every time carbon is processed by an organism the $\delta^{13}\text{C}$ value of its tissue increases owing to isotope fractionation (Smith & Epstein, 1971; Farquhar *et al.*, 1989; Ben-David & Flaherty, 2012). Thus, organisms that have higher $\delta^{13}\text{C}$ values have been observed to consume items that have processed carbon a greater number of times since it was first produced in a plant (Ben-David & Flaherty, 2012; the same is true of phytoplankton and marine organisms, McConnaughey & McRoy, 1979). The $\delta^{13}\text{C}$ values of species included in this study range from -30 to -20‰ (Butterfield *et al.*, 2021), making it possible that higher $\delta^{13}\text{C}$ values in this study are attributed to species that consume more plants with C_4/CAM photosynthesis, which are known to have average $\delta^{13}\text{C}$ values around -10‰ , compared with C_3 plants that average -27‰ (Smith & Epstein, 1971; Farquhar *et al.*, 1989). However, direct observation of turtles foraging in the field suggest that this is not the case because only two subspecies, *R. r. rubida* and *R. r. perixantha*, have ever been observed foraging on CAM plants in the wild. Three species have been observed consuming C_3 plants (*G. evgoodei*, *R. p. pulcherrima* and *R. p. rogerbarbouri*), and all other species that have been observed or are known to consume a mix of C_3 plants, insects, fungi and gastropods (T.G.B., personal observation; Legler & Vogt, 2013). If our findings can be corroborated in future studies, it would support the notion that evolutionary changes in the limb, shell and head are correlated with the continuum of aquatic to terrestrial and herbivorous to carnivorous ecologies.

There are several caveats that limit the interpretation of our results and that we hope will stimulate further investigation. One limitation to our study is taxonomic diversity. The turtles we studied belong to five different genera, and the species belonging to a genus all have similar ecologies (Fig. 1). Therefore, although we incorporated phylogeny in our analyses, the observations in our study could reflect the sampling of turtle lineages that have distinct ecologies. Future work should investigate whether the correlations between morphology, performance and ecology observed here hold true as more species from different families are incorporated into the analysis.

A second caveat of our study is the ecological diversity of the turtles at the field sites that we studied. Although each field site that we studied has a range of aquatic to terrestrial species, some localities across the globe are even more diverse; for example, there can be more than five aquatic species in the same locality (e.g.

turtle communities in India; Mital, 2016) or a locality can be dominated by terrestrial species (e.g. tortoise communities in Africa; Luiselli, 2003). Therefore, a future priority should be to compare morphology, performance and ecology in turtle communities that vary in their ecological diversity.

Lastly, the lack of biomechanical detail that we investigated limits our interpretation of swimming ability in different species of turtles. It has been shown that different turtle species have fundamentally different kinematic stroke patterns while swimming (Pace *et al.*, 2001; Young & Blob, 2015; Mayerl *et al.*, 2016, 2019; Young *et al.*, 2017). Our filming equipment was not sufficient to measure kinematics in detail, although one anecdotal observation we made that deserves further investigation occurred during swimming experiments in Sonora. During these experiments, it appeared that the rear foot in terrestrial emydid turtles (*Terrapene nelsoni klauberi*) might be more efficient at reducing drag during the recovery phase of the stroke in comparison to the semi-terrestrial geoemydid turtle (*R. p. rogerbarbouri*). In *Terrapene nelsoni klauberi*, during the recovery phase of the stroke, individuals maintained the rear foot in a plane parallel to the plastron, which appeared to reduce drag. In *R. p. rogerbarbouri*, the rear foot of individuals feathered during the recovery phase, which appeared likely to increase drag during the swimming stroke. This anecdotal observation suggests that comparison of kinematic patterns of the swimming stroke in emydid and geoemydid turtles could reveal fundamental differences in the swimming stroke between these families.

This study builds on previous work by combining multiple techniques and the is first field-based study aiming to understand the relationship between morphology, performance and ecology in turtles. Pace *et al.* (2001) and Joyce & Gauthier (2004) found that larger hands were associated with faster swimming speeds (increased thrust generation) and aquatic lifestyles. Stayton (2019) and Rivera (2008) demonstrated that turtles in more aquatic habitats had more streamlined and hydrodynamic shells. Herrel *et al.* (2002) and Claude *et al.* (2003, 2004) found that head morphology across turtles was significantly correlated with bite force and habitat. Our results tell a similar story to these previous studies and provide the first empirical evidence from the field that limb, shell and head morphology are correlated with turtle ecology and their ability to perform an ecologically relevant task. Furthermore, the significant results from 2B-PLS analysis and the patterns across linear models suggest that the evolution of turtles could occur along a specific continuum of variation, in which not all variables correlate significantly with ecology, but all make important contributions to the design of the overall organism.

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DATA AVAILABILITY

The data used in the analysis in this paper can be accessed at the Dryad Digital Repository ([Butterfield et al., 2021](#)).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Intra-individual variation between bite force and head width.

Figure S2. Inter-individual variation between swimming speed and shell height.

Table S1. Loadings from each two-block partial least squares (2B-PLS) analysis.

Table S2. Results from full and reduced linear models.

Supplementary Results

Figure S3. Two-block partial least squares (2B-PLS) test using contrasts of log-shape ratios.

Table S3. Results of linear models using contrasts of log-shape ratios.