

RESEARCH ARTICLE

Terrestrial feeding in aquatic turtles: environment-dependent feeding behavior modulation and the evolution of terrestrial feeding in Emydidae

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Accepted 30 August 2011

SUMMARY

Evolutionary transitions between aquatic and terrestrial environments are common in vertebrate evolution. These transitions require major changes in most physiological functions, including feeding. Emydid turtles are ancestrally aquatic, with most species naturally feeding only in water, but some terrestrial species can modulate their feeding behavior appropriately for both media. In addition, many aquatic species can be induced to feed terrestrially. A comparison of feeding in both aquatic and terrestrial environments presents an excellent opportunity to investigate the evolution of terrestrial feeding from aquatic feeding, as well as a system within which to develop methods for studying major evolutionary transitions between environments. Individuals from eight species of emydid turtles (six aquatic, two terrestrial) were filmed while feeding underwater and on land. Bite kinematics were analyzed to determine whether aquatic turtles modulated their feeding behavior in a consistent and appropriate manner between environments. Aquatic turtles showed consistent changes between environments, taking longer bites and using more extensive motions of the jaw and hyoid when feeding on land. However, these motions differ from those shown by species that naturally feed in both environments and mostly do not seem to be appropriate for terrestrial feeding. For example, more extensive motions of the hyoid are only effective during underwater suction feeding. Emydids evolving to feed on land probably would have needed to evolve or learn to overcome many, but not all, aspects of the intrinsic emydid response to terrestrial feeding. Studies that investigate major evolutionary transitions must determine what responses to the new environment are shown by naïve individuals in order to fully understand the evolutionary patterns and processes associated with these transitions.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/214/24/4083/DC1>

Key words: behavioral plasticity, suction feeding, environmental transition.

INTRODUCTION

Evolutionary transitions between aquatic and terrestrial environments are among the most challenging transitions that lineages can undergo. Almost every aspect of organismal function, from gas exchange to locomotion, requires different strategies in water and air (Denny, 1993). Despite this difficulty, transitions between these two environments are common in certain groups, although the frequencies of these transitions are variable across the tree of life (Vermeij and Dudley, 2000). An understanding of the evolutionary mechanisms and patterns accompanying these transitions can not only aid in the reconstruction of the history of such groups, but also improve our knowledge of the mechanisms by which major evolutionary transitions are accomplished.

Many studies have compared the physiology of organisms living in a novel environment with that of close relatives living in an ancestral environment in order to determine the changes that have occurred as a result of environmental transitions [see Wainwright and Reilly or Vermeij and Dudley for a catalogue of studies on aquatic–terrestrial transitions (Wainwright and Reilly, 1994; Vermeij and Dudley, 2000)]. However, many aspects of organismal phenotypes, such as behavior, can vary over an organism's lifetime (West-Eberhard, 2003). Such plasticity can greatly impact the evolutionary patterns and processes associated with transitions. Members of species undergoing an evolutionary transition between

environments may show consistent responses to the new environment; for example, aquatic individuals may all bite more slowly when feeding on land (e.g. Shaffer and Lauder, 1988). These responses may also be appropriate to the new environment; a slower bite might produce a greater success rate in the terrestrial environment than would a bite of the same speed used in the aquatic environment.

If the responses are appropriate, then plasticity can increase the tempo of evolution during the transition (West-Eberhard, 2003). Such responses can also shape future adaptation in the new environment by influencing the direction of evolution during the transition (Schluter, 1996; Hunt, 2007). Feeding in the terrestrial environment may be optimized by either taking slower bites or by using a wider initial gape, for example, but if the intrinsic response of a species is to bite more slowly, then this is the behavior that is likely to evolve. For these reasons it is crucial to evaluate performance in the novel environment, not only among species that have made this transition ('post-transition' species), but also among close relatives that remain in the ancestral environment (which may be considered representative of a naïve 'pre-transition' state).

This is particularly important for aquatic–terrestrial transitions, where the different physical properties of air and water present different challenges to animals attempting to feed in those media. For example, suction feeding can be effective in water, but the lower

viscosity and density of air precludes suction feeding to any significant degree (Summers et al., 1998; Reilly and Lauder, 1990; Lauder and Reilly, 1994). Organisms undergoing a transition between these environments must either employ the same set of feeding behaviors in both, and thus perform suboptimally in at least one, or alter their feeding behavior to accommodate the different physical properties they encounter (Bramble, 1973; Vogel, 1983).

Such studies of feeding behavior such as described here are rare, as they require study species that, despite feeding naturally in only one environment, can survive and function normally in both environments for moderate periods of time. More common are studies on species that will feed naturally in more than one environment, such as turtles (Summers et al., 1998; Natchev et al., 2009; Natchev et al., 2010), salamanders (Reilly, 1996; Deban and Marks, 2002; Shaffer and Lauder, 1988), snakes (Alfaro, 2002) and even fish (Sponder and Lauder, 1981; Van Wassenbergh et al., 2006), or studies on organisms that make this transition during metamorphosis, such as salamanders (Shaffer and Lauder, 1988; Reilly, 1996; Deban and Marks, 2002). In most of these cases, feeding behaviors and kinematics differ between different environments, usually in ways that are appropriate for the different fluid properties of water and air (Reilly, 1996; Alfaro, 2002; Deban and Marks, 2002; Natchev et al., 2009; Shaffer and Lauder, 1988). However, given that these species naturally feed in both environments, it is expected that they will have evolved appropriate responses to each environment. These species thus are uninformative about the intrinsic behavioral response in pre-transition species.

Aquatic emydid turtles, in contrast, do not naturally feed on land, but at least some species can be induced to feed in both environments. Thus, aquatic emydid turtles represent an excellent group for investigating feeding behaviors that may be representative of the earliest stages of the evolutionary transition between environments. Turtles are also an excellent group to use in feeding kinematic studies, as the turtle feeding system is extremely simple. Turtles possess no teeth and an akinetic skull, they show no lateral motion of the lower jaw, and all use the same skeletal elements (cranium, mandible and hyoid) during feeding. When feeding underwater, almost all turtles employ suction feeding (Lauder and Prendergast, 1992; Van Damme and Aerts, 1997; Lemell et al., 2002).

This study addresses the following question: do naïve aquatic emydid turtles modulate their feeding behavior in a consistent, appropriate manner when feeding in the terrestrial environment? There are two important components to this study's question. First, it is relevant to determine whether aquatic turtles modify their biting kinematics in a consistent manner. Evolution can still act upon variation even if it is inconsistent, but transitions between different environments will be greatly facilitated if the variation occurs in a consistent direction (West-Eberhard, 2003). Second, it is important to consider whether these aquatic turtles can modulate their feeding behavior in an appropriate manner. A difference in biting kinematics between the two environments is expected even if the turtles are not actively changing their behavior – the different viscosities of air and water will interact with conserved patterns of muscle activity to produce different movements. Thus, if differences are discovered, it will be important to consider whether these differences allow the turtles to feed more efficiently in the terrestrial environment, or at least reduce the use of behaviors that are ineffective in air.

Two previous studies on turtles that can feed in both environments provide information on what constitute 'appropriate' behavioral changes between water and air. When feeding underwater, *T.*

carolina usually employs rapid bites, accompanied by extension of the head towards the prey, a wide gape, and a large degree of hyoid depression (Summers et al., 1998). In air, *T. carolina* individuals took longer bites and generated a smaller amount of hyoid depression. Similarly, *Cuora amboinensis* uses a different pattern of hyoid protraction during aquatic feeding in order to generate compensatory suction (Natchev et al., 2009). In addition to these differences, turtles might also be expected to approach prey more closely before opening their jaws and to more rapidly depress the jaw underwater to maximize water flow velocity when biting (Summers et al., 1998; Reilly, 1996; Deban and Marks, 2002).

This study will provide important evolutionary information at two levels. First, it will provide information on the behavioral framework in which terrestrial feeding in emydids evolved. Second, this study will provide a general framework for studying other environment transitions. For example, many transitions in feeding environment in vertebrates represent returns to an ancestral state – all transitions to feeding underwater, for example, represent a return to the ancestral vertebrate environment. Given that species undergoing these transitions will not be completely naïve (in an evolutionary sense), it is important to establish protocols for assessing intrinsic, group-wide responses to feeding in novel environments, to better reconstruct the initial stages of these transitions.

MATERIALS AND METHODS

Specimens

Eight turtle species – six aquatic and two terrestrial – were employed in this study. The focus of this study was the behavior of the aquatic species – only a few terrestrial individuals were included for comparative purposes. The aquatic species were: *Chrysemys picta* (Schneider 1783) ($N=4$), *Deirochelys reticularia* (Latreille 1801) ($N=2$), *Graptemys pseudogeographica kohnii* (Baur 1890) ($N=1$), *Malaclemys terrapin* (Schoepff 1793) ($N=4$), *Pseudemys rubriventris* (LeConte 1830) ($N=2$) and *Trachemys scripta* (Schoepff 1792) ($N=3$). Specimens of *C. picta*, *G. p. kohnii* and *T. scripta* were captured from local ponds in Lewisburg, PA. *Chrysemys picta* is native to the area; *G. p. kohnii* and *T. scripta* are not native to central PA and these individuals likely represent released pets. *Malaclemys terrapin* individuals were donated by Dr Russell Burke of Hofstra University; these animals originated from a Long Island population. *Deirochelys reticularia* individuals were donated by the same source. The *Pseudemys rubriventris* individuals were pets temporarily loaned to the laboratory. Finally, a single individual each of *Glyptemys insculpta* (LeConte 1830) and *Terrapene carolina* (Linnaeus 1758) were also included in this study; these animals were captured in Lewisburg, PA. *Chrysemys picta* and *T. scripta* are aquatic generalist feeders (Ernst and Barbour, 1989; Bonin et al., 2006). *Deirochelys reticularia* is an aquatic specialist on mobile prey (Bonin et al., 2006). *Malaclemys terrapin* is an aquatic specialist on mollusks and crustaceans (Ernst and Barbour, 1989; Bonin et al., 2006); *G. p. kohnii* is also an aquatic species with some specialization to hard-shelled prey (Ernst and Barbour, 1989; Bonin et al., 2006). Both *G. insculpta* and *T. carolina* are terrestrial omnivores, with *T. carolina* showing a greater preference for the terrestrial environment (Ernst and Barbour, 1989; Bonin et al., 2006). *Terrapene carolina* was included for comparison with previous studies, and *G. insculpta* was included to establish the extent of feeding behavior modulation in another terrestrial species.

All animals were kept in glass-sided aquaria. For aquatic turtles, these were filled with water and turtles were provided with basking platforms. For terrestrial turtles, both aquatic and terrestrial areas were maintained in the aquaria. Prior to feeding trials, animals were

fed in their 'natural' feeding environment (underwater for aquatic turtles, on terrestrial substrate or underwater for terrestrial turtles). All animals were maintained under conditions approved by Bucknell's IACUC. Wild specimens were captured under permit no. 210 from the Pennsylvania Fish and Boat Commission. All aquatic species will only feed underwater in the wild. All donated or loaned specimens had never been fed on land. There is no way to determine whether the *G. p. kohnii* or *T. scripta* individuals had any experience feeding outside of water before these experiments.

Feeding behavior

Soft, non-mobile food items (pieces of earthworm or cat food) were presented to animals on forceps. The food item was always presented at a distance of approximately one to one-half head length away from the tip of the turtle's beak. Species were filmed while feeding at 125 frames⁻¹ using a Fastec Troubleshooter (San Diego, CA, USA) high-speed video camera. Only videos in which the turtle's head was oriented lateral to the camera were used. First, 10 videos per individual were taken while the turtles fed underwater. Second, 10 additional videos were taken of the turtles feeding above water. For terrestrial animals, the terrestrial feeding sessions required no special conditions. For aquatic turtles, terrestrial feedings were conducted in a tank containing 2–3 cm of water. This was necessary, as these aquatic turtles either could not swallow above the water's surface or only did so with great difficulty, and as turtles would not continue to feed if they could not swallow food items. All videos were edited so as to begin either when the jaws first began to open (most typically) or when the head began to extend towards the prey item and to end when both jaws contacted the prey item. In general, two to three feeding events were filmed per turtle, per day, with at least 2 days passing before feeding days. Aquatic turtles were not trained or allowed to acclimate to terrestrial feeding; hence, videos represent their first responses to feeding in this new medium.

Data extraction and ordination

All videos were converted to image stacks using ImageJ (Rasband, 2011) and digitized with tpsDig (Rohlf, 2004) (Fig. 1). The digitization scheme was chosen to capture motion of all of the mobile parts of the turtle's feeding apparatus (skull, lower jaw and hyoid) as well as movement of the turtle's body and of the food item.

First, the landmark coordinates were used to calculate a series of preliminary variables: gape angle (angle 4–3–5), gape distance (distance 4–5), distance to prey (distance 3–6) and head extension (distance 1–3). Additionally, landmarks for all frames were rotated such that landmarks 3 and 4 were aligned along a horizontal axis,

and the degree of hyoid depression was calculated according to these new coordinates as the difference between the initial *y*-coordinate of landmark 2 and the subsequent *y*-coordinates of this landmark. Next, a set of primary kinematic variables was derived from plots of these preliminary variables: (1) time to maximum gape; (2) time to maximum head extension; (3) time to minimum prey distance; (4) time to maximum hyoid depression; (5) time to initiation of head extension; (6) time to initiation of hyoid depression; (7) time to initiation of jaw opening (usually 0); (8) total bite time. Two additional variables were also calculated: (9) maximum gape angle and (10) maximum hyoid depression (measured relative to initial hyoid location).

Mean scores of all aquatic and terrestrial bites were calculated for each primary kinematic variable for each individual turtle. The differences between mean aquatic and terrestrial bites were also calculated for each primary kinematic variable for each individual turtle. Mean scores within individuals were then used to calculate mean scores of all aquatic and terrestrial bites for each primary kinematic variable within each turtle species; similarly, mean differences between aquatic and terrestrial bites were also calculated for each turtle species. Finally, species scores were used to calculate mean differences between aquatic and terrestrial bites, for each primary kinematic variable, for all turtles used in this study.

As the primary kinematic variables likely covary, a principal components analysis (PCA) was conducted on the correlation matrix of all variables in order to summarize multivariate variation in feeding behavior (Fig. 2). The correlation matrix was used as two of the variables (9 and 10) were measured on a different scale than the others. The broken stick method (Jackson, 1993) was used to determine the number of eigenvectors retained for analysis.

Mean scores for all significant PC axes were calculated for aquatic and terrestrial bites for each individual and species using the same procedures that were used for the kinematic variables. Mean differences between aquatic and terrestrial bites were calculated for each individual and species as well. Plots of these means were inspected to determine whether there was a consistent pattern of differences between aquatic and terrestrial bites among all turtles (Fig. 3). The primary focus of this study was on the behavior of aquatic species – terrestrial turtles were only plotted for comparative purposes.

Data analysis

Traditional data analysis would assume that the behaviors of all individuals in this study constitute statistically independent events. However, different species, because of different levels of relatedness, do not constitute statistically independent samples (Harvey and

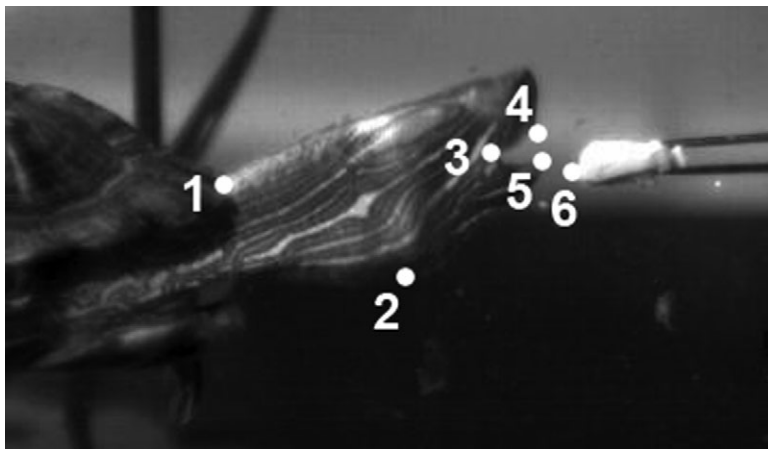


Fig. 1. Digitization scheme used in the analysis of turtle biting videos. Landmarks: (1) anterior margin of carapace, (2) ventralmost point on the second ceratobranchial (hyoid), (3) angle of mouth, (4) tip of upper jaw (premaxillae), (5) tip of lower jaw (dentary) and (6) most proximal point of food item.

Pagel, 1991). For this reason, the method of Blomberg et al. (Blomberg et al., 2003) was used to test whether the level of phylogenetic signal present in the data was sufficiently high to necessitate the use of phylogenetic comparative methods for data analysis. This method uses a permutation test to determine whether levels of phylogenetic signal (measured by the K -statistic) are less than would be expected for a given phylogeny. In this study, phylogenetic signal was tested for in all primary kinematic variables and significant kinematic PCs for both terrestrial and aquatic bites (note that the PC scores do not represent the results of a phylogenetically informed PCA) (Revell, 2009). A phylogeny of emydid turtles [fig. 3 in Wiens et al. (Wiens et al., 2009)] was used to describe the relationships among this study's six focal taxa, and species' mean scores (see above for calculation) were used as input. Scores for aquatic and terrestrial bites were treated as separate variables in these analyses; given that 10 primary kinematic variables and four PC variables were used, a total of 28 analyses were conducted. The PHYSIG program (Blomberg et al., 2003) was used to calculate K and perform the permutation tests. Again, as the focus was on the aquatic species, terrestrial species were not included in this analysis or subsequent evolutionary analyses.

A lack of phylogenetic signal was rejected for all variables ($P > 0.05$). For this reason, phylogenetically informed analyses were used to determine the significance of kinematic differences between aquatic and terrestrial bites. Species' mean scores for aquatic and terrestrial bites for all primary kinematic variables and significant PCs served as input. Species' differences between aquatic and terrestrial bites were summed for each variable. Larger sums of differences indicate larger and more consistent differences among all species. The significance of these differences was then assessed using an evolutionary modeling procedure.

Evolution of both aquatic and terrestrial biting behavior was simulated separately for all kinematic variables and PC axes (i.e. a total of 28 variables). Again, the phylogeny of Wiens et al. (Wiens et al., 2009) was used; means and variances at the tips were calculated from the original species mean data. Simulations were conducted as follows. All variables were assigned an ancestral state of 0. Different rates of evolution (derived from the variances of the tip data) (Garland et al., 1993) were used for each variable. The parameters of evolution could and did vary between aquatic and terrestrial bites for a single variable – for example, as total bite time for terrestrial bites showed more variation than did total bite time for aquatic bites, the evolution of total bite time for terrestrial bites was modeled using a higher rate of evolution. The evolution of aquatic and terrestrial bites was treated as independent (uncorrelated) for all variables.

The tip data resulting from each simulation were analyzed as with the actual data – differences between aquatic and terrestrial bites

on all kinematic variables were calculated for all species, and then summed. This procedure was repeated 999 times, and the number of times that the simulated data produced a sum of differences greater than or equal to that of the observed data was recorded and used to derive a P -value. If the observed sum of differences was greater than 95% of the simulated sums of differences, this was taken as evidence that the observed differences between aquatic and terrestrial bites were greater than expected by chance. All analyses were conducted using a program written in MATLAB (MathWorks, Natick, MA, USA) by the author.

The general question being addressed by these simulations can be summarized as follows: if aquatic and terrestrial bites are evolving independently, with no initial differences between aquatic and terrestrial bites and no correlated evolution between the two, what is the probability of evolving differences between aquatic and terrestrial bites as great as those in the observed data? A low probability would suggest the possibility of correlated evolution or the persistence of an ancestral difference, or both, between aquatic and terrestrial bites among the observed data.

Significant differences were found between terrestrial and aquatic bites for some variables. In these cases, the differences between aquatic and terrestrial feeding events were inspected to determine whether turtles were showing appropriate changes in feeding behavior.

RESULTS

All aquatic turtles fed readily in the terrestrial environment. The only exception was the single *G. p. kohnii* individual, which would make at most only a single bite per feeding session and often would not feed in air at all. Both terrestrial turtles fed readily underwater.

Data extraction and ordination

Means and standard deviations for all kinematic variables are shown in Table 1. The PCA produced four significant axes (Table 2). All time variables loaded positively on PC 1, with time to maximum gape, time to maximum head extension, time to minimum prey distance, time to maximum hyoid depression and total bite time loading most heavily. Maximum gape angle and maximum hyoid extension showed very small loadings on this axis. This axis thus primarily serves as a measure of bite duration. PC 2 contrasted maximum gape angle and maximum hyoid extension (positive loadings) with time to initiate hyoid depression (negative loading). This axis thus summarizes information on the magnitude of the movements of the feeding elements, as well as the delay in the initiation of suction. Maximum gape angle loaded heavily and positively on PC 3. Time to initiation of head extension and time to initiation of hyoid depression also loaded positively on this axis. PC 3, therefore, contrasts wide-gape bites in which head motions

Table 1. Means and standard deviations of all kinematic variables, averaged across species for the aquatic and terrestrial environments

Kinematic variable	Aquatic	Terrestrial
Time to maximum gape (s)	0.219±0.0398	0.319±0.118
Time to maximum head extension (s)	0.285±0.0691	0.367±0.139
Time to minimum prey distance (s)	0.279±0.0528	0.397±0.118
Time to maximum hyoid depression (s)	0.298±0.0570	0.409±0.130
Time to initiation of head extension (s)	0.0664±0.0188	0.160±0.101
Time to initiation of hyoid depression (s)	0.076±0.0133	0.165±0.0741
Time to initiation of jaw opening (s)	0.0196±0.0104	0.0248±0.0187
Total bite time (s)	0.337±0.0577	0.450±0.124
Maximum gape angle (rad)	0.917±0.142	1.201±0.105
Maximum hyoid extension (relative distance)	0.982±0.130	1.123±0.106

Table 2. Loadings of original variables on the first four principal components (PC) axes, and percent of total variance explained by each axis

Kinematic variable	PC 1	PC 2	PC 3	PC 4
Time to maximum gape	0.9908	-0.0082	0.0374	-0.0435
Time to maximum head extension	0.9767	0.0161	-0.0245	-0.0903
Time to minimum prey distance	0.9864	-0.0003	-0.0160	-0.0389
Time to maximum hyoid depression	0.9890	0.0108	-0.0419	-0.0314
Time to initiation of head extension	0.4846	-0.0672	0.1855	0.7409
Time to initiation of hyoid depression	0.6592	-0.1182	0.2163	0.6558
Time to initiation of jaw opening	0.1261	0.0626	0.0134	0.1146
Maximum gape angle	0.0903	0.2645	0.9548	-0.1011
Maximum hyoid depression	-0.0032	0.9950	-0.0948	0.0296
Total bite time	0.9925	0.0035	-0.0321	-0.0336
Variance explained (%)	58.576	25.746	9.7116	3.5301

initiated after jaw depression had begun with relatively small bites where head and jaw motions began simultaneously. Finally, time to initiation of head extension and time to initiation of hyoid depression also loaded highly and positively on PC 4. This axis

therefore summarized variation in the timing of these movements independent of gape angle.

In general, terrestrial bites from aquatic turtles produced higher scores on PC 1, 2, and 3 (Figs 2, 3). The pattern on PC 4 seemed

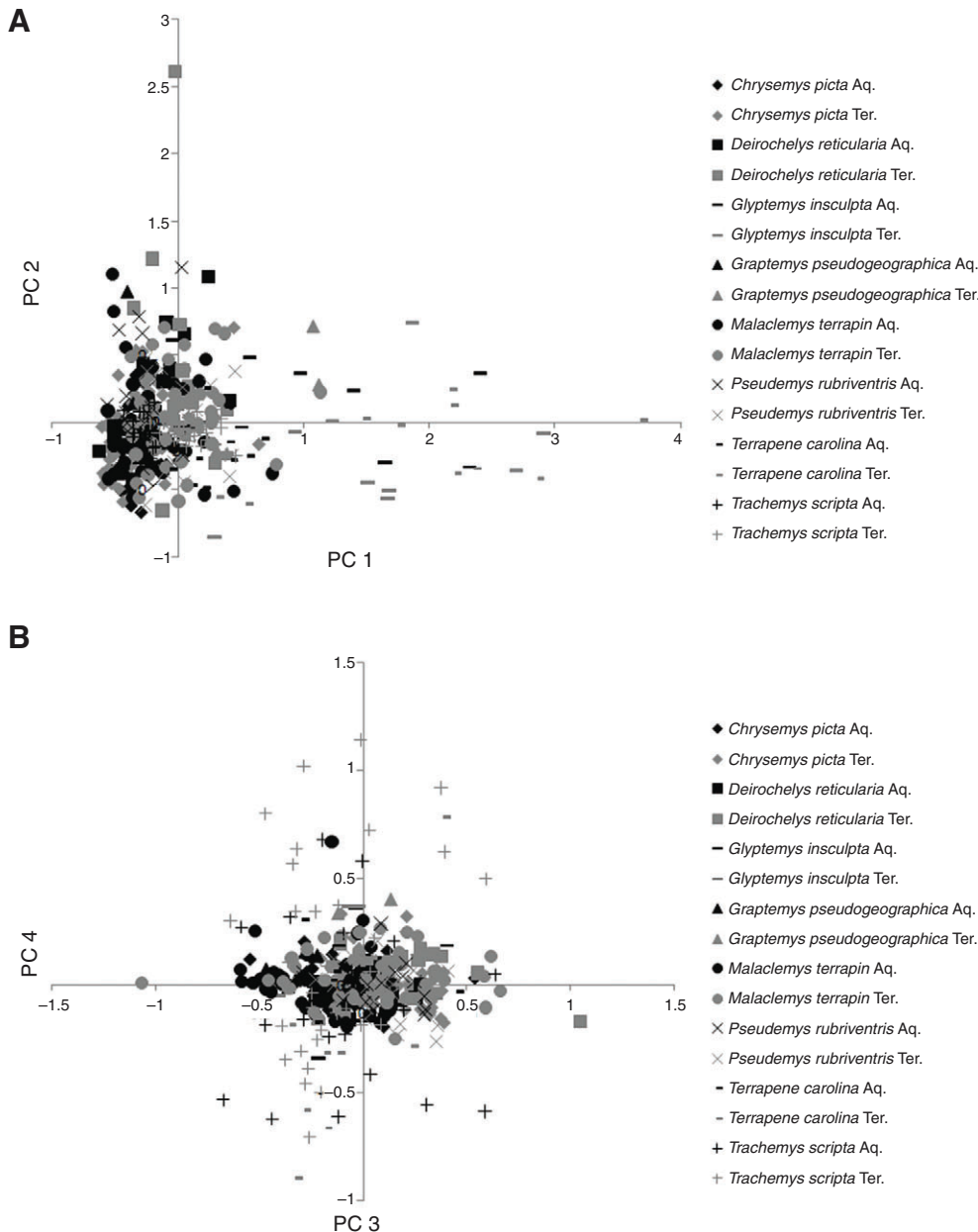


Fig. 2. Principal components (PC) scores of all bites. (A) PC 1 and 2. (B) PC 3 and 4. Mean differences between aquatic and terrestrial bites for all kinematic variables, for both observed and permuted data.

less consistent but also showed overall higher scores on this axis. Terrestrial bites of terrestrial turtles also showed higher scores on PC 1 but did not show any consistent differences with aquatic bites on the other PC axes. Note that, although only a single individual of *T. carolina* was used in this study, all changes observed match those found by Summers et al. (Summers et al., 1998) and are likely representative of the behavior of the species overall.

Phylogenetic analyses

Phylogenetic tests were necessary for all kinematic variables and PC axes. Simulations confirmed that the observed differences between aquatic and terrestrial bites were greater than would be expected if there were no ancestral differences between these bites and if the evolution of aquatic and terrestrial bites was uncorrelated ($P < 0.001$). Time to initiation of jaw opening ($P = 0.351$) and PC 4 scores were the only variables that did not show significant differences between aquatic and terrestrial feeding behaviors (Figs 4, 5).

DISCUSSION

Aquatic turtles clearly vary their feeding behavior in response to feeding environment (supplemental material Fig. S1). This variation is consistent among aquatic turtles. The differences between aquatic and terrestrial bites along PC 1, the axis explaining the greatest amount of variation in the data set, are potentially appropriate (Figs 3, 5). Differences along this PC axis indicate that aquatic bites took less time. This decrease in bite duration would, all else being equal, result in a greater amount of water displacement per unit time and thus would produce greater suction. This suction would be useful in prey capture underwater, but in air, suction is far less effective and no such rapid motions are necessary. It is possible that biting more slowly on land might reduce capture rates of agile terrestrial prey, which would make this change in feeding behavior inappropriate for the aquatic species. However, given that only immobile prey items were presented to the turtles in this study, the differences in feeding kinematics at least seem appropriate within the context of this study. In addition, species that feed in both

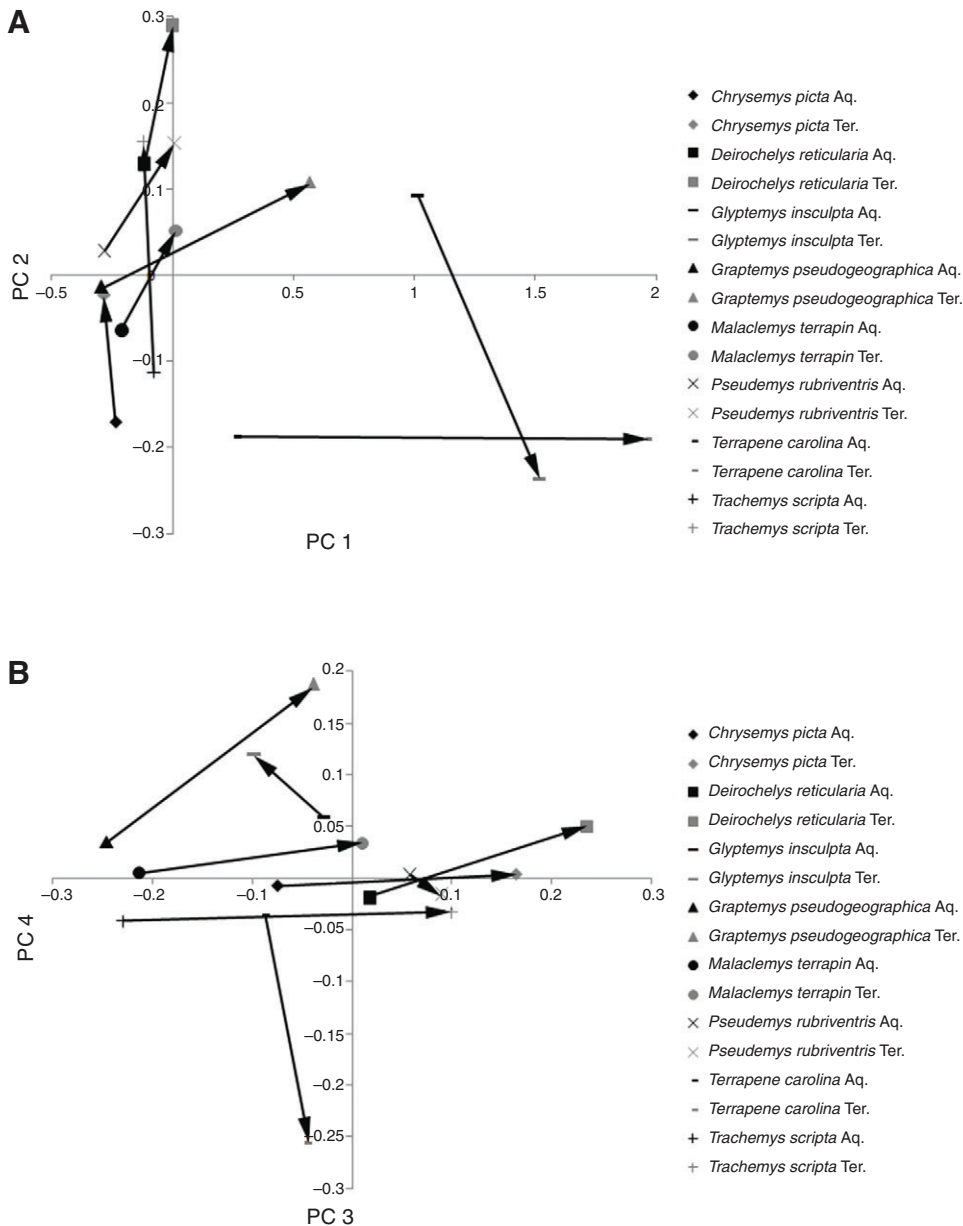


Fig. 3. Mean PC scores of all aquatic and terrestrial bites for each species, with arrows drawn between aquatic means and terrestrial means. (A) PC 1 and 2, (B) PC 3 and 4.

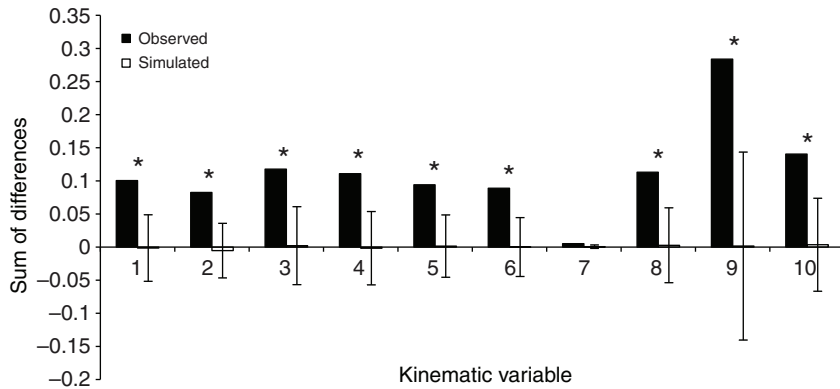


Fig. 4. Mean differences between aquatic and terrestrial bites for all kinematic variables, for both observed and evolutionarily simulated data. (1) Time to maximum gape; (2) time to maximum head extension; (3) time to minimum prey distance; (4) time to maximum hyoid depression; (5) time to initiation of head extension; (6) time to initiation of hyoid depression; (7) time to initiation of jaw opening (usually 0); (8) total bite time; (9) maximum gape angle; and (10) maximum hyoid depression (measured relative to initial hyoid location). Variables 1–8 are measured in seconds, variable 9 is measured in radians and variable 10 is measured in relative distance. *Significant difference between observed and permuted values at $\alpha=0.05$ after Bonferroni correction. Error bars indicate ± 1 s.d. for simulated data. *Significant difference between observed and simulated values at $\alpha=0.05$ after Bonferroni correction.

environments also displayed this same difference between aquatic and terrestrial bites (both in the present study and in others) (Summers et al., 1998; Natchev et al., 2009) (supplemental material Fig. S2), providing support for the idea that these differences are ultimately appropriate for the two environments.

In contrast, the differences observed along PC 2 and 3 are not obviously appropriate (Figs 3, 5). As suggested by these PC axes and confirmed by an analysis of the individual kinematic variables, aquatic turtles consistently used larger gapes, showed a greater magnitude of hyoid depression, and delayed jaw opening to a greater degree while feeding in air, relative to water. An increase in hyoid depression does not provide any benefit in air. An increased gape angle may be useful for capturing prey in a novel environment, but both behaviors would involve some increase in energy expenditure (although the cost might be minimal) and would provide no benefit in terms of suction. In addition, a delay in jaw opening would seem to confer no benefit when feeding in air; if anything, such a delay would probably be more useful in water. A closed buccal cavity would produce much lower drag on a rapidly moving head than an open mouth, and a delay in jaw opening might permit more rapid buccal expansion once the turtle's head approaches within suction distance of a prey item. It is again noteworthy that the terrestrial species showed the opposite pattern of changes on PC 2, towards smaller hyoid and jaw movements while feeding in air. That these species showed apparently appropriate changes to their feeding kinematics is understandable, given that they will naturally feed in both environments.

Thus, although aquatic turtles show consistent changes between feeding environments, these changes seem to be only partially appropriate. If a group of these turtles were to begin feeding on terrestrial prey, their intrinsic feeding behavior modulation would guide them to a more appropriate, longer bite, but also towards inappropriately large motions of the jaw and hyoid and a likely inappropriately early jaw opening. Some other mechanism would have to operate to refine this intrinsic response towards less hyoid depression or smaller gapes. This other mechanism could be natural selection, but it is also possible that turtles could learn to use more appropriate feeding behaviors, if they fed frequently enough in the terrestrial environment. The ability to learn more appropriate behaviors can greatly facilitate transitions into new environments (West-Eberhard, 2003), but this aspect of organismal adaptation to new feeding environments has been studied very little.

It is, in fact, unknown whether the more appropriate responses of the terrestrial turtle species are entirely the result of natural selection. It would be very surprising, however, if natural selection had played no part in shaping their behavioral response. Both of these turtles belong to a clade of mostly terrestrial or semi-terrestrial

turtles (Stephens and Wiens, 2003), indicating an extended evolutionary period of living in terrestrial or semi-terrestrial habitats and, presumably, terrestrial feeding. Still, given the long lives of these animals (Gibbons and Semlitsch, 1982; Gibbons, 1987), learning could potentially play a role in shaping feeding behavior during the shift from an aquatic to a terrestrial lifestyle.

Why do aquatic turtles show such a consistent behavioral response to feeding in air? One possibility is that the turtles use the same intrinsic motor patterns when feeding in both media, but that the different physical properties of air interact with these inputs to produce a different but consistent output. However, the changes seen in terrestrial feeding do not match those that would be expected if the same muscle inputs were used in both environments. A given input muscle force would produce quicker motions in air than in water, for example, given the greater density of the latter medium, but in general bites took longer in air than water. Future studies could use electromyography of the feeding musculature to determine whether the muscle input patterns are similar in both environments.

Another possibility is that the terrestrial feeding behaviors are simply those used by turtles when a more 'typical' feeding behavior fails. This could explain why greater motions of the jaw and hyoid were used, as well as longer bites: if the turtles are not receiving feedback that water (or the prey item) is being pulled into the buccal cavity, then they may continue or exaggerate those movements to try to generate the expected suction. This kind of response to failed or delayed prey capture is seen in at least two species of suction-feeding fish (Aerts, 1990; Van Wassenbergh and De Rechter, 2011). Such a response would result in longer bites with greater motions of all elements of the feeding apparatus.

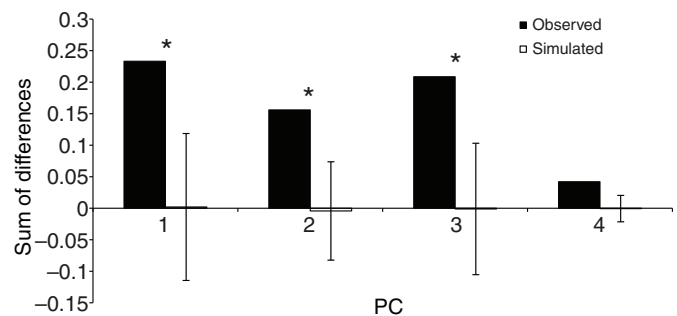


Fig. 5. Mean differences between aquatic and terrestrial bites for all PC axes, for both observed and evolutionarily simulated data. Error bars indicate ± 1 s.d. for simulated data. *Significant difference between observed and simulated values at $\alpha=0.05$ after Bonferroni correction.

Finally, the consistent response could represent the vestiges of an ancestral motor pattern that is activated when feeding on land. Though the ancestor of crown-group turtles was probably aquatic, as was the ancestral emydid (Gaffney et al., 1987; Gaffney et al., 1991; Shaffer et al., 1997; Stephens and Wiens, 2003; Joyce and Gauthier, 2004; Scheyer and Sander, 2007), turtles (and, indeed, all amniotes) (Carroll, 1988) ultimately derive from a terrestrial ancestor, so the aquatic turtles are not 'naïve' in a strict evolutionary sense [indeed, Summers et al. used a variety of this logic to argue that terrestrial turtles are probably not a good model for ancestral tetrapod feeding (Summers et al., 1998)]. However, several pieces of evidence suggest that this explanation is unlikely. First, the probability of this scenario – that emydid turtles have retained at least a partially appropriate response to feeding on land – depends on how long these species have lived and fed exclusively in water. Emydid fossils may occur as early as the Late Cretaceous or Early Paleocene (Bona and de la Fuente, 2005). Although the feeding environment of these species is unknown, this suggests a potentially long interval between the present and the last terrestrially feeding ancestor of these species. Second, it is certain that some important aspects of terrestrial feeding have been lost: most individuals could not swallow unless underwater, though some managed with apparent difficulty. Finally, as this study has illustrated, many aspects of aquatic turtles' response to terrestrial feeding are not appropriate. An appropriate response could have degraded over time, but if so, such consistent responses on the part of aquatic turtles would also not likely be present. It would, however, be interesting to study terrestrial feeding in other turtle groups that have been aquatic for much longer (such as the Trionychidae, or even Cheloniidae) to see whether the same pattern of responses is observed.

In summary, a consistent but only partially appropriate response to terrestrial feeding has been documented in aquatic emydids. It has been shown that the terrestrial emydids would have been able to use some aspects of the response (i.e. longer bites) and would have had to modify others (greater jaw and hyoid motions) to feed effectively on land. These findings illustrate the need for studies such as these when investigating evolutionary transitions among environments. Transitions by naïve species (those whose ancestors have never lived in the new environment) are quite common, but reinvasions of ancestral environments are also common (Vermeij and Dudley, 2000). In the former case, consistent and appropriate responses are not expected, but it will be important to establish the responses of the species making the transition (usually by documenting the responses of extant, related naïve species) and thus determine which aspects of behavioral variation would be useful during the transition and which would have to be modified by natural selection or learning. Studies of the variation in responses can also provide clues to the direction of evolution during such transitions (Schluter, 1996; Hunt, 2007). In the latter case, it is also important to establish the intrinsic response of the group to the new environment, not only for the above reasons, but also to determine whether any vestiges of an older and appropriate response remain.

ACKNOWLEDGEMENTS

Many students contributed to all aspects of this project: J. Kariuki, J. Linetty, J. MacFeeters, H. Ruiz and R. Taylor all deserve mention. P. Wainwright and D. Adams provided useful advice regarding the analysis of kinematic variables. R. Burke provided several of the specimens used in this analysis. P. Caloz, L. Diehl, S. Eikenberry, A. Goodfriend, M. Haines, P. Kannor, H. Ruiz, Y. Siegel-Richmond, S. Toothman, C. Vega and A. White all provided useful comments on previous versions of this manuscript. At least two anonymous reviewers also provided useful comments on an earlier version of this manuscript.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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