COADAPTATION OF PHYSIOLOGY AND BEHAVIOR: VARIATION IN ESTIVATION AMONG MUD TURTLES (*KINOSTERNON* SPP.)

By

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MOVEMENTS AND ESTIVATION IN A POPULATION OF SONORAN MUD TURTLES (*KINOSTERNON SONORIENSE*) IN SOUTHWESTERN NEW MEXICO

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ABSTRACT – Movement patterns and drought-related survival strategies were examined in a population of Sonoran mud turtles inhabiting an ephemeral stream in the Peloncillo Mountains, New Mexico. Movements within the study canyon were measured using traditional mark-recapture methods coupled with radio-tracking of a subset of the population. Extensive terrestrial movements along the canyon bed, some in excess of 2 km, and across steep terrain, were recorded. Use of the main water body in the canyon (a cattle pond) fluctuated with water levels, and male turtles were found in canyon pools below the pond more frequently than were females. All turtles that were radio-tagged before the summer monsoon season were observed engaging in terrestrial dormancy. Estivation sites were located 1 – 79 m from the dry streambed, and estivation lasted 11 – 34 days.

INTRODUCTION

Many species of freshwater turtles inhabit environments in which water availability varies seasonally (Mahmoud 1969, Bennett et al. 1970, Wygoda 1979, Christiansen et al. 1985, Grigg et al. 1986, Morales-Verdeja and Vogt 1997). When faced with drying of a local pond or stream, turtles have two options: migrate to another body of water, or become dormant until the local water supplies are replenished by rain. An alternative option employed by several desert-dwelling species is to restrict activity year-round to permanent bodies of water (oases).

The Sonoran mud turtle (*Kinosternon sonoriense*) ranges from the Gila River in New Mexico to the lower Colorado River in California and Arizona, southward into the Rio Yaqui basin in Sonora, and eastward into northwestern Chihuahua, Mexico (Ernst et al. 1994). Reports of the terrestrial proclivities of Sonoran mud turtles (*Kinosternon sonoriense*) are inconsistent. In central and southeastern Arizona, they are reported to be highly aquatic, inhabiting permanent creeks, streams and ponds, converging in water holes during periods of drought, and only venturing onto land during bouts of heavy rain and the nesting season (Hulse 1974, Ernst et al. 1994, van Loben Sels et al. 1997). Research in the eastern portion of its range provides a different impression of this species' terrestrial habits. Given the existence of populations in southwestern New Mexico that inhabit farm ponds as far as five miles from permanent water, extensive terrestrial activity must occur (Degenhardt and Christiansen 1974). Also in southwestern New Mexico, a population of *K.*

sonoriense in the Peloncillo Mountains is apparently thriving in an ephemeral stream that dries completely in some years (Stone 2001). During periods when water was scarce, capture rates were low, an indication that turtles were not congregating in available pools. Sampling and recapture efforts over six years revealed extensive long-term movements by many turtles, some of which exceeded 1000 m (Stone 2001). However, no migrations to or from stock tanks outside of the canyon were recorded.

Simulated estivation trials in the laboratory were used to measure the physiological response of *K. sonoriense* from New Mexico to extended water deprivation (Peterson and Stone 2000). These results indicated that *K. sonoriense* are capable of surviving many weeks without food and water, and that their response to such conditions are similar to *K. flavescens* (a well documented estivator) treated identically (Peterson and Stone 2000).

My first objective was to better document within-season movement patterns and habitat occurrence in the population of Sonoran mud turtles studied by Stone (2001) and Peterson and Stone (2000), and relate them to water availability. Whereas extensive movement data based on biannual sampling for five years have been reported for this population (Stone 2001), such measurements do not address responses of turtles to the extreme within-season water fluctuations that are known to occur in the Peloncillo Mountains (B. Brown, pers. comm.). The second objective was to identify the range of behavioral strategies used by individual Sonoran mud turtles in this population in response to drought, and to gauge the relative importance of each strategy. Water

supplies within the study area are known to dry completely in some years (Stone 2001), so the turtles in this canyon are not restricted to areas of permanent water. Therefore, only migration to water outside of the canyon and estivation are possible drought survival strategies.

METHODS

Study Area – The study area was in the Peloncillo Mountains, Coronado National Forest, Hidalgo County, New Mexico (precise location intentionally omitted). It was located on the extreme eastern edge of the species' range, and was the same as that studied by Stone (Stone 2001). The main study area was a gently sloping canyon approximately 3.75 kilometers long. A stock tank (dammed pool) was located approximately 0.75 kilometers from the top of the canyon (elevation ca. 1700m). Below the tank was a string of discrete ephemeral pools (Fig. 1). The stock tank was situated in a naturally narrow stretch of canyon, and was contained by a concrete dam. When full, the tank is 1.9 m deep, 15 m wide and 25 m long (ca. 375 m^2 surface area). Above the dam, the canyon becomes steeper and rockier, and pools become increasingly scarce. Two permanent impounded tanks, a small permanent spring, and at least four ephemeral tanks lie 2 – 4 km from the stock tank in the main study canyon (Fig. 2).

Stone (2001) mapped the canyon using a 50-meter tape and a compass. Sixty-seven numbered points were painted at fifty-meter intervals (with a few exceptions) along approximately 3.2 km of the canyon bed. In 1999, these

marks were extended to include a total of 5.3 km along the main canyon bed, as well as 375 m along a small branch entering the main canyon (Fig. 1). Turtle locations were mapped according to their distance above or below one of these points. Precise mapping of turtle locations was possible, as turtles were always within 25 m of one of these numbered points.

Seasonal Water Variability – Water levels in the stock tank and pools were monitored from 22 May to 4 August 1999. Short trips to the area in May and July 2000 offered snapshots of water levels in the study canyon and in surrounding watersheds. In the main study canyon, water levels in the stock tank and the canyon pools are not closely correlated (Stone 2001). To quantify water availability, water depths in the stock tank were measured daily from 27 May to 4 August 1999. After canyon pools filled in late July, water depths of 15 major pools were measured at their maximum levels. Two rain gauges were placed near the lower end of the canyon, and were used to measure rainfall between 22 May and 4 August. Rain events that were too small to be measured with the gauges (< 0.1 cm) were recorded as traces.

Turtle Sampling – Turtles were captured using hoop nets of various sizes and by hand. In stock tanks, hoop nets baited with sardines, chicken livers, or corned beef (in order of frequency of use) were used to capture turtles when water levels permitted. Intensive noodling (hand sampling) of the primary stock tank was performed on 10 June after water levels had dropped to levels too low to set a net. The canyon bed was noodled regularly when water was present in the canyon pools. Four stock tanks outside of the study area were also sampled by

noodling and trapping to identify migrants from the main study canyon. Trapping effort in the primary stock tank was 3,240 net-hours over the course of the summer, and ranged from 528 to 600 net-hours in tanks outside of the study canyon.

Upon capture, turtles were measured and weighed, and their locations mapped. Unless previously marked, a small triangle file was used to notch a unique combination of marginal scutes for future identification. Sex and number of growth rings also were recorded. Males were identified by the presence of a plastral concavity or by their long, thick tails. Turtles with midline carapace length (MCL) greater than 86 mm that possessed a flat plastron and had a small tail were classified as females; those under 86 mm were classified as juveniles (Hulse 1974). Annuli were counted on the scute(s) that showed the least wear, most frequently one of the two pectoral scutes.

Movements – Radio transmitters (L. L. Electronics, Mahomet, IL) were attached to seven male and four female adult turtles. Each transmitter weighed 7.8-8.0 g, ranging from 3.53 – 7.88% body weight. Transmitters were attached to the rear margin of the carapace with silicone sealant, and antennas, 25 cm in length, dragged freely behind the turtles. The sealant was allowed to dry overnight prior to each turtle's release at the site of capture, after which locations were recorded at least once daily. Whenever possible, visual or tactile confirmation of each location was made. Locations were then plotted onto maps of the study area. Precise locations of turtles in the stock tank were difficult due to reflection of signals from rocks and the dam.

Activity ranges of tracked turtles were measured from maps of the study area, and were defined as the distance moved between linearly terminal locations along the canyon bed. This method was chosen over straight-line distances, as turtles were seldom found outside of the streambed, except during bouts of estivation. I therefore felt that distances along the canyon were better estimates of the area utilized by turtles (Stone 2001 reached a similar conclusion). Estivation movements were measured as the minimum linear distance from the streambed to the estivation pallet. Movements of 42 non-radio tagged turtles that were captured two or more times were calculated as the distance along the creek bed between terminal locations.

RESULTS

Water fluctuations in the study canyon – Upon arrival at the study site on 22 May 1999, the stock tank was just over half of its maximum depth (Fig. 3). Despite a number of trace rain events in late May, the water level dropped 0.5 – 4.0 cm per day until mid-July. At its lowest level, the tank consisted of a puddle 10 cm deep with a surface area of ca. 2 m^2. On 15 July, 2.5 cm of rain fell, causing the stock tank to refill to a depth of 0.32 m (17% of maximum depth). An additional 9.0 cm fell during 18 – 23 July, filling the stock tank and causing water to flow over the dam and down the canyon. The tank was still full when I left the study area on 4 August 1999.

The canyon above and below the stock tank was dry on 22 May when I arrived at the study area, with the exception of one small, algae-filled pool (ca.

There were temporal differences in use of canyon pools by males and females. Sex ratios of turtles caught in the tank were close to 1:1 throughout the summer, whereas ratios in the canyon were consistently skewed in favor of males during periods when water was present (Table 1; chi-square goodness of fit, $X^2 = 4.791$, $P = 0.029$). From 20 July to 4 August, the only period when both habitats could be adequately sampled, over three times more males than females were caught in canyon pools, but the sex ratio of turtles caught in the stock tank remained close to 1:1 (Table 1). Approximately 42% more males than females were caught exclusively in canyon pools (Table 2).

There were 89 occasions when turtles were recaptured. Of these, 55 were recaptured in the same location as the previous capture (predominantly in the stock tank); 13 movements were 1 – 100 m, 15 were 101 – 500 m, and 4 were 501 – 1,000 m (Fig. 4). Among the animals that moved more than 10 m, male turtles averaged (mean $\pm$ SD) 201 $\pm$ 50 m between captures, and female turtles averaged 467 $\pm$ 188 m (Fig. 4). Although the majority of turtle activity ranges were less than 500 m (Fig. 5), two turtles, both females, made within-season movements that exceeded 1,000 m: one moved 1,259 m, and the other moved 2,331 m (Fig. 6).

Radio transmitters were fixed to eleven turtles for periods ranging from 7 to 68 days (a twelfth turtle was equipped with a transmitter that failed shortly after release; this turtle's movements were removed from the data set). Four of the tagged turtles were originally caught in the stock tank, and 7 were caught in canyon pools. Activity ranges were from 27 to 1,395 m (Table 3). Maximum

single-day movements for radio-tagged turtles ranged from 23 to 609 m. Three tagged turtles spent time in both the stock tank and canyon pools. Two were originally tagged and released into the stock tank, and the third was released in the canyon 1,340 m below the tank.

Estivation – All turtles that were carrying radio transmitters during the dry portion of the summer engaged in bouts of terrestrial dormancy (Table 3). The duration of observed estivation events ranged from 11 to 34 days; however, it is likely that many turtles in the canyon estivated for much longer periods, as dry conditions prevailed before my arrival in May (B. Brown, pers. comm.). The duration of observed bouts of estivation were highly dependent on when each turtle was equipped with a radio tag; all turtles emerged from estivation and returned to water during 15 – 18 July.

Turtles showed little consistency in the estivation sites they chose. Shallow pallets (in which the turtle's posterior was sometimes visible) were formed under clumps of bear grass (*Nolina microcarpa*), sotol (*Dasylirion wheeleri*), pointleaf manzanita (*Arctostaphylos pungens*), and large rocks. Estivation sites varied in distance from the dry streambed from 1 to 79 m (Table 3), and were located in areas ranging from dense pine canopy with a thick layer of litter to the top of a sparsely vegetated rocky ridge with full exposure to the afternoon sun.

Sampling outside the study area – Turtles were trapped in three cattle tanks outside of the main study canyon. A fourth tank was sampled, but no turtles were caught. Trapping success was highly variable between the three sites. A

tank 4 km north of the main canyon yielded seven turtles, two of which were caught twice; a tank 1.5 km south of the study canyon yielded 89 captures of 45 turtles; and four turtles were caught by hand in a tank 3 km south of the study canyon. The latter tank was silted in above the dam, resulting in a string of puddles rather than a proper pond. No turtles were caught in multiple watersheds in 1999. However, two turtles that had previously been marked in the study canyon were found in the tank 1.5 km to the south. One was a male originally marked in August 1997 and caught a second time in May 1998; the other was a male originally marked in May 1998. These migrations must have included extensive overland movements, as the two watersheds are separated by very steep, rocky terrain.

DISCUSSION

Drought survival strategies – One conclusion can clearly be drawn from my field observations: *K. sonoriense* in this study population rely heavily on estivation as a means of surviving extended droughts. Thus, they are confirmed to make behavioral use of the physiological capacity for estivation that has been demonstrated in the laboratory (Peterson and Stone 2000, Chapter 2). All animals equipped with radio transmitters before the rainy season engaged in terrestrial dormancy. In addition, the short lag time between the filling of pools in the canyon with rainwater and the arrival of turtles at these pools suggests that they were estivating nearby. The alternative, that they migrated from the nearest

permanent water, would have required an overnight movement in excess of 2.5 km over difficult mountainous terrain, which seems unlikely.

Little evidence supports the two alternatives to estivation for surviving droughts: migrating between bodies of water, and converging in any remaining water holes. Two turtles originally marked in the study area were found to have migrated over rough terrain to a tank in a watershed 1.5 km to the south. However, these long-range movements do not appear to be a response to the problem of dealing with within-season fluctuations in water levels. Water availability in this tank was lower than in the tank in the study area. Upon my arrival in May, the tank to the south of the study site was dry, while the tank in the primary study canyon was close to half full. Both tanks filled on 18 July.

A small puddle of water was present in stock tank no. 1 in mid-July prior to its refilling with rain water, and two turtles remained in this puddle, apparently never going onto land to estivate. These two turtles were in the extreme minority, however; the turtle population in the study site is estimated at 300 individuals (Stone 2001). I am confident that no other small bodies of water persisted through the summer in the canyon, suggesting that only 0.7% of the population remained entirely aquatic throughout the summer.

My findings are in stark contrast to descriptions of this species' terrestrial habits in other parts of its range. Based on five years of sampling a population in the Chiricahua Mountains, Arizona, van Loben Sels et al. (1997) reported that 90% of all captures occurred in stock tanks using baited hoop nets, whereas only 10% were caught in the associated stream or on land. However, the relative

sampling efforts in the different habitat types were not reported. Hulse (1974, p. 16) stated in his broad autecological study of the species that, "*Kinosternon sonoriense* is totally aquatic, seldom venturing onto land except to lay eggs. In several years of collecting I observed only one specimen, a basking male, out of the water." The only reports of significant amounts of terrestrial activity are based on circumstantial evidence, and come from the extreme eastern portion of the species' range (Degenhardt and Christiansen 1974, Stone 2001).

Differences in terrestrial habits of *Kinosternon sonoriense* populations in the Peloncillo and Chiricahua mountain ranges likely stem from differences in water availability. The Peloncillo mountain range has fewer permanent springs and less snow pack than the Chiricahua Mountains, so streams are highly seasonal. As a result, selection for drought tolerance is likely greater in the Peloncillo Mountains.

Movements and habitat selection – Radio tracking and repeated sampling revealed that Sonoran mud turtles are capable of long aquatic and terrestrial movements, yet the use of this ability is highly variable among individual turtles. At one extreme, 50% of recaptured turtles were caught exclusively in the stock tank, the most permanent body of water immediately available to them. At the other extreme were turtles that used more than 2 km of the canyon's length over the course of the summer, and radiotagged turtles that made daily movements in excess of 500 m. It is clear that Sonoran mud turtles in the study area are capable of moving distances sufficient to move between the tank and the canyon pools, offering the opportunity to exploit the unique resources each offers.

That filling of canyon pools and the stock tank is not synchronous means that relative food availability in the two habitats varies temporally. The canyon pools were filled early by small rain showers, offering breeding sites for red-spotted toads (*Bufo punctatus*) and canyon tree frogs (*Hyla arenicolor*). The very high tadpole densities achieved in some pools appeared to be an important food source for black-necked garter snakes (*Thamnophis cyrtopsis*), and a variety of aquatic beetles, as well as resident Sonoran mud turtles. In addition, a mud turtle was observed consuming an adult male toad. Large quantities of chitin from the exoskeletons of aquatic beetles were observed in turtle feces. Upon arrival at a pool, a turtle would need to spend little energy foraging on the resident prey. However, turtles are extremely exposed while active in canyon pools, and several potential turtle predators inhabit the area, including zone-tailed hawks (*Buteo albonotatus*), red-tailed hawks (*Buteo jamaicensis*), ravens (*Corvus corax*), black bears (*Ursus americanus*), and coatis (*Nasua nasua*). Four turtle shells were found in the canyon, three of which were perched upside down atop rocks. The location of these shells, and that they had been cleaned out without damaging the shells, suggests avian predation.

After the stock tank filled in mid-July, anurans were heard calling around its perimeter, and tadpoles were observed shortly thereafter. However, the densities in the tank were much lower than in the canyon pools; presumably, the amount of time spent by turtles searching for prey would be much higher in the tank than in the canyon pools. Deep turbid water, thick aquatic vegetation, and many dead tree limbs in the water diminished visibility, so the risk of predation in

the stock tank is likely much lower than in the stock tank. That said, a radiotagged turtle that had been inhabiting the stock tank was found dead on 2 August with its forelegs and head partially torn from its shell.

The costs and benefits associated with inhabiting the canyon pools versus the stock tank likely fluctuate on a seasonal basis. Based on recapture data from this summer and from previous years (Stone 2001), it appears that most turtles opt for the relatively low but stable availability of resources in the stock tank over the abundant but short-lived resources available in the canyon. Females in particular were found in greater numbers in the stock tank than in canyon pools. The ratio of males to females inhabiting canyon pools did not differ significantly from the overall male-biased sex ratio (1.4 : 1) reported in this population (Stone 2001). This biased ratio enforces the fact that relatively few male mud turtles were caught in the stock tank. Males of polygynous species increase their fitness by maximizing the number of females with which they copulate, and by copulating with females unlikely to be encountered by other males. Extensive aquatic and terrestrial movements have been observed by males of several freshwater turtle species (*Clemmys marmorata*, Bury 1972; *Trachemys scripta*, Morreale et al. 1984, Parker 1984). Whereas male *Kinosternon sonoriense* may benefit from searching for mates in the canyon where intrasexual competition for dispersed females is low, females may increase survival of their hatchlings by laying eggs near the relatively reliable water of the stock tank. Although females were never observed nesting, all hatchlings encountered (two in July 1999,

eleven in July 2000) were within 100 m of the stock tank. Stone (2001) reported a similar trend in the distribution of hatchlings.

Conclusion – The widespread belief that *Kinosternon sonoriense* is obligately aquatic and almost never ventures onto land (Hulse 1974, Ernst et al. 1994, van Loben Sels et al. 1997) clearly does not apply across the species' range. Research in the Peloncillo Mountains has consistently concluded that turtles exist in canyons where water is ephemeral, and that extensive overland movements occur (Degenhardt and Christiansen 1974, Stone 2001). Published reports of this species have stemmed from only a few studied populations, and the western and southern portions of the species' range have been largely neglected. Desertification of the southwestern United States is a continuing trend that began ca. 12,000 years ago (Fredrickson et al. 1997), and water availability is presently highly variable across the range of *K. sonoriense*, perhaps resulting in varying degrees of selection for drought tolerance (Chapter 2). A broad comparative study of populations from across their range could reveal further variability in terrestrial habits within this species and others, e.g., *Kinosternon integrum* (J. Iverson pers. comm.).

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Table 1. A comparison of sex and age classes of Sonoran mud turtles in the Peloncillo Mountains during summer 1999, describing temporal patterns of space use. Turtles caught multiple times were counted only once per sampling period. In the last three periods, more males than female were caught in canyon pools.

Sampling Period	Habitat	Male	Female	Juvenile	Hatchling	Total
22-May -- 5-Jun	Tank	9	13	3	0	25
	Canyon Pools	0	0	0	0	0
6 -- 20 Jun	Tank	7	7	0	0	14
	Canyon Pools	0	0	1	0	1
21-Jun -- 5 Jul	Tank	0	0	0	0	0
	Canyon Pools	11	5	4	0	20
6 -- 20 Jul	Tank	1	2	2	0	5
	Canyon Pools	19	11	4	0	34
20-Jul -- 4-Aug	Tank	20	24	4	0	48
	Canyon Pools	16	5	1	2	24

Table 2. Number of Sonoran mud turtles caught in each habitat type in the Peloncillo Mountains in 1999. Brackets indicate the subset of turtles in each category that were caught two or more times.

	Stock Tank	Canyon Pools	Both
Males	30 [9]	26 [10]	[3]
Females	29 [16]	15 [4]	[4]
Juveniles	9 [2]	9 [1]	[0]
Hatchlings	0 [0]	2 [0]	[0]
Total	68 [27]	52 [15]	[7]

Table 3. Activity ranges and estivation movements of 11 radio tagged turtles in the Peloncillo Mountains, New Mexico. Days is the total number of days for which each turtle carried a tag, Activity Range is the distance in meters along the canyon bed between terminal locations, Max. Movement is the greatest distance moved in a 24-hour period, and Estivation Movement is the distance in meters from the canyon bed to the estivation pallet. Bold font represents animals originally released into the stock tank.

Individual	Sex	Days	Activity Range	Max. Movement	Estivation Movement
62	m	29	1,395	609	1
18	f	35	575	567	29
259[A]	m	7	545	218	---
227	**f**	**67**	**509**	**186**	**79**
2300	m	42	369	208	1
172	m	26	315	123	8
179	m	20	310	184	1
250	f	41	291	112	5
161	**m**	**65**	**55**	**49**	**15**
7	**m**	**67**	**27**	**23**	**27**
104	**f**	**68**	**33**	**33**	**24**

[A] Turtle 259 was equipped with a transmitter in late July after the stock tank and canyon pools filled with water.

FIGURE LEGENDS

Fig. 1: Map of the study canyon in the Peloncillo Mountains of southwestern New Mexico. The large dot near the south end of the canyon represents the stock tank, and smaller dots along canyon's length represent major pools.

Fig. 2: Water depth in the stock tank located in the primary study canyon in the Peloncillo Mountains, New Mexico. Depths are based on daily measurements. Rain events (right axis) over the course of summer 1999, are indicated by arrows. Trace showers are excluded from this figure.

Fig. 3: Distance between all consecutive captures of Sonoran mud turtles caught two or more times in 1999 in the Peloncillo Mountains, New Mexico. Variation in the amount of time elapsed between captures is not accounted for. There was no difference between sexes ($P= 0.37$). Of those turtles that moved ≥ 1 m, male distances averaged 201 m and females averaged 461 m ($P= 0.07$).

Fig. 4: Activity ranges of non-radiotagged Sonoran mud turtles that were caught two or more times in 1999. Activity ranges were calculated as the distance between terminal points of capture.

Fig. 5: Captures of two non-radiotagged Sonoran mud turtles in 1999. Turtles 70 and 101 exhibited the largest within-season activity ranges recorded.

Figure 1

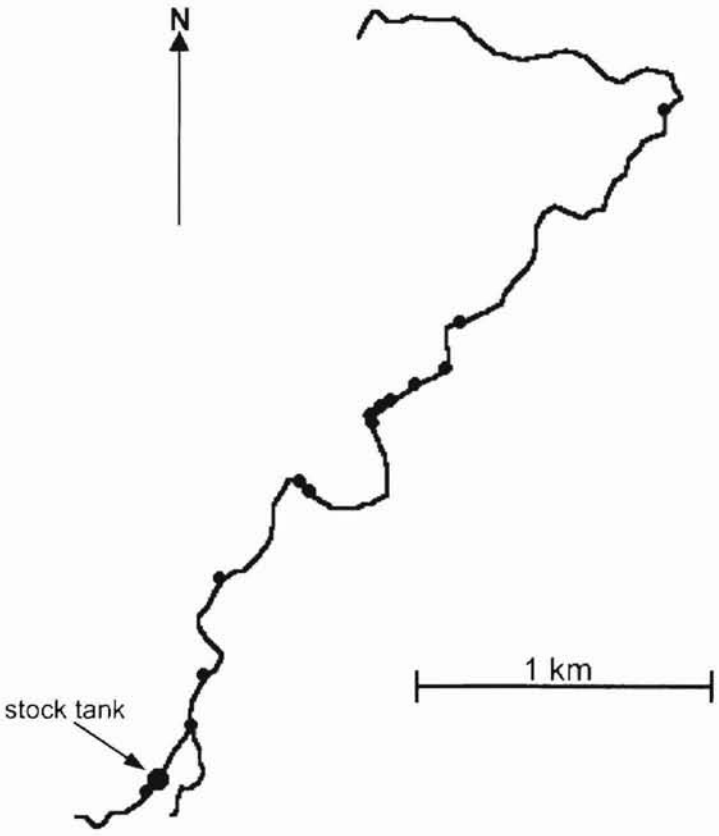


Figure 2

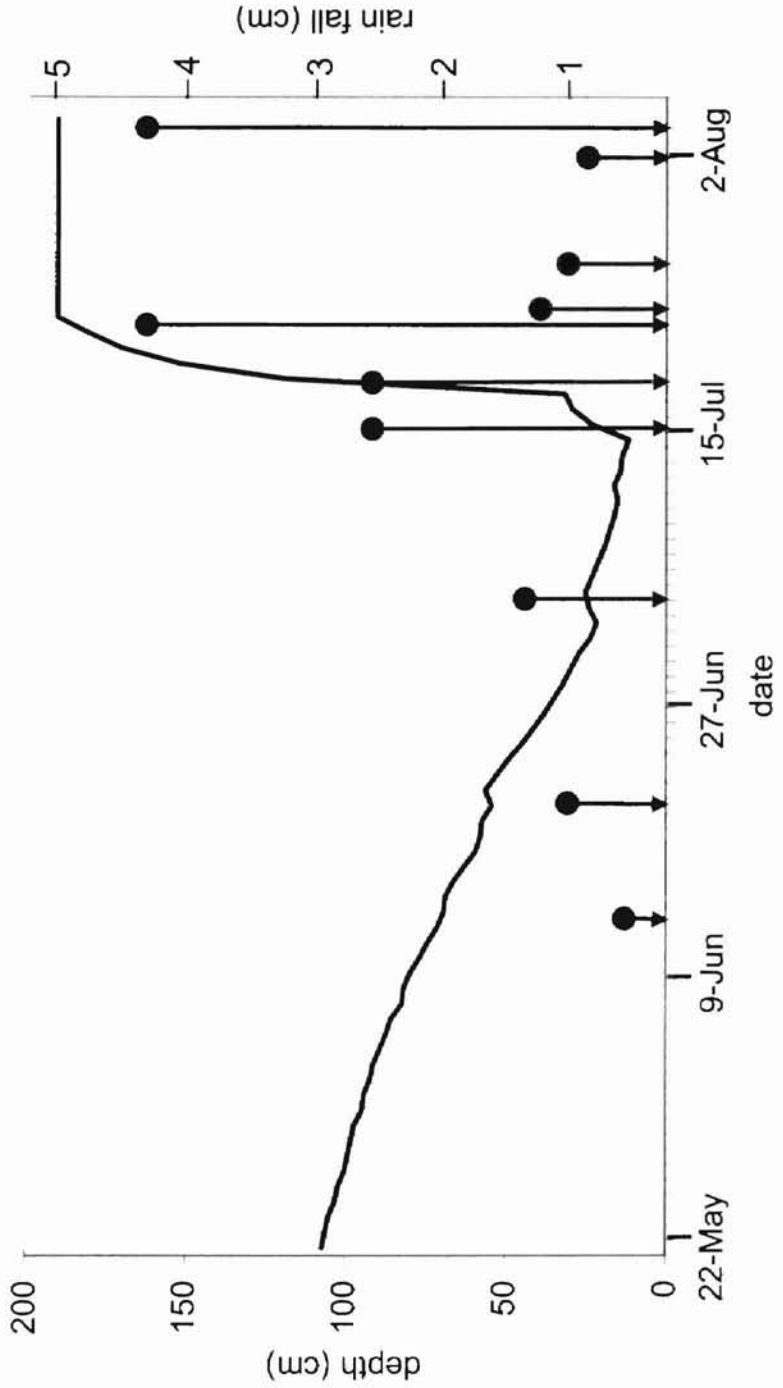

rain fall (cm)
5
4
3
2
1
depth (cm)
200
150
100
50
0
22-May
9-Jun
27-Jun
15-Jul
2-Aug
date

Figure 3

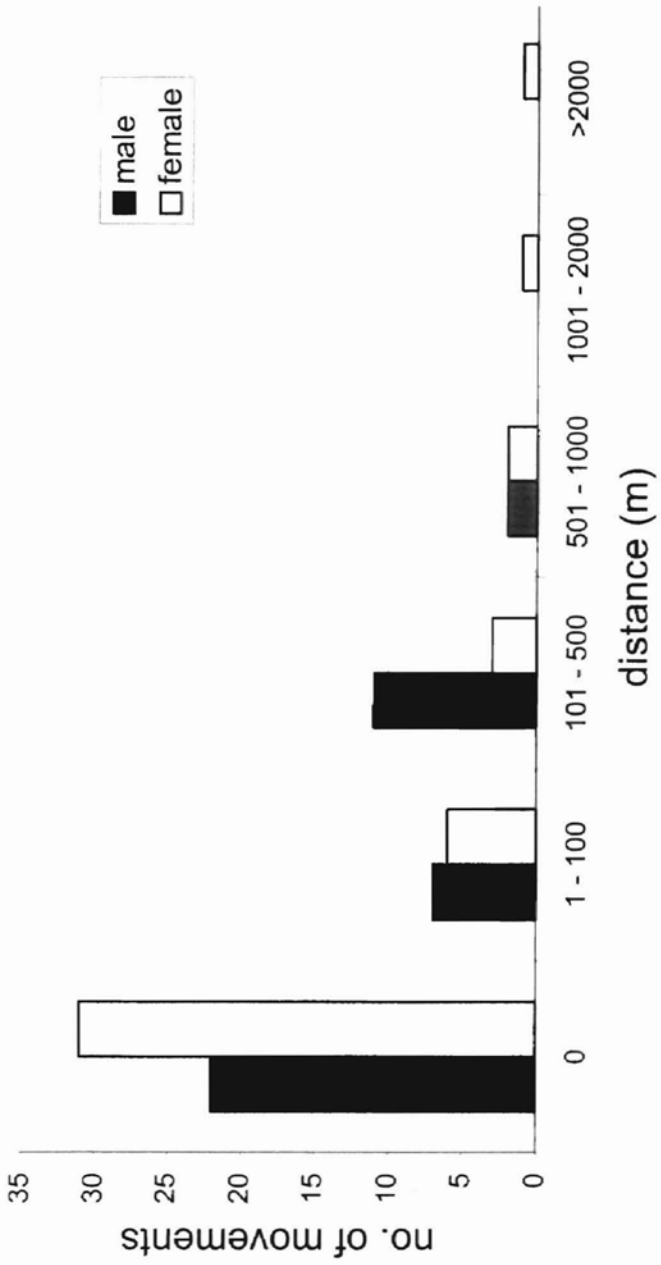
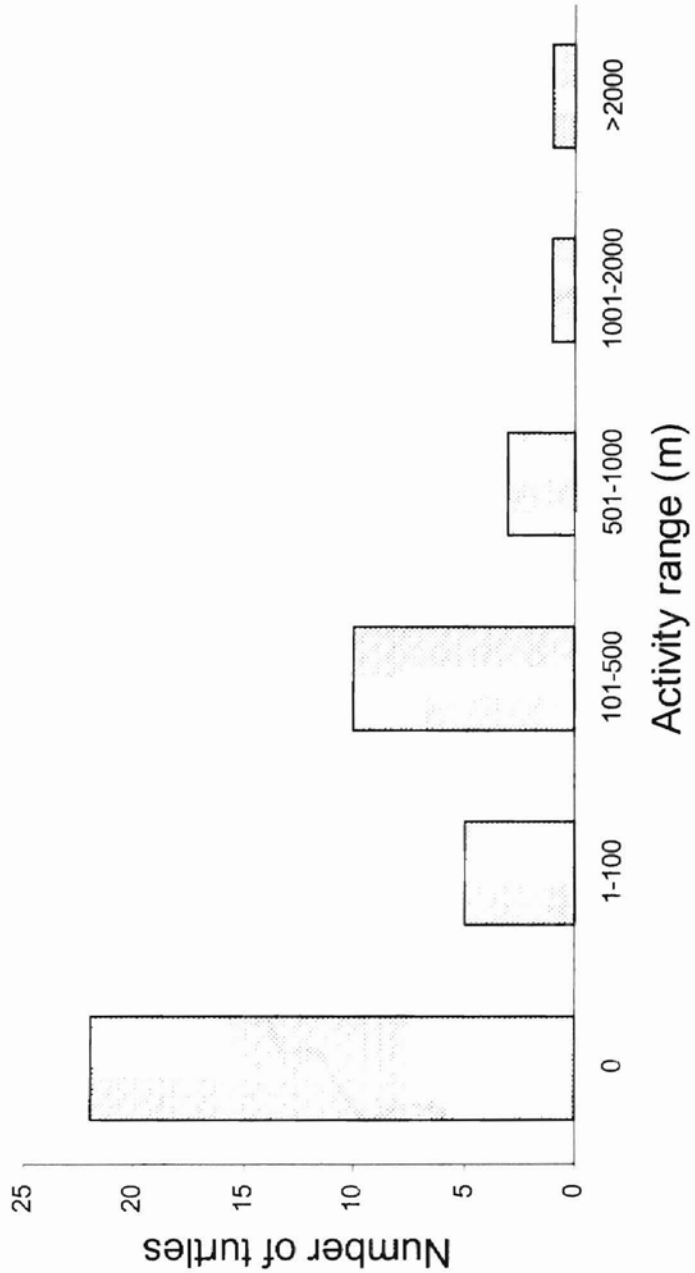

male
female
no. of movements
35
30
25
20
15
10
5
0
0
1 - 100
101 - 500
501 - 1000
1001 - 2000
>2000
distance (m)

Figure 4


Number of turtles
25
20
15
10
5
0
0
1-100
101-500
501-1000
1001-2000
>2000
Activity range (m)

Turtle no. 70
15 Jul, p.m
21 Jul
15 Jul, a.m.
3 Jul
1 km

Turtle no. 101
21 Jul
25 Jul

Figure 5

VARIATION IN PHYSIOLOGICAL AND BEHAVIORAL RESPONSES TO DEHYDRATION AMONG MUD TURTLES (*KINOSTERNON* SPP.)

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ABSTRACT – Sonoran mud turtles (*Kinosternon sonoriense*) have long been believed to be obligately aquatic, yet recent evidence suggests that populations in the extreme eastern portion of the species' range thrive in habitats that experience annual drying. I compared the physiological response to dry conditions of *K. sonoriense* from two populations: one from Arizona, in which turtles have been described as primarily aquatic, and one from New Mexico, in which extensive estivation has been documented. For comparative purposes, the responses of groups of yellow mud turtles (*K. flavescens*, a well-documented estivator) and Mexican rough-legged mud turtles (*K. hirtipes*, a highly aquatic species) also were measured. All four groups were subjected to simulated dry-season conditions in the laboratory, and behavioral and physiological responses were recorded. Dehydration trials revealed substantial differences in response to dry conditions among

populations of southwestern kinosternids. Yellow mud turtles were good estivators. They exhibited the lowest activity levels, the slowest increases in plasma osmolality, and the lowest blood urea levels. With the exception of urea, Sonoran mud turtles from New Mexico tended to be more similar to yellow mud turtles than to their Arizona conspecifics. Compared to those from Arizona, Sonoran mud turtles from New Mexico had lower activity levels, slower increases in plasma osmolality, and higher concentrations of uric acid. High activity levels and rapid increases in plasma osmolality suggest that Mexican rough-legged mud turtles and Arizona Sonoran mud turtles are relatively poor estivators. Large numbers from both groups had to be rehydrated after only 30 days out of water. Also, Mexican rough-legged mud turtles had higher rates of evaporative loss than the three other turtles studied. No evidence of metabolic depression during dehydration was demonstrated by any of the four populations.

INTRODUCTION

The present-day deserts of the southwestern United States were cool and wet during the Late Pleistocene (ca. 18,000 ya), and dominated by coniferous forests (Schmidt 1979, Van Devender et al. 1987). Desertification of the region began between 12,000 and 11,000 years ago during the late glacial period, and by the late Holocene (ca. 6,000 ya), many of the modern desert species had become established in their present ranges (Van Devender et al. 1987,

Fredrickson et al. 1998). These dramatic changes in water availability and vegetation composition significantly affected much of the vertebrate fauna, perhaps contributing to the extinction of about two-thirds of indigenous large mammals (Martin 1984). Trends toward increasing desertification continue today due to changing climatic conditions, and grazing and agricultural mismanagement in the region (Fredrickson et al. 1997, Thompson and Anderson 1997).

The shift toward an increasingly arid climate undoubtedly had a profound impact on aquatic flora and fauna. Probably, many such organisms disappeared from the region; those that remained either became restricted to areas where permanent water persisted, or possessed or evolved behavioral and physiological characteristics that facilitated survival in the drier conditions.

Extant mud turtles (Kinosternidae) are primarily aquatic, though substantial terrestrial activity has been reported in several species (Carr 1952, Skorepa and Ozment 1968, Mahmoud 1969, Bennett et al. 1970, Scott 1976, Rose 1980, Pritchard and Trebau 1984, Iverson 1989, Stone et al. 1993, Morales-Verdeja and Vogt 1997). Three species of mud turtles are indigenous to the Sonoran and Chihuahuan deserts: the Sonoran mud turtle (*Kinosternon sonoriense*), the yellow mud turtle (*K. flavescens*) and the Mexican rough-footed mud turtle (*K. hirtipes*) (Iverson 1992, Ernst et al. 1994). Though all three species inhabit areas where water is scarce and rainfall highly seasonal, responses to such conditions reportedly vary substantially among species (Ernst et al. 1994), and even among conspecific populations (Chapter 1). Variable

selective pressure for drought tolerance resulting from regional variation in water availability (both temporally and spatially) may account for the range of drought tolerance found among desert-dwelling kinosternids.

The degree of drought tolerance in *Kinosternon sonoriense* appears to be highly variable. In general, they are reported to be aquatic, only occurring where water is available year round, and rarely venturing onto land (Hulse 1974, Ernst et al. 1994, van Loben Sels et al. 1997). However, in the Peloncillo Mountains of New Mexico along the extreme eastern edge of their range, populations inhabit canyons where water is highly seasonal (Stone 2001, Chapter 1). The physiological response to dry conditions by *K. sonoriense* was similar to that of *K. flavescens*, a well-known estivator (Peterson and Stone 2000).

Kinosternon flavescens frequents temporary farm ponds in semi-arid grasslands and deserts (Ernst et al. 1994, Mahmoud 1969), and has long been recognized as a champion estivator capable of surviving as long as two years without water (Rose 1980). During extended periods of estivation, yellow mud turtles become inactive and reportedly exhibit a decrease in metabolic rate (Seidel 1978). *K. hirtipes*, on the other hand, is generally regarded as highly aquatic, limiting its activity to seeps and springs where water is present year-round (Scudday and Miller 1986, Iverson et al. 1991, Ernst et al. 1994). In laboratory trials, *K. hirtipes* exhibited rates of evaporative water loss nearly three times greater than *K. flavescens*, and failed to become quiescent when placed on dry substrate (Seidel and Reynolds 1980).

My first objective was to compare physiological and behavioral responses to dry conditions by Sonoran mud turtles from two populations: one in which surface water is absent for part of the year and estivation by turtles has been documented (Chapter 1), and one from an area where spring-fed streams hold water year-round and *K. sonoriense* have been described as obligately aquatic (van Loben Sels 1997). However, whether different patterns in terrestrial behavior are founded on fundamental physiological differences between these populations or are simply behavioral responses (with important physiological consequences) to variation in water availability remains undetermined (see Garland and Adolph1991 for a review of physiological differences among conspecific populations). I also tested responses of *K. flavescens* and *K. hirtipes* to dry conditions to give meaningful context to differences observed among *K. sonoriense*, and to more completely describe the range of physiological and behavioral responses to drought of all kinosternids indigenous to the arid southwestern United States.

Coadaptation (the evolution of particular combinations of distinct phenotypic traits in response to natural selection [Hertz 1988, Huey et. al 1989, Bauwens et. al 1995]) predicts that behavioral and physiological correlates of terrestrial activity should co-occur in populations where water availability is seasonally intermittent. Estivation has been documented in *Kinosternon flavescens* and *K. sonoriense* in the Peloncillo Mountains; thus, these populations are expected to exhibit both behavioral and physiological traits that enhance drought survival, and hence fitness. Examples of such traits include: 1)

quiescence and/or burrowing during drought, 2) tolerance to anhomeostasis (an increase of solute concentrations in body fluids [Peterson 1996, Peterson and Stone 2000]), and concomitant loss of body water, 3) reduced rates of evaporative water loss, 4) reduced metabolic rate, and 5) storage of excretory wastes to minimize loss of water as a solvent (Peterson and Stone 2000). An examination of interpopulational differences in these traits should determine whether variation in behavioral responses to drought by mud turtles in the desert southwest are purely a function of water availability, or are driven by physiological constraints on terrestrial activity.

MATERIALS AND METHODS

Animal Acquisition – Mud turtles were collected at four locations in the southwestern United States. Ten *Kinosternon hirtipes* were collected on 17 May 2000 from a permanent seep in Presidio County, Texas (precise location omitted at landowner's request). Ten *K. flavescens* were collected on 27 May 2000 from an ephemeral cattle pond in Greer County, Oklahoma. The pond was located 1.6 km north of the Elm Fork of the Red River, and 1.2 km east of State Route 34.

Kinosternon sonoriense were collected from two populations, distinguished hereafter by their state of origin (Arizona and New Mexico). Ten turtles were collected on 15 May 2000 from a small spring in the Chiricahua Mountains, Coronado National Forest, Cochise County, Arizona. The spring was located in Tex canyon (Sec. 2, T20S, R29E) on the eastern side of the mountain

range. The second population was collected on 13-14 May 2000 from the Peloncillo Mountains, Coronado National Forest, Hidalgo County, New Mexico. Extremely low turtle capture rates resulting from nine months without rain (G. Helbing, pers. comm.) forced me to take turtles from three sites within 5.5 km of one another. Seven *K. sonoriense* were taken from Maverick Spring, a small permanent spring located in Miller Creek Canyon (Sec. 31, T32S, R21W); two turtles were taken from an ephemeral cattle tank located near the top of Miller Creek Canyon (Sec. 36, T32S, R22W); and one was taken from Geronimo Seep Tank, a large impounded tank on Geronimo Trail Road (Sec. 18, T32S, R21W).

Experimental Protocol – All turtles were transported to Oklahoma State University within five days of capture, and maintained until experiments began in June 2000. During this time, turtles were kept in 5 – 10 cm of deionized water at room temperature (20.5 – 23.0° C, Hobo Temperature Logger, Onset Computer Corporation, Bourne, MA), and fed super worms (*Zophobas morio*), crickets (*Acheta domestica*), and thawed fish *ad libitum*. Light was provided by incandescent and fluorescent sources, and was set on a 16:8h light:dark cycle. Turtles were held under these conditions for at least ten days to allow them to normalize their activity patterns to laboratory light and temperature conditions.

Turtles were weighed and 300 – 500 μL blood samples were drawn on 21 and 22 June, following a 15- or 16-day fast. Resting metabolic rates and evaporative water loss were measured on the afternoons of 23 June – 1 July. Following these measurements, turtles were placed in population-specific groups of three or four in opaque plastic tubs filled to 15 centimeters with dry vermiculite.

All incandescent lights were turned off, but ambient light from overhead fluorescent fixtures was left on a 16:8 light cycle. Additional vermiculite was added to each tub as the substrate settled to maintain depths sufficient for the turtles to bury themselves completely. Aside from the exceptions described below, turtles were left undisturbed for 55 days.

Activity levels of each turtle were observed frequently throughout the estivation trial, and turtles were removed and weighed six times during estivation. In addition to pretrial measurements, blood samples were drawn on 22 July (day 31, mid-trial), on 15 August (day 55, at the end of the trial), and on 18 August following rehydration. Evaporative water loss and resting metabolic rates were measured at the end of the trial between 10 and 15 August, and following rehydration between 25 and 31 August.

Behavior – Each turtle's identification number was written on its carapace with a red permanent marker so that identification was possible with minimal disturbance. Activity levels were scored on 37 days of the 55-day trial. Turtles were recorded as on the surface or buried. If on the surface, I noted whether eyes were open or closed, and whether the turtle was sedentary or active. These activities were then scored as follows: 0 = buried or inactive on surface with eyes closed, 1 = sedentary on surface with eyes open, 2 = eyes open, moving around on surface. Individual scores were summed, then divided by the total number of observations to calculate each turtle's mean activity level for the entire trial.

Body Mass – Turtles were weighed at 7 –16 day intervals throughout the trial. Masses were converted to percent of maximum pre-trial mass; turtles that lost 30% of hydrated mass before the completion of the trial were returned to water. Reported desiccation limits for turtles are 30 – 35% of hydrated mass (Ernst 1968, Minnich 1979, Mautz 1982, Peterson and Stone 2000).

Blood samples – Blood was drawn on four occasions: immediately prior to removal from water, after 31 days, at the end of the trial (55 days), and following rehydration. All samples were drawn from a sinus located dorsal to the seventh cervical vertebrae (Kuchling 1999, Peterson and Greenshields 2001). This location was chosen for two reasons. First, samples of adequate volume (300 – 500 μL) could be drawn reliably, though dilution with lymph was common. In addition, turtles could remain retracted into their shells, thereby reducing the stress caused by the procedure.

Two to three capillary tubes were filled with blood and centrifuged to measure hematocrit. Osmolality of the centrifuged plasma was measured using a Wescor vapor pressure osmometer. With the exception of the 31-day sample, the remaining blood was placed on ice in a heparinized microtainer and transported to the Oklahoma State University Veterinary Clinical Pathology Laboratory for additional analyses. Samples were analyzed for plasma concentrations of sodium, potassium, blood urea nitrogen (BUN), glucose, total protein, and circulating uric acid with a Kodak Ektachem 750 autoanalyzer. The 31-day blood samples were small (<100 μL) and many contained large quantities

of lymph. The supernatant was heat sealed and later used to measure extracellular fluid osmolality.

Evaporative water loss – Measurement of evaporative water loss followed methods described by Peterson and Stone (2000). Turtles were weighed (Acculab V-2000 balance, ± 0.1g), then placed in plastic chambers (955 or 1,835 mL depending on turtle's size) between 1000 and 1045h on the days that EWL was to be measured. Each chamber was fitted with two stopcocks on opposing sides of the lid. The rim of each container was lined with Teflon tape prior to pressing the lid on to ensure an airtight fit. The containers were placed in a 28°C ± 0.5°C environmental chamber with the stopcocks open, and a tubing manifold was connected to one stopcock on each chamber. Air was pumped from outside the building through a column of Drierite, and then to each chamber at a rate of 100 ml/min. Turtles were left in the dark for 4 – 6.5 hours to allow body temperatures to equilibrate.

Water collection columns were constructed by connecting two 3-cc syringe bodies in series. Each syringe was equipped with a stopcock, and then filled with Drierite. With stopcocks closed, each column was weighed twice (± .0001 g, Scientech analytical balance) prior to each trial, and the mean of the two measurements was used for the pre-trial mass.

A Drierite column was attached to each turtle chamber, and dry air was pumped into the chambers and through the columns at 100 mL/min. Evaporative water loss trials lasted 1.5 – 2.0 hours, after which columns were reweighed. The difference between pre- and post-trial mass was assumed to equal the

volume of water lost from cutaneous and pulmonary surfaces. Evaporative water loss trials were rerun the following day on two occasions when turtles urinated in their chambers.

Metabolic rate – Methodology for measurement of metabolic rates was as described by Peterson and Stone (2000). Resting metabolic rates were measured as oxygen consumption by closed system respirometry (Vleck 1987). Metabolic trials were conducted at 28°C ± 0.5°C, and were initiated between 1608 and 1831h, ca. 0.5 hours after evaporative water loss measurements.

The tubing manifold was disconnected, and the turtle chambers were carefully removed from the environmental chamber into a dimly lit room. With both stopcocks open, 15-ml air samples were drawn into 20-ml syringes equipped with stopcocks. The stopcocks on the syringe and chamber were then closed, the syringe removed, and the time of sampling recorded. After samples were drawn and all chambers were sealed, the turtles were returned to the dark environmental chamber. The chambers were again removed after 57 to 65 minutes, and post-trial samples were drawn with only one stopcock on the chamber opened to permit air extraction.

Oxygen concentrations of all air samples were analyzed in 10-ml aliquots with a Sable Systems FC-1 oxygen analyzer. Air was drawn from outside the building at a regulated flow rate of 100 ml/min and passed through a column of Drierite and into the oxygen analyzer. Each sample was injected into the airflow tube where it passed through a small column of Drierite and Ascarite to remove water and carbon dioxide, respectively, from the sample. Oxygen consumption

for each turtle was calculated as the difference between the initial and final volumes of oxygen after correcting for chamber volume (Peterson 1990).

Data analysis – Turtles from all populations were separated into two groups: those that remained without water for the duration of the 55-day estivation trial (Group A), and those that were rehydrated approximately midway through the trial due to rapid decreases in body mass (Group B). With the exceptions of activity and plasma osmotic pressure, only turtles that remained out of water for the entire trial were included in analyses. Plasma osmolality was analyzed for two data sets: one comprising only the full-trial estivators over the course of the entire trial, and the second including pooled data for both groups over the first thirty days of estivation, before any turtles were returned to water. Activity levels were analyzed for pooled data from both groups; mean activity levels for Group A turtles only are also reported for comparison with other variables. Because I obtained insufficient blood samples from a few turtles during one or more sampling periods, I was unable to apply a repeated measures analysis to the entire data set. Therefore, body mass and blood chemistry variables were analyzed using a two-way ANOVA (date and population as grouping variables) to test for differences between populations. All results were confirmed by repeated measures ANOVA on a data subset that allowed such analysis. Post-hoc pairwise comparisons were conducted using the Student-Newman-Keuls method. Resting metabolic rate and evaporative water loss were analyzed using ANCOVA of log-transformed variables with log mass as a covariate to eliminate the effects of body size.

Pearson correlations were calculated for a dataset of individuals' rate of change for each variable during dehydration (end-of-trial – before-trial measurements divided by n days) to look for effects of activity and dehydration on physiological responses to drought.

Statistical procedures were performed using Excel, Sigmastat, or SYSTAT. Results are reported as mean ± one standard error.

RESULTS

Grouping – Turtles were divided into two groups: those that completed the entire 55-day trial (Group A), and those that were rehydrated after just 30 – 33 days (Group B) (Fig. 1). Group B comprised sixteen turtles: eight *K. sonoriense* from Arizona, five *K. hirtipes*, two *K. flavescens*, and one *K. sonoriense* from New Mexico. Two turtles died during the trial: an Arizona *K. sonoriense* upon losing 32% of hydrated body mass after 25 days out of water, and a *K. hirtipes* upon losing 28.7% of hydrated mass after 51 days. A *K. hirtipes* that exhibited symptoms thought to be indicative of extreme dehydration (sunken eyes and dulled response when disturbed) was returned to water after having lost only 18% of hydrated mass. This individual was subsequently eliminated from the study. Except where indicated otherwise, all results are based on Group A turtles only.

Behavior – Activity levels ranged from very low (turtles that remained sedentary for the duration of the trial), to very high (frequent restless movement

and attempts to escape). Early in the study, six turtles escaped from their tubs, after which wire mesh lids were installed.

The scale used to measure levels of activity during the experiment was a relative one, useful for comparing activity between groups. When turtles from groups A and B were pooled, *Kinosternon flavescens* exhibited the lowest levels of activity, while *K. sonoriense* from Arizona and *K. hirtipes* showed similar high levels of activity. Activity levels of *K. sonoriense* from New Mexico were approximately intermediate to the two extremes (Fig. 2). Among Group A turtles, a very different pattern emerged (Fig. 2). The few Arizona *K. sonoriense* and *K. hirtipes* that remained out of water for the duration of the trial exhibited activity levels comparable to those of *K. flavescens*, much lower than averages for their respective populations.

Body mass – Body mass was analyzed as absolute mass and as proportion of hydrated mass. *Kinosternon hirtipes* were larger than turtles from the three other populations, yet no differences in proportion of hydrated mass were evident among populations (Table 1). Pair-wise comparisons on period confirm that each turtle's mass decreased at each weighing, and that masses following rehydration were not different from pre-estivation masses (Fig. 3). Group A turtles lost an average of 0.38 $\pm$ 0.03 %/day over 55 days without water. Group B turtles lost mass at a faster rate before rehydration (0.49 $\pm$ 0.12 %/day during the first 30 days of the trial). Pearson correlation analyses showed a strong correlation between activity levels and relative mass loss, but not absolute mass loss (Table 2).

Blood variables – *Effects of estivation and dehydration*. Protein, potassium and BUN demonstrated a date-by-population interaction, whereas other blood variables were affected only by date (Table 1). With the exception of glucose, all blood variables measured (hematocrit and plasma osmolality, sodium, BUN, uric acid, potassium, and protein) followed a similar general pattern: an increase during dehydration, followed by a return to near pre-trial levels following rehydration (Figs. 4 – 7). Glucose was highly variable among populations at the beginning of the trial, converged during dehydration, then decreased following rehydration (Fig. 7B).

Population Effects. Pooling data for all turtles over the first 30 days of the dehydration trial revealed that plasma osmolality of Arizona Sonoran mud turtles increased faster than for Sonoran mud turtles from New Mexico or for *K. flavescens* and *K. hirtipes* (Table 1, Fig. 4A). When only Group A turtles were considered, population differences disappeared (Table 1). Pearson correlation analyses suggested that plasma osmotic pressure was influenced by behavior, as well as plasma sodium, glucose, BUN and circulating uric acid concentrations (Table 2).

Plasma sodium (Fig. 5) and uric acid (Fig.6B) showed similar patterns among populations. New Mexico *K. sonoriense*, *K. flavescens* and *K. hirtipes* demonstrated nearly identical rates of change in response to dehydration and rehydration, though levels in *K. hirtipes* were consistently slightly higher than the other two populations. Arizona *K. sonoriense* had much lower rates of increase during dehydration than did the three other populations. The lack of population

or interaction effects is likely due to the small sample size of Arizona *K. sonoriense* in Group A.

Sonoran mud turtles from Arizona and New Mexico exhibited similar increases in BUN during dehydration (Fig. 6A). *Kinosternon flavescens* showed the smallest increase in BUN during the course of the trial, while *K. hirtipes* was intermediate. Following rehydration, BUN decreased more quickly in both Arizona and New Mexico *K. sonoriense* than in *K. hirtipes* or *K. flavescens* (it was these differences that resulted in a date x population interaction [Table 1]).

Plasma potassium (Fig. 5B) and protein (Fig. 7A) concentrations exhibited very similar population differences. All four populations exhibited comparable levels at the beginning of the trial and following rehydration. At the end of dehydration, concentrations in *K. sonoriense* from Arizona had increased the most, followed by those in *K. sonoriense* from New Mexico, then *K. flavescens*. *Kinosternon hirtipes* showed no change in plasma potassium, and a slight decrease in protein concentrations.

Both populations of *K. sonoriense* showed decreases in plasma glucose during dehydration, whereas *K. flavescens* exhibited increases (Fig. 7B). *K. hirtipes* showed no change in plasma glucose during dehydration, but, like *K. flavescens*, levels decreased sharply upon rehydration.

Hematocrit increased most among *K. hirtipes* during dehydration (Fig. 7C). A pre-trial value was not available for *K. sonoriense* from Arizona because all of those blood samples were contaminated with lymph.

Evaporative water loss – Evaporative water loss scaled to body mass (Fig. 8A), with *K. hirtipes* higher than the three other populations (Table 1). No date effect was observed, and EWL did not decrease during estivation, though all four groups showed a decrease in EWL following rehydration (Fig. 9A).

Resting metabolic rate – RMR followed a pattern similar to that of EWL: Measurements scaled to body mass (Fig. 8B), and with the exception of *K. hirtipes*, RMR was higher at the end of estivation than at the beginning (Table 1). Following rehydration, Arizona *K. sonoriense* showed a slight increase in RMR while the three other populations decreased (Fig. 9B).

DISCUSSION

Animals inhabiting aquatic habitats that experience seasonal fluctuations in water availability may be forced to adopt an amphibious lifestyle (Chilian 1976). Terrestrial activity under these circumstances generally involves migration to other bodies of water, or estivation until local water supplies are replenished (Morales-Verdeja and Vogt 1997, Stone 2001). When engaging in terrestrial activities, tolerance to desiccation (Grigg et al. 1986, Peterson 1996) and retardation of loss of body water (Seidel and Reynolds 1980, Chessman 1984) are of primary concern. As dehydration progresses, anhomeostasis occurs. The results of the dehydration trials show that, though exposed to similar large-scale desertification trends, species and populations of mud turtles in the southwestern United States exhibit an array of strategies to cope with these changes.

A reduction in cutaneous permeability and metabolic depression (which decreases water loss from pulmonary surfaces and conserves energy stores) are physiological mechanisms that may minimize water loss (Seidel 1978, Chessman 1984, Kennett and Christian 1994). Evaporative water loss also can be reduced behaviorally by minimizing activity, reducing operative surface area (Wygoda and Chmura 1990), and selecting estivation sites that maintain low temperatures and high relative humidity. The populations of southwestern kinosternids studied displayed some of the traits predicted to facilitate terrestrial estivation, but not others.

The physiological response of turtles to water deprivation evidently was highly dependent on behavior. Mean activity levels of *Kinosternon hirtipes* and Arizona *K. sonoriense*, the two groups in which most turtles reached near-lethal levels of dehydration within 30 days, were higher than those of the New Mexico *K. sonoriense*, and much higher than those of *K. flavescens*. However, the few *K. hirtipes* and *K. sonoriense* from Arizona that completed the trial (Group A) had extremely low activity levels, comparable to those of *K. flavescens* (Fig. 2).

Metabolic rate can be influenced by activity. At the end of the trial, New Mexico *K. sonoriense* had the highest RMR among Group A turtles, whereas the other three populations were somewhat lower (Fig. 9B), a pattern similar to that of Group A activity (Fig. 2). Notably, metabolic rates of hydrated *K. flavescens* both at the beginning and end of the trial were substantially lower than those exhibited by the three other populations. Activity levels of turtles while in the metabolic chambers likely affected measurements (thus, measurements may not

have been of true resting metabolic rates). The data shown for *K. hirtipes* and Arizona *K. sonoriense* may not be representative of their populations, as few turtles from these populations finished the trial. That metabolic rates were lowest in hydrated turtles can be interpreted as evidence against metabolic depression during estivation. However, this pattern may simply be further evidence that within-chamber activity influenced measurements, a well-documented phenomenon among animals disturbed during estivation (Seidel 1978, Kennett and Christian 1994, Dunlap 1995, Peterson and Stone 2000).

Plasma solute concentrations generally followed a characteristic pattern. With the exception of glucose, pre-estivation levels were similar to those previously reported for fasted, normally hydrated turtles (Seidel and Reynolds 1980, Peterson and Stone 2000). Dehydration was accompanied by an anhomeostatic increase in plasma concentrations, followed by steep decreases, indicative of dilution, following rehydration.

Much of the blood chemistry data suggest that *Kinosternon flavescens* and New Mexico *K. sonoriense* were more tolerant of dry conditions than were Arizona *K. sonoriense* and *K. hirtipes*. Over the first 30 days of the trial, Arizona *K. sonoriense* showed the greatest increases in plasma osmolality, followed by *K. hirtipes*; New Mexico *K. sonoriense* and *K. flavescens* showed the smallest changes (Fig. 4). In apparent contrast to my results, Peterson and Stone (2000) found that New Mexico *K. sonoriense* and *K. flavescens* regulated plasma osmotic concentrations over the first thirty days of estivation, then began increasing only after plasma and bladder urine osmolality became isosmotic. It

should be noted, though, that plasma osmolality was substantially lower at the outset of the present study (~250 versus ~280 mOsm/L), possibly because the turtles were fasted in deionized water before the dehydration trial. If 300 mosm/L is the upper limit of the range of ECF osmolality for hydrated turtles (Peterson and Stone 2000), three of the four populations of Group A turtles maintained, on average, normal levels up to the thirtieth day of dehydration, whereas the highly aquatic *K. hirtipes* likely reached this upper limit earlier (Fig. 4B). If the pooled data are considered, only *K. flavescens* maintained normal osmotic concentrations for 30 days (Fig. 4B).

The patterns I observed fit the prediction that the more aquatic populations should show higher rates of increase in osmolality than those that occur in habitats where water availability is sporadic. Differences among populations were obscured when only Group A turtles were considered (Fig. 4), but *K. hirtipes* still showed the highest average rates of increase, and *K. flavescens* showed the smallest increases after 55 days out of water. Rehydration resulted in the return of plasma osmotic concentrations to pre-estivation levels.

In the absence of excretory sodium loss, plasma sodium concentrations should increase in proportion to water loss, and thus should be a reliable index of dehydration. With the exception of Arizona *K. sonoriense*, changes were as predicted: *K. hirtipes* exhibited a high rate of increase while out of water, while *K. flavescens* and New Mexico *K. sonoriense* showed similar, lower increases (Fig. 5A). Surprisingly, the two Group A *K. sonoriense* from Arizona had the lowest rate of sodium increase, a result not expected of highly aquatic turtles. As was

evident with plasma osmotic concentrations, this anomaly likely resulted from the fact that Arizona *K. sonoriense* were represented by only the two most successful estivators. Though not measured before rehydration, Group B turtles probably had rates of increase of plasma sodium proportional to their high rates of water loss.

Nitrogenous waste resulting from protein catabolism can be packaged in different forms, each of which carries trade-offs between energetic costs of production and water conservation. Turtles are primarily ureotelic, though desert species (e.g., *Gopherus agassizii*) tend toward greater levels of uricotelism than those with consistent access to water (Dantzler and Schmidt-Nielsen 1966). Though energetically more costly to produce than urea, uric acid contains twice as much nitrogen per molecule, and can precipitate out of solution in the bladder and be excreted as a solid. For turtles inhabiting areas where water is limited, uric acid production can result in substantial water savings; thus, preferential production of uric acid may be an adaptation to inhabiting dry environments.

Measuring nitrogenous waste production from plasma concentrations is problematic, as blood urea nitrogen (BUN) and circulating uric acid concentrations are influenced by a number of variables. As with other solutes, dehydration has a concentrating effect, resulting in increased levels during estivation. These increases are compounded as additional nitrogenous wastes resulting from protein catabolism are produced. Reductions in BUN can be accomplished by maintaining a large reservoir from which water can be reabsorbed to dilute extracellular fluids (additionally, urea in solution can be

sequestered in the bladder). Uric acid can precipitate out of solution in the urinary bladder, resulting in lower plasma concentrations. Thus, circulating levels are not necessarily indicative of actual production of nitrogenous wastes (Peterson and Stone 2000).

Patterns of increase of blood urea were similar to those described by Peterson and Stone (2000). Both populations of *Kinosternon sonoriense* showed similar, high rates of increase in BUN, but increases among *K. flavescens* were noticeably lower. The high rates of increase among *K. sonoriense may* be indicative of relatively higher metabolic rates during estivation. Alternatively, these results may suggest that *K. sonoriense* had lower fat stores than the two other species, forcing them to metabolize protein instead. *K. flavescens* have extremely high lipid stores (Chilian 1976, Rose 1980, Long 1985), and were relatively inactive in this study; thus, it was not surprising that they showed the smallest increases in BUN during estivation.

Circulating uric acid levels increased much less over the course of the dehydration trial in the two Arizona Sonoran mud turtles than in turtles from the three other populations. Because uric acid is a form of nitrogenous waste that conserves water, an inability to package large quantities of nitrogen in this form suggests that this population may be less efficient at conserving water. If this is the case, however, it is surprising that *K. hirtipes*, the other highly aquatic group, did not have similarly low uric acid levels. Without knowing the total amount of waste nitrogen produced by turtles in each population, it is impossible to compare urea versus uric acid production rates.

Plasma glucose concentrations have previously been shown to decrease during estivation, presumably as a result of starvation and the catabolism of glycogen stores (Seidel and Reynolds 1980, Peterson and Stone 2000). In this study, pre-estivation glucose concentrations in *K. hirtipes* and Arizona *K. sonoriense* were similar to those previously reported for post-absorptive, normally hydrated turtles (Seidel and Reynolds 1980, Peterson and Stone 2000), whereas initial concentrations were higher in New Mexico *K. sonoriense* and lower in *K. flavescens* than have previously been reported. Over the course of estivation, mean glucose concentrations of all four populations converged. Dilution of blood plasma solutes following rehydration resulted in decreases in glucose concentrations. That all four populations did not begin the trial with similar glucose levels may be an indication that not all turtles were of equivalent nutritional condition, which could have influenced their overall response to estivation. However, *K. flavescens* started with the lowest plasma glucose levels, yet in most respects proved to be the least affected by dry conditions. That turtles in this population showed an increase in plasma glucose after 55 days without food is puzzling; a similar pattern was reported in *K. hirtipes* following 12 – 14 days of dehydration (Seidel and Reynolds 1980).

Concentrations of plasma protein and potassium followed nearly identical patterns, and reached extremely high levels among Arizona *K. sonoriense*, compared to published ranges (Figs. 5B, 7A) (Seidel and Reynolds 1980, Peterson and Stone 2000). That these extreme values are mirrored in the two solute groups suggests that hemolysis may have occurred. It is perplexing that

K. hirtipes, the other highly aquatic group, exhibited no increases in plasma protein and potassium following dehydration. In view of the large changes in other variables, it seems doubtful that these were tightly regulated.

It has been suggested that *K. flavescens* and New Mexico *K. sonoriense* regulate plasma protein concentrations during dehydration (Peterson and Stone 2000), but those in the present study exhibited a significant increase after 55 days of dehydration (Table 1). As with plasma osmotic pressure, however, these differences may reflect the fact that pre-trial concentrations were lower in the present study, and increased to levels comparable to those previously reported in kinosternids (Seidel and Reynolds 1980, Peterson and Stone 2000).

Conclusion – Clearly, the two populations of *Kinosternon sonoriense* differed in their responses to drought-like conditions. Turtles from the New Mexico population had lower activity levels, slower increases in plasma osmolality (when all turtles were included over the first 30 days of the trial), and higher concentrations of uric acid. The *K. sonoriense* from Arizona faired poorly: one turtle died after just 25 days out of water, and another seven had to be rehydrated after 30 days (compared to just one from the New Mexico population). The two Arizona turtles that did complete the trial performed well in many respects (slow increases in sodium and plasma osmotic concentrations, coupled with very low levels of activity); however, those responses were atypical of the population.

Kinosternon hirtipes showed responses in keeping with their highly aquatic lifestyle. As with Arizona *K. sonoriense*, one turtle from this population died

during the trial, and four were rehydrated after 30 days. Activity levels were high, and plasma osmotic concentrations and plasma sodium reached the highest levels of the four populations after 55 days of dehydration. Finally, even after correcting for their larger mass, *K. hirtipes* displayed rates of evaporative water loss substantially higher than the other populations studied.

Kinosternon flavescens and the New Mexico *K. sonoriense* responded very similarly to dry conditions. Both maintained low levels of activity (*K. flavescens* was the lowest of the four populations), had the smallest increases in plasma osmolality, and exhibited nearly identical changes in levels of circulating uric acid. These conclusions are consistent with those of Peterson and Stone (2000) for this *K. sonoriense* population.

Some of the variables measured support the notion of coadaptation of behavioral and physiological components of drought-survival strategies, and may result from population-level adaptation to local differences in water availability. *Kinosternon hirtipes* appear to be both behaviorally and physiologically adapted to aquatic habitats; individuals in this population exhibited relatively high activity levels during dehydration, as well as rates of evaporative water loss substantially greater than the other three populations studied (Table 1).

A greater body of evidence supports the alternative possibility, that the physiological differences observed between populations were simply consequences of differences in behavior. There were strong correlations between individual activity levels and relative mass loss and changes in plasma osmotic concentrations during the trial (Table 2), whereas patterns of change in

these variables were not attributable to population differences (Table 1). The different levels of activity exhibited by each population may be indicative of their typical responses to drought: perhaps individuals that remained quiescent normally estivate when local water supplies disappear, whereas those that were very active normally migrate to new bodies of water. This hypothesis is supported by many of the descriptions of these turtles in wild populations. Estivation has been identified as the primary drought-survival strategy among Sonoran mud turtles in the Peloncillo Mountains (Chapter 1), but across much of the rest of their range (including the Chiricahua Mountains) they have been described as highly aquatic and congregating in water holes during dry periods, suggesting terrestrial migration during drought (Hulse 1982, Ernst et al. 1994, Van Loben Sels et al. 1997). The nature of these interpopulational differences could best be determined by raising hatchlings using a common-garden approach to eliminate the effects of long-term acclimatization.

A natural extension of this study would be to increase its scope to include more species and more populations. Estivation has been confirmed in several kinosternids (see Peterson and Stone 2000 and references therein), and is suspected of others (e.g., *Staurotypus salvini*, Morales-Verdeja and Vogt 1997, and *K. integrum*, J. Iverson, pers. comm.). The habitats in which estivating kinosternids occur vary widely, and likely so, too, do their capacities for long-term estivation. That estivation has a strong behavioral component is beyond doubt; the question of whether or not physiological adaptations for increased drought tolerance exist remains open.

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Table 1. Results of two-way ANOVA on population and date. Shown are F-values followed by degrees of freedom and P-values in parentheses. "Date effect" and "Population effect" columns show relationships among samples according to post-hoc pairwise comparisons by the Student-Newman-Keuls method on repeated measures for a data subset. B = before trial, M = mid-trial, E = end of trial, and R = following rehydration.

Variable	N	Date	Population	Date x Population	Date effect	Population effect
Mass (g)	95	1.989(3, 79, 0.122)	5.919(3, 79, **0.001**)	0.049(9, 79, 1.000)	---	*K.h.*>*K.f.*=*K.s.* $_{AZ}$ =*K.s.* $_{NM}$
Relative mass (% max.)	95	147.784(3, 79, **0.001**)	0.097(3, 79, 0.961)	0.479(9, 79, 0.883)	B=R>M>E	---
Osmolality (mOsm) Grp A[a]	89	38.52(3, 73, **< 0.001**)	2.041(3, 73, 0.116)	0.287(9, 73, .0976)	E>M>B=R	---
Osm. (mOsm) Grps A&B[a]	77	57.874(1, 69, **< 0.001**)	4.412(3, 69, **0.007**)	2.744(3, 69, 0.050)	B<M	*K.s.* $_{AZ}$>*K.s.* $_{NM}$=*K.h.* =*K.f.*
Sodium (mmol/L)	68	55.276(2, 56, **< 0.001**)	1.344(3, 56, 0.269)	0.526(6, 56, 0.786)	E>B>R	---
Urea nitrogen (mg/dL)	68	101.79(2, 56, **< 0.001**)	2.43(3, 56, 0.075)	2.802(6, 56, **0.019**)[b]	E>B=R	Interaction[b]
Uric acid (mg/dL)	68	13.182(2, 56, **< 0.001**)	0.851(3, 56, 0.472)	0.479(6, 56, 0.821)	E>B=R	---
Potassium (mmol/L)	68	49.328(2, 56, **<0.001**)	6.353(3, 56, **0.001**)	6.458(6, 56, **<0.001**)[c]	Interaction[c]	*K.s.* $_{AZ}$>*K.s.* $_{NM}$>*K.f.*>*K.h.*

Table 1 (continued)

Protein (g/dL)	68	55.739(2, 56, **<0.001**)	6.300(3, 56, **0.001**)	6.943(6, 56, **<0.001**)[d]	Interaction[d]	*K.s.* $_{AZ}$>*K.s.* $_{NM}$>*K.f.*>*K.h.*
Glucose (mg/dL)	68	6.614(2, 56, **0.003**)	1.113(3, 56, 0.344)	1.272(6, 56, 0.285)	B=E>R	---
Hematocrit (%)[e]	54	6.355(3, 47, **0.001**)	.0631(3, 47, 0.599)	---	E>B=R	---
log EWL (g/h)[f]	116	0.821(2, 103, 0.443)	13.20(3, 103, **<0.001**)	1.749(6, 103, 0.117)	---	*K. h.* >*K.s.* $_{AZ}$=*K.s.* $_{NM}$=*K.f.*
log RMR (ml O_2/h)[f]	116	5.263(2, 103, **0.007**)	14.97(3, 103, **<0.001**)	1.079(6, 103, 0.380)	E>B=R	*K. f.* <*K.s.* $_{AZ}$=*K.s.* $_{NM}$=*K.h.*

[a] Osmolality Grp A includes turtles that completed 55 days of dehydration and spans the entire trial; Osmolality Grps A&B includes data from all turtles over the first 30 days of dehydration (from Before trial to Mid-trial).

[b] Source of interaction. Following dehydration, *K. s.* $_{AZ}$ = *K. s.* $_{NM}$ > *K. h.* = *K. f.* Following rehydration, *K. h.* > *K. s.* $_{AZ}$ = *K. s.* $_{NM}$ = *K. f.*

[c] Source of interaction. *K. s.* $_{AZ}$, *K. s.* $_{NM}$, *K. f.*, E > B = R; for *K. h.*: E = B = R.

[d] Source of interaction. *K. s.* $_{AZ}$, *K. s.* $_{NM}$, *K. f.*: E > B > R. *K. h.*: B > E = R.

[e] Too many missing data to perform repeated measures analysis.

[f] Log mass was included as a covariate in log EWL an log RMR analyses.

Table 2. Pearson Correlation coeffiecients. Analyses were performed on a dataset of rates of change during dehydration (days 0 – 55) of Group A turtles.

	Activity	Absolute mass (g/d)	Relative mass (% max/d)	Osmolality (mOsm)
Absolute mass loss (g/d)	0.174	---		
Relative mass loss (% max/d)	0.663*	0.358*	---	
Osmolality (mOsm)	0.446*	.674*	0.792*	---
Sodium (mmol/L)	0.226	0.537*	0.703*	0.896*
Urea nitrogen (mg/dL)	0.443*	0.503*	0.615*	0.839*
Uric acid (mg/dL)	0.264	0.597*	0.592*	0.649*
Potassium (mmol/L)	0.233	-0.153	0.048	-0.032
Protein (g/dL)	0.301	-0.08	0.171	0.002
Glucose (mg/dL)	0.127	0.467*	0.297	0.479*
EWL[A] (mL H_2O/h)	0.044	0.096	0.262	0.323
RMR[B] (mL O2/d)	0.295	-0.050	0.060	0.346

* $P \leq 0.05$

[A] Evaporative water loss

[B] Resting metabolic rate

FIGURE LEGENDS

Fig. 1: Grouping of mud turtles (*Kinosternon* spp.) according to rate of dehydration. Turtles in Group A completed the 55-day dehydration trial, and Group B turtles were rehydrated in 30 days after losing ~30% of hydrated mass. *K. s.*$_{NM}$ = New Mexico *Kinosternon sonoriense*, *K. s.*$_{AZ}$ = Arizona *K. sonoriense*, *K. flav.* = *K. flavescens*, *K. hirt.* = *K. hirtipes.*

Fig. 2: Mean activity levels of each mud turtle population (see text for calculation of activity scores). Black bars are pooled data for Group A and B turtles; white bars represent activity levels of Group A turtles only. Error bars are $\pm$ one standard error.

Fig. 3: Changes in body mass in mud turtles resulting from dehydration and starvation, followed by rehydration after 55 days. A: Mean body mass of Group A turtles in each population. B: Percent of maximum hydrated mass of Group A turtles (connected points) and Group B turtles prior to rehydration. Open circles: Arizona *K. sonoriense*, closed circles: New Mexico *K. sonoriense*, open triangles: *K. flavescens*, closed inverted triangles: *K. hirtipes*. Some points are offset 1-2 days for clarity.

Fig. 4: Changes in plasma osmotic concentrations of A) all mud turtles over the first 30 days of dehydration, and B) Group A mud turtles during the 55-day period of water and food deprivation, followed by rehydration. Dotted lines indicate the upper limit of osmotic concentrations of hydrated kinosternids (*from* Peterson and Stone 2000). Symbols as in Fig. 3.

Fig. 5: Changes in A) plasma sodium and B) potassium concentrations during water deprivation, and following rehydration in mud turtles from four populations. Symbols as in Fig. 3.

Fig. 6: Changes in plasma concentrations of A) blood urea nitrogen (BUN) and B) circulating uric acid in mud turtles from four populations. Symbols as in Fig 3.

Fig. 7: Changes during water deprivation followed by rehydration of A) plasma protein, B) plasma glucose, and C) hematocrit Concentrations in mud turtles from four populations. Symbols as in Fig. 3.

Fig. 8: Rates of evaporative water loss and B) metabolic rates measured in kinosternid turtles. Symbols as in figure 3.

Fig. 9: Changes in A) evaporative water loss (EWL) and B) resting metabolic rate (RMR) during water and food deprivation, and following rehydration in kinosternid turtles. Residuals from log-log graphs on mass (shown in Fig. 8) are plotted to correct for intra- and inter- populational differences in body size. Symbols as in Fig. 3.

Figure 1

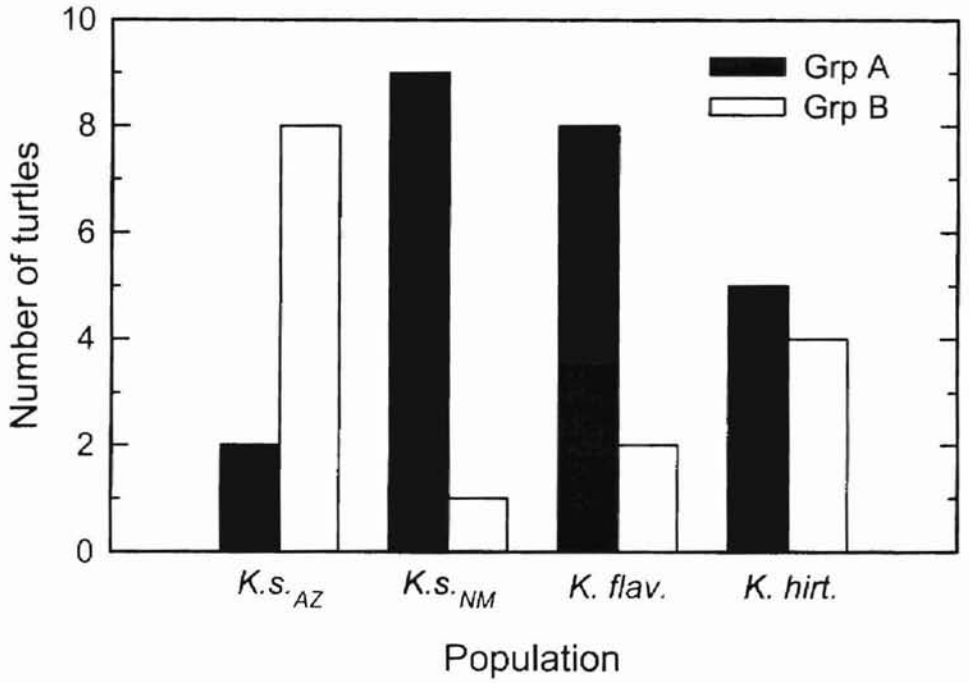

10
8
6
4
2
0
Number of turtles
Grp A
Grp B
K.s.AZ
K.s.NM
K. flav.
K. hirt.
Population

Figure 2

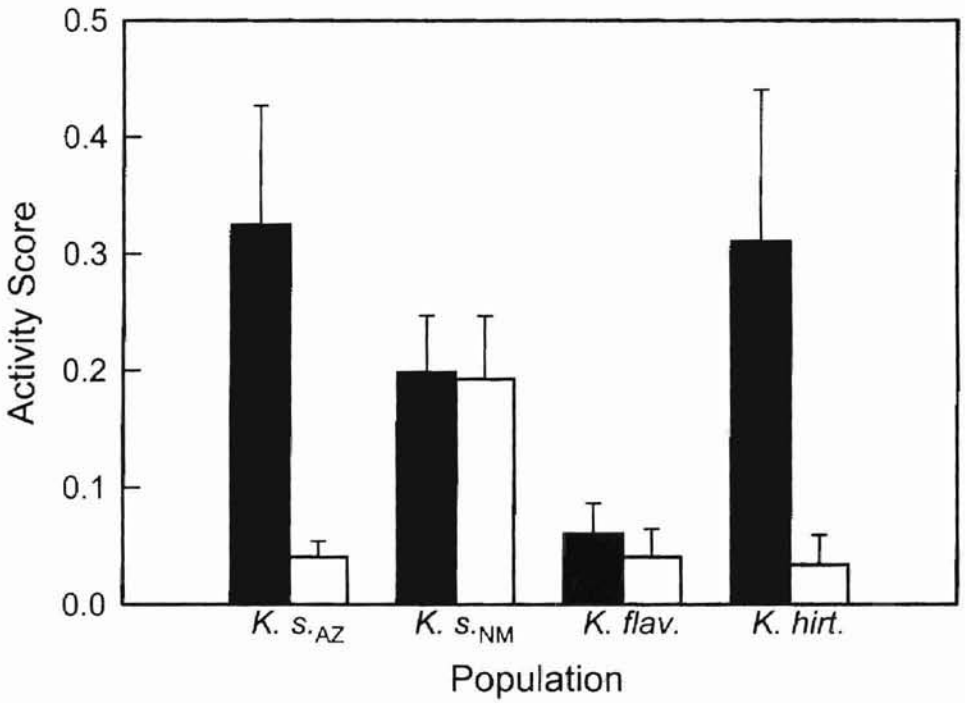

0.5
0.4
0.3
0.2
0.1
0.0
Activity Score
K. s.AZ
K. s.NM
K. flav.
K. hirt.
Population

Figure 3

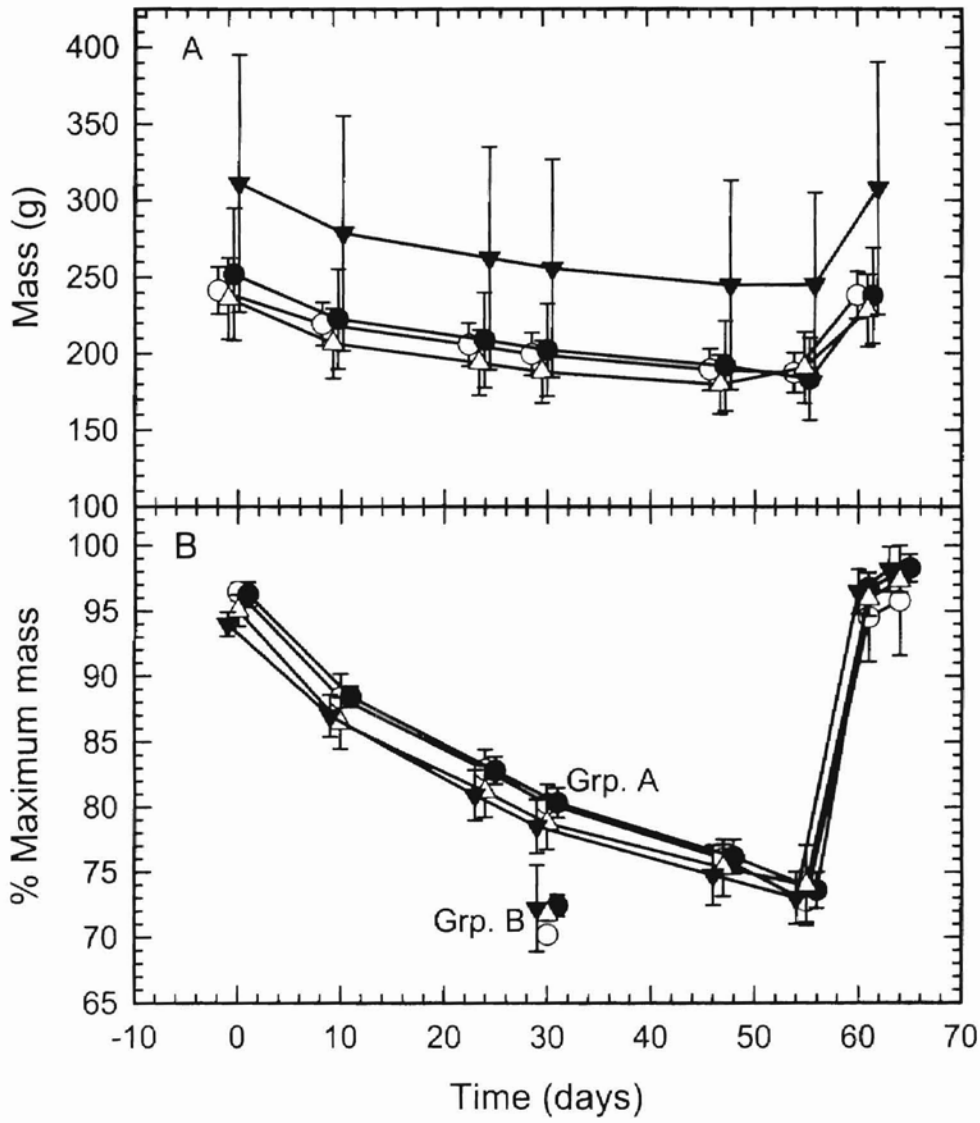

A
B
Mass (g)
% Maximum mass
Time (days)
Grp. A
Grp. B
400
350
300
250
200
150
100
100
95
90
85
80
75
70
65
-10
0
10
20
30
40
50
60
70

Figure 4

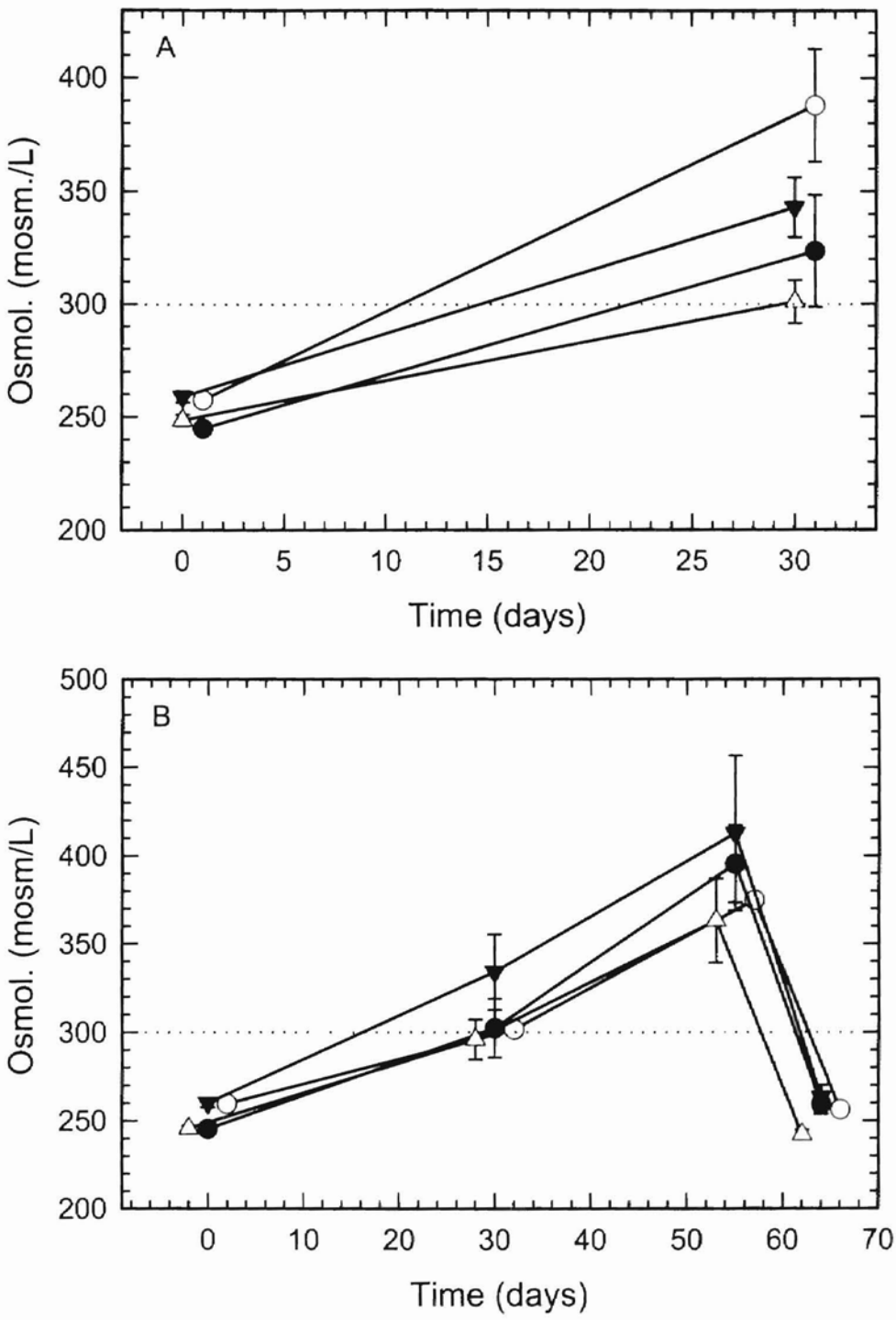

A
Osmol. (mosm./L)
400
350
300
250
200
0
5
10
15
20
25
30
Time (days)
B
Osmol. (mosm/L)
500
450
400
350
300
250
200
0
10
20
30
40
50
60
70
Time (days)

Figure 5

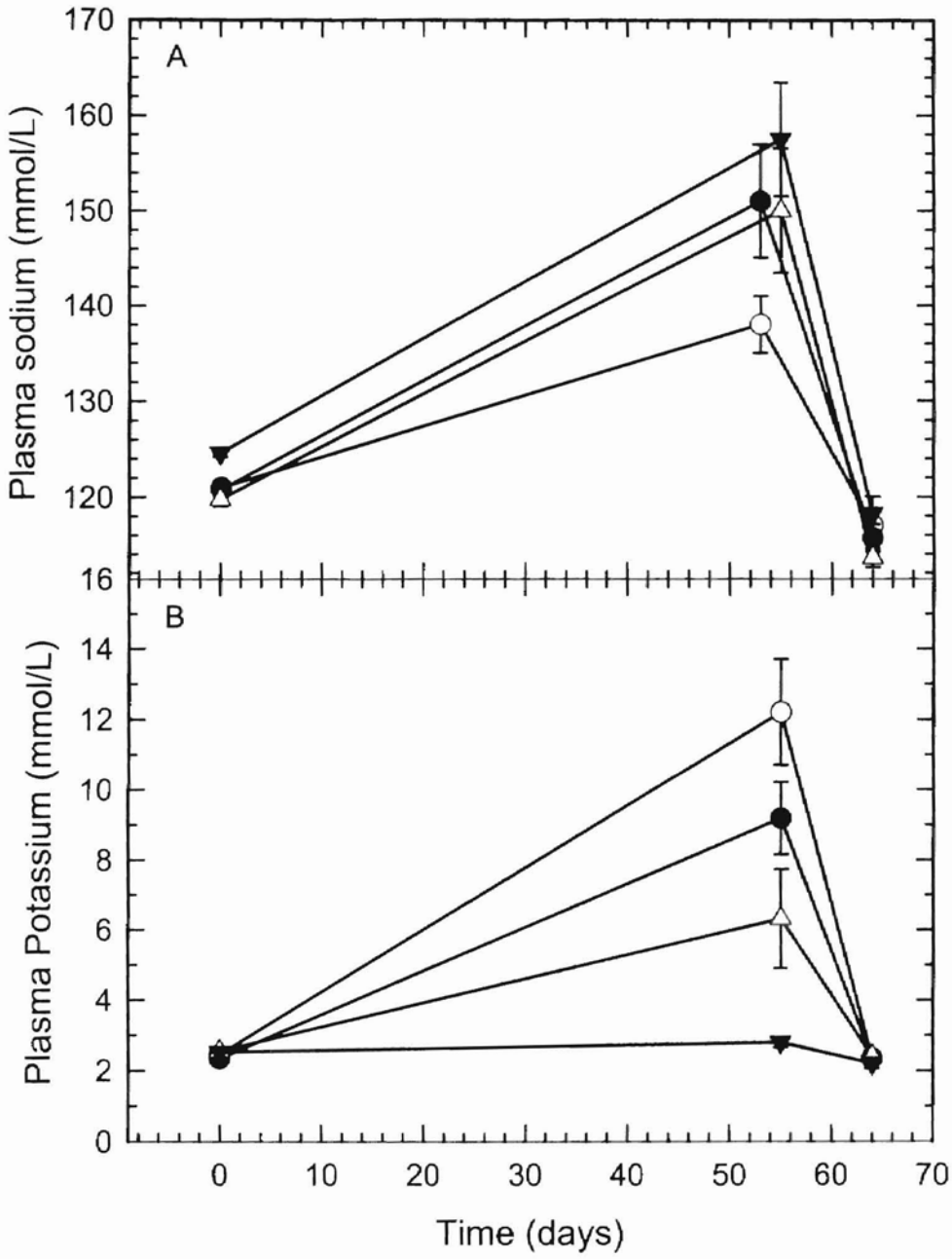

A
B
Plasma sodium (mmol/L)
Plasma Potassium (mmol/L)
Time (days)
170
160
150
140
130
120
16
14
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70

Figure 6

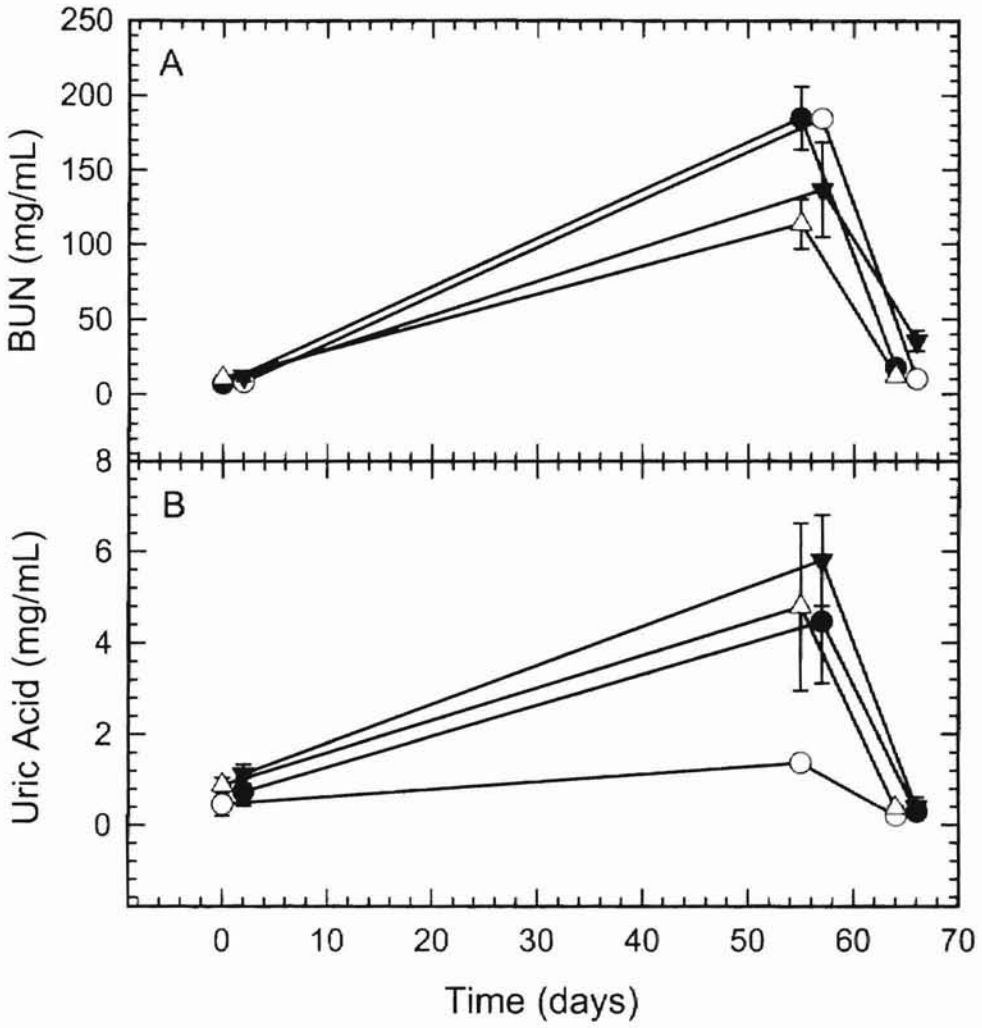

A
B
BUN (mg/mL)
Uric Acid (mg/mL)
250
200
150
100
50
0
8
6
4
2
0
0
10
20
30
40
50
60
70
Time (days)

Figure 7

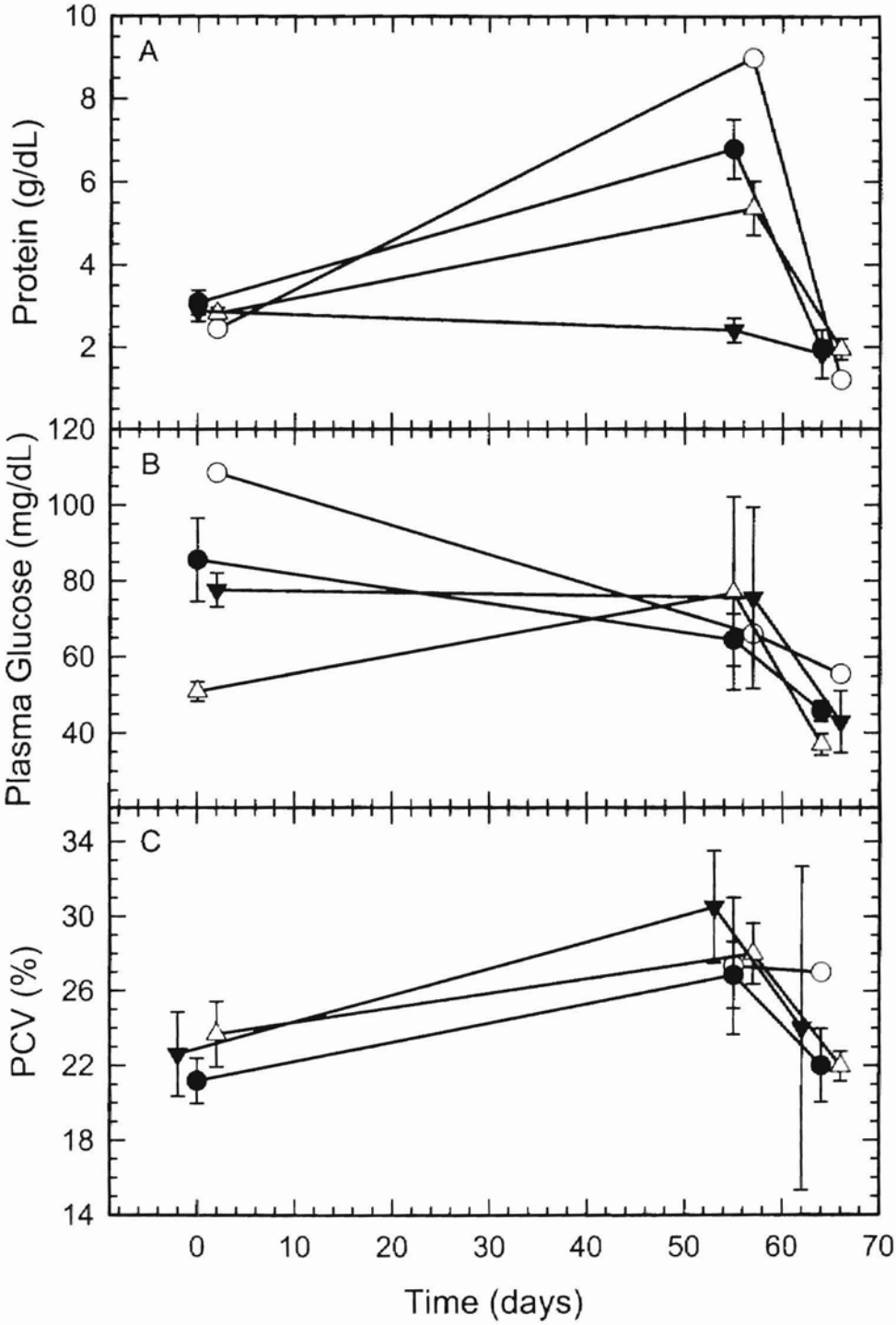

A
B
C
Protein (g/dL)
Plasma Glucose (mg/dL)
PCV (%)
Time (days)
10
8
6
4
2
120
100
80
60
40
34
30
26
22
18
14
0
10
20
30
40
50
60
70

Figure 8

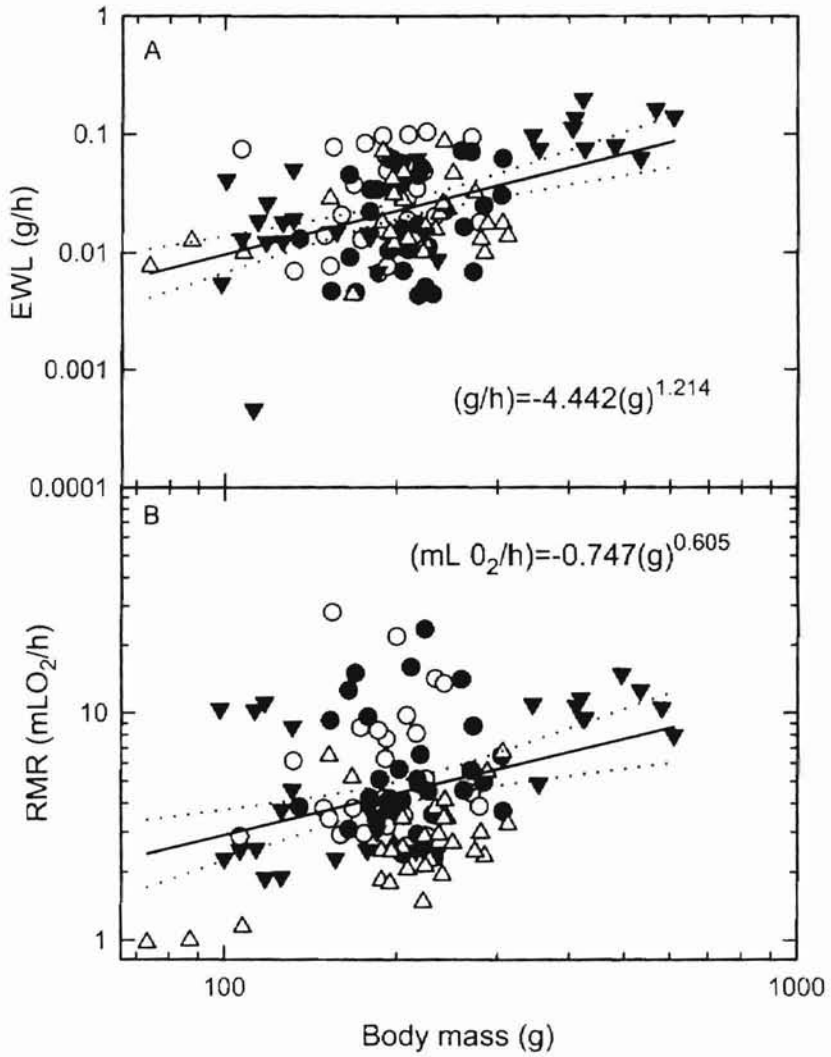
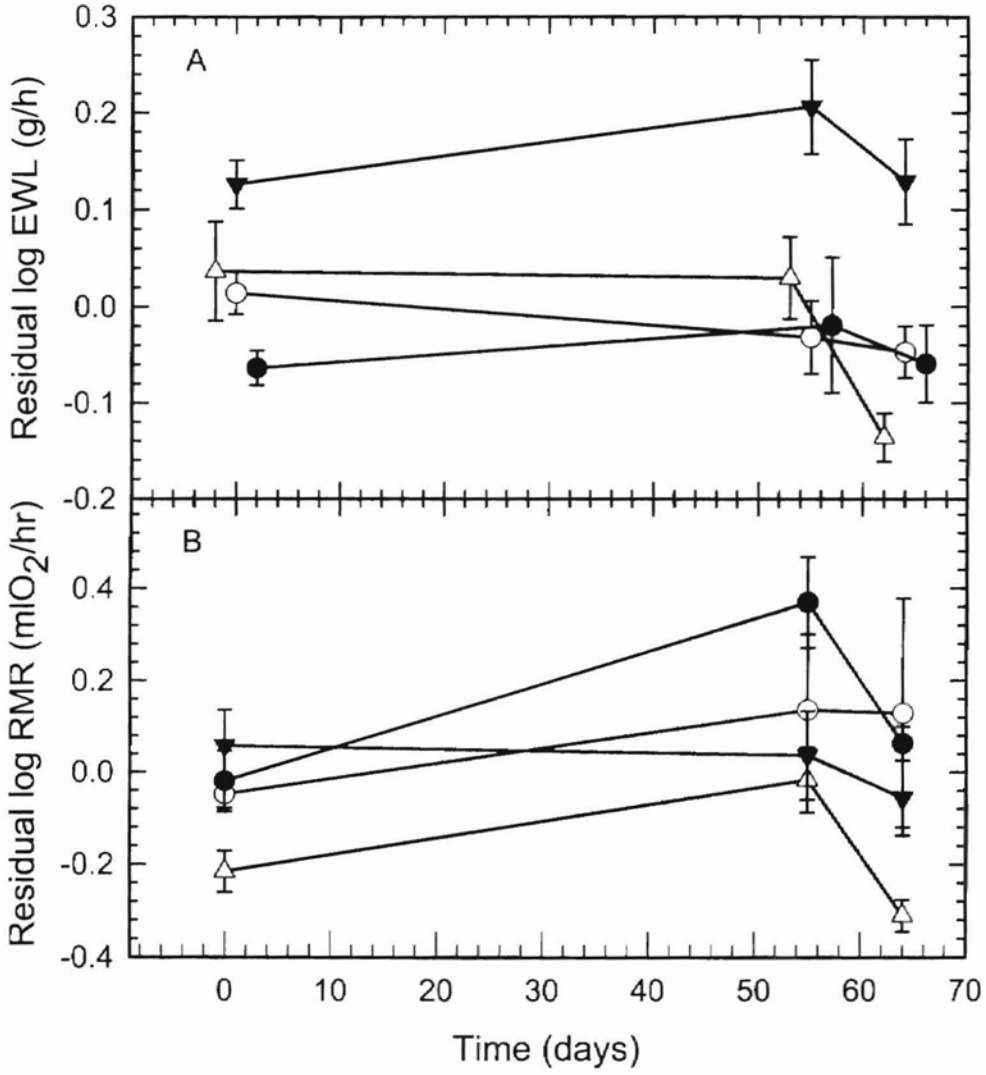

A
B
EWL (g/h)
RMR (mLO2/h)
(g/h)=-4.442(g)1.214
(mL O2/h)=-0.747(g)0.605
1
0.1
0.01
0.001
0.0001
10
1
100
1000
Body mass (g)

Figure 9


A
B
Residual log EWL (g/h)
Residual log RMR (mlO_2/hr)
0.3
0.2
0.1
0.0
-0.1
-0.2
0.4
0.2
0.0
-0.2
-0.4
0
10
20
30
40
50
60
70
Time (days)

VITA

Day Briggs Ligon

Candidate for the degree of

Master of Science

Thesis: COADAPTATION OF PHYSIOLOGY AND BEHAVIOR: VARIATION IN ESTIVATION AMONG MUD TURTLES (*KINOSTERNON* SPP.)

Major Field: Zoology

Personal Information: Day Briggs Ligon was born to Thomas and Linda Ligon in Fort Collins, Colorado, on 29 January, 1975. He was married to Elicia Rochelle Foster on 15 June, 2000.

Education: Entered Lewis and Clark College in Portland, Oregon in September 1993, and received a Bachelor of Science degree in Biology in May, 1997. Completed the Requirements for the Master of Science degree at Oklahoma State University in August, 2001.

Experience: Teach Assistant in Human Physiology, Oklahoma State University Department of Zoology, 1998 – 2001. Laboratory Research Assistant to Dr. Charles Peterson, developing a shelf-stable commercial box turtle diet. Contract Biologist, monitoring nesting patterns of western painted turtles, Northwest Ecological Research Institute, 1998. Research Assistant, studying turtle seasonal movements and space use of western painted turtles, Oregon Department of Fish and Game, 1997-98.

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