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Sexually Dimorphic Growth in the Western Swamp Tortoise, *Pseudemydura umbrina* (Testudines: Chelidae)

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Abstract
Captive breeding of the Critically Endangered western swamp tortoise (WST; *Pseudemydura umbrina*) has been taking place at Perth Zoo as part of the species' recovery plan since 1988. The first release of captive bred individuals took place in 1994. Individuals are released into protected wild reserves at approximately three years of age, when they typically reach the mandatory minimum body mass of 100 g. They are sexually immature at this age, and sex determination is not possible by external physical examination. For sustainable reintroduction programs, it is desirable to know the sex ratio of individuals prior to release, and also the sex of individuals being retained for future captive breeding purposes. Utilising body mass and size morphometrics from zoo-bred individuals retained until sexual maturity, we evaluated sex-related changes in growth rates and body size over time. Sexual dimorphism in favour of males was first detected at four years of age, with significant differences (P<0.05) observed in both the body mass (121.9±6.8 g vs. 105.0±4.4 g) and carapace length (89.5±2.0 mm vs. 83.2±1.4 mm). Age at which sexual maturity was attained varied between 5 to 13 years, but on average occurred earlier in males (8.0±0.5 years) than females (8.7±0.4 years). Data confirm a faster growth rate and earlier age of sexual maturity in males than females, and smaller size (carapace length) at sexual maturity (~110 mm for males and ~100 mm for females) than previously reported for wild WST (110–131 mm). It is suggested that using growth rate data, body size (mass and length) and plastron shape may be useful to determine sex at 4–5 years of age, and prior to release to the wild, to better monitor sex ratios at release and during future in situ monitoring of microchipped zoo-bred WST.

Introduction

The western swamp tortoise (WST; *Pseudemydura umbrina*) is listed as Critically Endangered (IUCN 2016). It is the sole member of its genus and is so distinct from other freshwater chelids that it is placed in its own subfamily, Pseudemydurinae (Burbidge et al. 1974). It was first collected in 1839, and thought to be extinct for over 100 years until its rediscovery in 1953 (Burbidge 1981). WST were in low numbers and limited to two small ephemeral winter-wet swamp areas of the Swan coastal plain of southwestern Australia, leading to the acquisition and protection of that habitat in the early 1960s (Burbidge 1981; Kuchling and DeJose 1989). The WST has been kept in captivity in very low numbers since the late 1950s, but breeding was limited and inconsistent (Kuchling 2002). Captive breeding at Perth Zoo began in 1988 (Harley et al. 2018) as it was estimated that only ~50 individuals were left in the wild (Kuchling et al. 1992). In 1991, Perth Zoo became the primary site for captive breeding (Kuchling 2002). Between 1988 and 2015, 938 tortoises were produced, 688 of these reintroduced into protected reserves (Mawson and Lambert 2017) in the Swan Valley, 25 km north of Perth, and two nature reserves ca. 100 km north of Perth, Western Australia (Kuchling et al. 2018).

WST are the smallest of the Australian chelids, and are unique as they exhibit male-biased sexual dimorphism (Kuchling and DeJose 1989). Males have been reported to reach a maximum mass of 550 g and straight carapace length (CL) of 155 mm, while females typically only reach a maximum

mass of 410 g and CL of 135 mm (Burbidge 1981; Kuchling 2002). Males exploit this, exhibiting a promiscuous mating system and "forced insemination", while other tortoises exhibit female based mate choice (Berry and Shine 1980). Hatchlings typically weigh 3.2–6.8 g and have a CL of 24–29 mm (Kuchling 2002). They are thought to require a CL of 110–120 mm (corresponding to a mass of approximately 180–200 g) before becoming sexually mature (Burbidge 1981; Kuchling and Bradshaw 1993). The overall growth rate of WST is very slow, most likely due to their need to aestivate during the dry austral summer months as their natural habitat consists of shallow ephemeral swamps (Burbidge 1981).

Considerable annual variability exists in the seasonal duration of swamp life, and as such the annual growth of WST is highly variable in the wild. Depending on the growth rate, wild individuals can become sexually mature between 6 and 15 years of age (mean ~10 years) (Burbidge 1981; Kuchling and Bradshaw 1993). Captive bred WST are released at a minimum mass of 100 g, typically around three years of age, and when sexually immature (Burbidge and Kuchling 2004) to minimise predation risk. As a result, the sex of individuals is not known at the time of release.

This study aimed to determine if the sex of juveniles could be predicted based on growth rates and body morphometrics. Specifically, this study retrospectively examined the growth rates and body sizes of male and female WST from hatching through to sexual maturity (confirmed by the production of young) to 1) determine if sexually dimorphic growth rates exist; 2) evaluate body size differences at sexual maturity; and 3) determine the average age at which sexual maturity occurs.

Methods

In the captive breeding program at Perth Zoo, tortoises are housed in in-ground ponds (approximately 1 m x 2.5 m, with maximum depth approximately 0.4 m) from May until December and moved into shaded aestivation pens with deep leaf litter from late December until May. In the early days of the breeding program (1989 onwards) a captive diet was developed which consisted of components such as minced prawns, fish, mice, rats, beef hearts,

squid, marron (*Cherax cainii*), carrots, *Daphnia*, algae powder, multi-vitamin supplement and calcium carbonate in gelatin. This was fed daily through the active season, however amounts are unknown due to a lack of records (Kuchling 2002). For their first five months, WST hatchlings are fed a diet of black-worm twice a day and either *Daphnia*, live brine shrimp, or mosquito larvae (dependent on availability). For the remainder of their first year, they are fed invertebrates in the morning and finely cut pieces of the adult diet in the afternoon. The sub-adult and adult diets are prepared meat mixtures consisting of gelatin, marron, beef heart, white-fleshed fish and rats, supplemented with calcium and Reptivite® (Zoo Med Laboratories, USA).

To identify significant differences in the growth rates of male and female WST, we used historic records of body mass (g) and carapace length (mm) from 48 individuals (24 females, 24 males) of known age and sex, born and housed at Perth Zoo for a minimum of six years. Due to the nature of the species' slow growth rate and the release of large numbers of animals into the wild, it is not possible to find suitable records from enough males and females from any given year to perform analyses on. As such, individuals born across a range of years (from 1989 to 2008) were used, with roughly equal numbers of each sex in each year. To maintain adequate genetic diversity of the breeding program, most WST retained in the program had limited relatedness. Body mass and carapace lengths were measured upon hatching and continued to be measured on a yearly basis, typically in late December as the WST was transferred into aestivation pens (Table 1).

Growth and development of tortoises bred at Perth Zoo were recorded annually from the time of hatching. Mass (g) and straight carapace length (CL) were used in this analysis as they were deemed the most reliable across records. Tortoises were weighed on digital scales to the nearest 0.1 g immediately after being hand-caught from holding ponds (wet mass). This is important as individuals can expunge all fluids in their bladder if stressed, resulting in substantially different mass readings. The annual mass values for each individual were those taken closest to aestivation (around 20th December) as this is when tortoises achieve their maximum mass, however dates vary due to variations in yearly operations (see Table 1.1). This also ensures that body mass measures for females are post follicular development or egg laying. However, the first mass and carapace length measurement (year 0) is an exception, taken on the day of hatching. Carapace length (measured in a straight line from the furthest point of the nuchal shield to between the two most posterior marginal shields) was measured to the nearest 0.1 mm using a set of hand calipers.

Sex and confirmation of sexual maturity were determined for individuals through the evaluation of reproductive function. For females, they were confirmed by the detection of growing ovarian follicles by ultrasound (Kuchling and Bradshaw 1993) and the subsequent production of eggs. For males, confirmation occurred through the observation of mating activity followed by the production of fertile (confirmed by egg candling) or viable (confirmed by the hatching of offspring) eggs by the female after mating. As sex determination and age of sexual maturity were dependant on animal management practices, ultrasound evaluation and provision of mating opportunities for males, which may have varied from year to year, it is acknowledged that accuracy may be variable. As such, these data will be primarily used to determine the potential minimum and average age and mass at which male and female WST reach sexual maturity in captivity for comparison against published records of sexual maturity of wild tortoises.

Statistics
Statistical analysis was completed using GenStat 16.0 (VSN International). Due to the nonparametric nature of the data, a

Table 1. Mean, minimum and maximum dates (dd/mm) when mass samples were collected for each age group. Age 0 is hatching mass, and age 1 refers to the mass taken closest to the individual's first aestivation period.

	Male			Female		
Age	Min	Max	Average	Min	Max	Average
0	11/03	19/05	16/04	21/03	16/06	20/04
1	13/12	27/01	29/12	11/12	05/02	05/01
2	03/12	04/02	28/12	14/12	04/02	29/12
3	23/11	10/02	28/12	26/11	04/02	24/12
4	15/11	31/01	21/12	15/11	18/01	27/12
5	05/12	17/01	25/12	01/12	18/02	27/12
6	29/11	07/01	22/12	26/11	19/01	19/12
7	15/11	10/02	24/12	29/11	19/01	21/12
8	05/12	16/01	24/12	24/10	10/02	21/12
9	29/10	15/01	19/12	31/07	31/01	04/12

Table 2. Mean (± SE) carapace lengths for male and female WST regardless of date of birth. Significant difference between sexes are denoted by * (P<0.05).

	Overall Mean Carapace Length (mm)		Length difference between means	Percentage difference between means
Year from Birth	Males	Females	(mm)	%
0	26.6±0.4	26.9±0.3	-0.3	-1%
1	66.3±1.2	65.8±1.3	0.5	1%
2	73.7±1.7	74.0±1.4	-0.3	0%
3	80.5±1.4	79.2±1.5	1.3	2%
4	89.5±2.0	83.2±1.4	6.3*	7%
5	97.1±2.7	87.6±1.7	9.5*	10%
6	100.4±3.0	93.9±1.7	6.5*	6%
7	106.1±2.6	96.6±1.8	9.5*	9%
8	110.3±2.2	101.0±1.3	9.3*	8%
9	114.8±2.1	102.8±1.3	12.0*	10%
10	115.8±2.0	104.8±1.4	11.0*	9%

Table 3. Mean (±SE) body mass measures for WST regardless of date of birth. Significant differences in mass between sexes are denoted by * (P<0.05).

	Overall Mean Body Mass (g)		Difference between mean mass	Percentage difference between mean mass
Year from Birth	Males	Females	(g)	%
0	5.1±0.2	5.3±0.2	-0.2	-4%
1	52.6±3.0	51.8±3.1	0.7	1%
2	76.5±4.3	75.7±3.6	0.8	1%
3	93.9±4.7	91.9±4.4	2.1	2%
4	121.9±6.8	105.0±4.4	16.8*	14%
5	156.3±9.6	123.5±6.2	32.8*	21%
6	183.7±11.0	146.7±6.0	37.0*	20%
7	212.8±11.0	169.2±6.6	43.6*	20%
8	231.9±9.9	185.8±5.3	46.0*	20%
9	256.8±9.8	198.1±3.9	58.8*	23%
10	278.6±.5	209.8±3.8	68.8*	25%

restricted maximum likelihood (REML) of a linear mixed model of repeated measures was used to evaluate the differences of mass (g) and carapace length (mm) between sexes during the first 6–10 years of each individuals' life.

As individuals sampled were hatched from different egg clutches and maintained over such a long timeframe, data sets were subsequently split into three different groups (for each sex). To give relatively equal sample sizes study individuals hatched between 1989 and 1994, 1995 and 2000, and between 2001 and 2008 were grouped as cohorts and analysis was completed again. This was performed to highlight any differences over time which may be the result of changing climate or husbandry regimes.
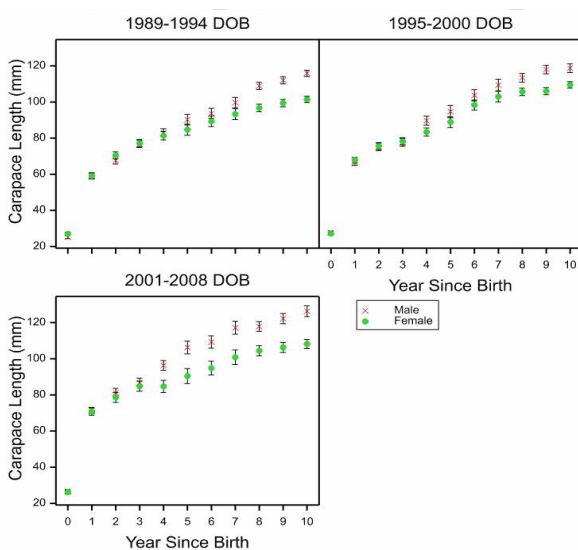

Figure 1. Changes in carapace length (mm) over years between the early, mid and late born cohorts. Mean (±SE) are given for both male and female western swamp tortoise. Significant differences were detected at year 8, year 8, and year 4 for each age cohort respectively (P<0.05).
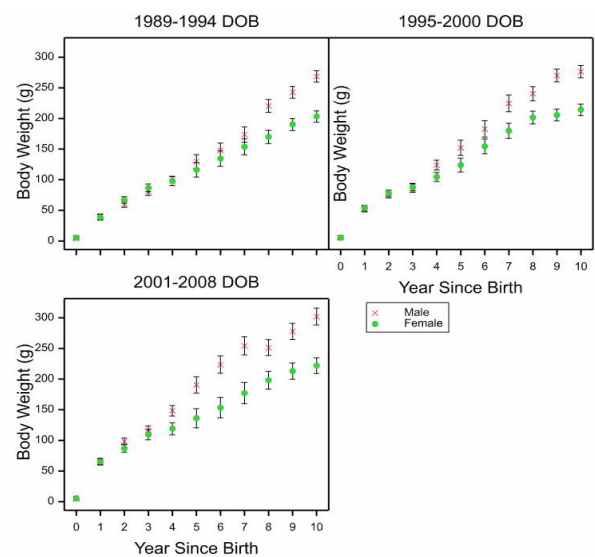

Figure 2. Changes in body mass (g) over years between the early, mid and late born cohorts. Mean (±SE) are given for both male and female western swamp tortoise. Significant differences were detected at year 8, year 7 and year 5 for each age cohort respectively (P<0.05).

Table 4. Overall mean age, carapace length (CL) and body mass (g) at which sexual maturity was confirmed for WST as determined for each year of birth cohort (Mean±SE). The three females in the 1995-2000 cohort which were initially believed to be male were excluded from this data as it is unknown when they first achieved sexual maturity due to a lack of appropriate monitoring. Significant differences between sexes are denoted by * (P<0.05).

	Males	Females
Year of Birth Cohort	Mean Age (yr) of Sexual Maturity	
1989-1994	9.0±0.7	9.3±0.6
1995-2000	7.8±0.8	8.2±0.6
2001-2008	7.0±0.7	8.2±0.7
	Mean CL (mm) Length at Sexual Maturity	
1989-1994	112. ±1.0*	100.8±1.8
1995-2000	115.0±3.0*	105.2±1.2
2001-2008	118.4±1.1*	105.0±2.0
	Mean Body Mass (g) at Sexual Maturity	
1989-1994	213. ±11.4*	186.0±4.9
1995-2000	221.6±26.0*	179.8±10.1
2001-2008	227.5±7.0*	185.8±9.2

Table 5. The age, mass and carapace length (CL) of the male and female western swamp tortoise necessary to achieve sexual maturity at lowest body mass within each date of birth cohort.

Date of Birth Cohort		Male	Female
1989-1994	Age (yr)	8	9
	Mass (g)	155.1	139.8
	CL (mm)	111.1	90.1
1995-2000	Age (yr)	5	6
	Mass (g)	143.3	143.4
	CL (mm)	105.2	106.2
2001-2008	Age (yr)	7	7
	Mass (g)	204.7	155.5
	CL (mm)	115.5	103.4

Results

All WST were healthy during the study period with no nutrient related health conditions noted. The number of WST per date of birth cohort were as follows; the 1989–1994 cohort included nine males and nine females; the 1995–2000 cohort included eight males and 10 females, and the 2001–2008 cohort included seven males and five females for a total of 24 male and 24 female WST evaluated in this study. All individuals of confirmed sex born between 1989 and 2008 with adequate mass and carapace data were included in this study.

Physical sexual dimorphism
Overall, carapace length (CL) became greater in males (n=24) than females (n=24) at 4 years of age (Table 2; P< 0.05). If examined by year groups, sexual dimorphism of shell size occurred earlier in the third cohort and the first cohort (Figure 1). On average, the first significant difference in mean body mass between males (n=24) and females (n=24) also occurred at four years of age (Table 3; P<0.05). Again, if examined by year groups, sexual dimorphism of mass occurred earlier for individuals born in the 2001–2008 cohort (Figure 2).

Sexual maturity
For three males and four females, insufficient information was available to estimate age of sexual maturity accurately. For three of those females, age of sexual maturity is likely over-estimated as these individuals were initially assumed to be male and therefore were not monitored for follicle development or paired with males for mating for several years (2–5 years) after they exceeded body mass of 200 g. For this reason, body mass and carapace length data

were only used in the growth rate analysis and these individuals were excluded from the sexual maturity analysis.

The age at which WST achieved sexual maturity was similar between males (n=21) and females (n=20) for each year cohort (Table 4) with an overall average of 8.0±0.5 years (range 5–13 years) for males and 8.7±0.4 years (range 6–13 years) for females. Mean age at sexual maturity, between cohorts for each sex were similar but males were significantly larger than females (Table 4; P>0.05). The youngest and lightest individual male WST to become sexually mature was 5 years old with a body mass of 143.3 g (Table 5). The youngest female (6 years old, 143.4 g) to attain sexual maturity was not the lightest (9 years old, 139.8 g) (Table 6). The minimum body mass required to obtain sexual maturity was similar between males and females suggesting a minimum body mass threshold beyond ~140 g is required.

Discussion

Whilst it has been known for some time that male WST typically attain a larger size than females (Burbidge 1967), the present study sheds some light on several aspects of the size dimorphism and sexual maturity in this species. No significant differences were found in the average mass or shell size of males and females until the end of their fourth year. This confirms that sexual dimorphism is not present at birth, but rather becomes apparent at four years of age and continues over time. It is achieved through a faster growth rate in males than females, and increased size is then maintained in adulthood.

Sexual size dimorphism (SSD) describes species in which males and females are dimorphic in body size at both sexual maturity and at maximum size. All Australian chelid turtles exhibit SSD,

but aquatic species typically favour females as the larger sex, an adaptive feature related to habitat and mating strategy (Berry and Shine 1980; Kennett 1996). However, the WST, the smallest chelid species, is the only one in which males are larger than females. In aquatic species, female mate choice and pre-coital displays by the males are typical, whereas the semi-aquatic WST are considered forcible inseminators. This is possibly related to the poor swimming ability and "bottom-walking" nature of the species, which gives females limited ability for avoidance of males (Berry and Shine 1980). For WST, males may also need to be larger to be able to compete for food and mates in the limited time that the swamps are filled. Females need to utilise nutrients to produce eggs and regain condition before entering aestivation. Interestingly, Australian male turtles appear to reach sexual maturity earlier than females regardless of which direction the SSD occurs (Spencer 2002), and this was confirmed to be true of WST. Captive WST also exhibit a similar range of age at maturity to other Australian chelid species (Kennett 1996) suggesting that our data may accurately reflect the growth rates and age of sexually maturity of wild WST, if habitat conditions are good during the first several years of their lives.

From the current results, it is probably conservative to estimate that sexual maturity for most WST occurs around 7–8 years of age for males and around 8–9 years of age for females within the captive population which is likely more accurate than previous reports for wild WST (10–15 years of age) (Burbidge 1981; Kuchling and Bradshaw 1993). This also suggests that conditions in captivity are optimal for growth and that they facilitate sexual dimorphism, and therefore sexual maturity, at a younger age than previously thought. Changes in growth rates and body mass of males and females over the study period also suggest that age at which sexual maturity is attained is nutritionally dependant. It is estimated that the differences in growth rate between the first and third cohorts is likely, at least in part, to be due to improvements made to their captive diet. Growth rates in the wild are likely to be less predictable and more variable due to annual differences in temperature and rainfall (Burbidge 1981). Although early estimates of age of sexual maturity were 10 years of age, that study did have a single male that reached sexual maturity at the age of 6.5 years (Burbidge 1981). This individual was born the year before a stretch of three good years of rainfall, constituting very favourable habitat conditions, which may have facilitated optimal growth rate and faster sexual maturation (Burbidge 1981). Therefore, if good conditions prevail in early life, wild WST are likely to reach sexual maturity at similar ages to our captive population. Unfavourable conditions may delay this, suggesting previous reports of 10–15 years of age to be more accurate under variable or poorer habitat conditions.

Previous research on factors affecting within season WST growth rate in captivity found that increased pond temperature was correlated with increased food intake and growth rate in both hatchlings and juveniles (Mitchell et al. 2012). In the wild shorter wet seasons, especially if associated with warmer temperatures, are likely to reduce hydro-periods (the number of days of standing water) of ephemeral swamps. This would shorten the growing period and limit food availability, counteracting any benefits warmer temperatures might have (Mitchell et al. 2012; Arnall et al. 2018). These effects would be especially detrimental to hatchlings as a delayed wet season would increase the chance of desiccation and predation, and limitations on growth and body size would also compromise their ability to survive their first aestivation. The present study also found that individuals with slower growth rates in the first four of five years of life were more likely to reach sexual maturity later as demonstrated by the overall size and age at sexual maturity of the first cohort of WST, but this delay was also present in some individuals in all cohorts. This suggests that poor

habitat conditions in the wild during the first few years of life could slow growth and delay sexual maturation. The study has shown that the current captive breeding and head-start program at Perth Zoo facilitates optimal growth during the first four years of life, which dually benefits released WST by increasing their potential survival rates and facilitating sexual maturity at an earlier age so they can contribute to the wild population's growth sooner.

Shell size or CL appears to be a stronger factor than body mass in predicting sexual maturity. Although age at which males achieved sexual maturity decreased from the first to the last cohort, the average shell size increased. The youngest male (5 years of age) to achieve sexual maturity was also the smallest at 143.3 g and a CL of 105.2 mm. Although the youngest female (6 years of age) had the smallest body mass (143.3 g), her shell size was around the average size at 106.2 mm CL. The smallest female (139.8 g and 90.1 mm CL) to achieve sexual maturity was 9 years old at the time. Her growth rate over the first two years was similar to that of other hatchlings but became slower after this for reasons unknown, which likely delayed her sexual maturation.

Previous studies of WST found the smallest female confirmed to have produced eggs was 120 mm CL in the wild (Burbidge 1981) or that females required a minimum CL of 113 mm for egg production or 108 mm CL for follicular growth without ovulation in an early captive breeding program (Kuchling and Bradshaw 1993). This is much larger than the average size of the present captive study animals at the 10-year mark, several years after many had reached sexual maturity. The authors of the initial wild study did acknowledge that the observed sizes may not be representative of the minimal size required for sexual maturity due to low observation levels of copulation and egg laying in the wild, suggesting it might be closer to that which they observed sexual dimorphism (~110 mm) (Burbidge 1981). For the current captive population, it appears likely that male WST become sexually dimorphic with a CL of ~90 mm and achieved sexual maturity at a CL of 110 mm. However, it appears that females achieve sexual maturity at a smaller body size than males. Females appear to reach sexual maturity closer to a CL of 100 mm, but this is due to slower growth rates rather than earlier age of sexual maturity.

Although captive WST fit the typical Australian chelid turtle growth rate models with a pattern of rapid juvenile growth followed by slow, possibly indefinite, adult growth (Kennett 1996; Spencer 2002), published data on growth rates in carnivorous or omnivorous turtles in Australia are from mark-recapture studies of wild populations limiting our understanding of captive growth rates and influences. It is important to understand the potential impacts of captive diets and environmental conditions on growth rates, health and reproduction of captive species in order to develop and optimize captive breeding programs for endangered species. One study on the herbivorous Mediterranean spur-thighed tortoise, *Testudo graeca*, reported challenges in optimizing the captive diet (Lapid et al. 2005). A lack of protein produced soft shell syndrome (osteomalacia) but too much protein caused gout, both conditions increasing mortality rates. This highlights the need to find a balanced captive diet that fits the requirement of the species not only for healthy growth, but also for reproduction (Lapid et al. 2005). Drought conditions, resulting in limited nutrients, have also been found to inhibit reproduction in wild Eastern long-necked turtles (Kennett and Georges 1990). Early in the WST captive breeding program, females were not producing eggs and it was determined that this was likely due to inadequate nutrient intake (Kuchling and DeJose 1989). As such, the captive WST diet was modified and an increase in volume provided to females from the time of insemination to laying which resulted in improved egg production (Kuchling and DeJose 1989). Shell ulceration was also observed in captive WST prior to the changes in diet which was attributed to a lack of calcium, now supplemented in their diet

(Spence et al. 1979). No nutrient related health conditions were reported in the present study animals and female WST produced eggs typically annually after reaching sexual maturity suggesting that the current captive diet is suitable.

One concern of accelerated growth rate and sexual maturity in captive animals is the potential impact on reproductive success associated with a precocial breeder. If females are becoming sexually mature younger at smaller body sizes, this may have a negative impact on clutch size (Kennett 1996). For a male, becoming sexually mature at a younger age and smaller size may impact on his ability to catch and restrain larger, older females (Kennett 1996). Interestingly, despite the average age of sexual maturity declining as the WST captive breeding program has developed, no associated decrease in body size at sexual maturity has been observed. This suggests that there is likely a minimum body size threshold that is required to attain sexual maturity in WST regardless of age or growth rate. Accelerated growth rate may facilitate decreased intergenerational intervals in captive breeding programs, increasing the rate at which young are produced. This could increase the number of individuals available for reintroduction programs, increasing the rate at which wild populations could be rebuilt. Although improvements in captive management and diet have increased overall growth rate in captive WST, we believe the life history of the species prohibits further acceleration of growth rates and precocial breeding. Aestivation and the cessation of food consumption halts growth and imposes a period of declining body condition, before growth can occur again during the following hydro-period. As such, we believe that the current captive growth rates are optimal, and monitoring will continue to ensure no deleterious effects on health or reproduction occur over time.

A delay of sexual maturity until 8 years of age and small egg clutch sizes (2–6 eggs) confirms that this species of tortoise has a large intergenerational period. This means over the 19-year span of the DOB of the study animals, the second generation of WST had only just begun to be born. This increases the challenges associated with maintaining high levels of genetic diversity and the strategic choosing of which offspring to retain for future breeding. As all offspring are retained for a minimum of 3 years and 10 breeding females could produce 30–50 eggs per year, this means the space requirements to maintain this breeding program for reintroduction purposes is exceptional. Although it has been indicated that sex can be determined through differences in plastron shape and tail length in adult WST above the carapace length of ~110 mm (Burbidge 1981) our results show that it takes males around 7–8 years of age to reach that size and females another 5 years more, which would further increase space requirements to retain unsexed offspring for the breeding program.

This study has found that individual variation, especially at younger ages, prohibits differences in these external features from being foolproof prior to sexual maturation (unpublished data). Tail length differences are minor if any (even in adults) and difficult to determine due to challenges in measuring without the animal retracting its tail into the shell cavity. Males do tend to have a much more concave plastron than females (which generally have flat plastrons) and we believe that if this particular physical trait is combined with growth rate, CL and body mass between 4 and 5 years of age, sex should identifiable in captive WST with some confidence before sexual maturity is reached. This would limit the number of WST that need to be retained beyond the age of 4 or 5 for future breeding. This means that by retaining juvenile WST for an extra year (3 vs. 4 years) prior to release, it may be possible to get a reasonable estimate of the sex ratio of the released cohort without the need or cost of genetic analysis. Unfortunately,

as growth rates slow after sexual maturity in WST and nutrient availability in the wild is more variable, the asymptotic nature of the growth rate model if developed for WST based on the current data, would prohibit accurate estimations of age of older or wild animals (Spencer 2002). Therefore, to monitor growth rates and age demographics of wild WST population, mark-recapture methods are the only reliable means of aging older animals over time.

As sex determining genetic analysis techniques become more reliable and less costly, these may replace prediction of sex using growth rates and size. Regardless, information on growth rate and sexual maturity is invaluable to captive management and breeding programs and also has the potential to provide indices of health and growth of young wild individuals to improve in situ conservation management.

Acknowledgements

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