J Comp Physiol B (2013) 183:235–241
DOI 10.1007/s00360-012-0695-2

ORIGINAL PAPER

Salinity of incubation media influences embryonic development of a freshwater turtle

Deborah S. Bower · Kate M. Hodges · Arthur Georges

Received: 11 March 2012 / Revised: 30 June 2012 / Accepted: 13 July 2012 / Published online: 2 August 2012

Abstract Variations in water potential have marked effects on aspects of embryological development in reptiles. Therefore variation in the salinity of the incubation environment is likely to have significant consequences on the early life stage. The combination of an extended incubation period, coupled with the real threat of soil salinisation within their range makes *Chelodina expansa* an ideal model to assess the influence of salinity on turtle embryology. We quantified the influence of salt on the development of *C. expansa* hatchlings in four substrate treatments varying in salinity. Embryos incubated in higher salinities had 39 % less survival than those incubated in substrates with freshwater. Hatchlings that emerged from eggs in saline treatments were smaller with higher concentrations of plasma sodium, chloride, urea, and potassium. The physiological effects of salinity mirror those of turtles incubated in drier media with low water potential. Salinisation of river banks has the potential to reduce hatching success and fitness of nesting reptiles.

Keywords Salt · Egg · Developmental plasticity · Hydric · Moisture

Communicated by I.D. Hume.

D. S. Bower · K. M. Hodges · A. Georges
Institute for Applied Ecology, University of Canberra, Canberra, ACT 2601, Australia

Present Address:
D. S. Bower (✉)
School of Environmental and Life Sciences, University of Newcastle, Callaghan Road, Newcastle, NSW 2308, Australia
e-mail: Deb.Bower@newcastle.edu.au

Introduction

Dry land salinisation is a human mediated threatening process that affects landscapes globally (Rengasamy 2006). This environmental problem commands attention because models project an increase in both the extent and intensity of salt affected areas (Pannell and Ewing 2006). Increases in salinity cause a cascade of effects through the ecosystem and have the capacity to change entire community structures [reviewed in Hart et al. (1991)]. In combination with climate change, dry land salinisation may have significant consequences on the moisture availability and osmotic potential available to organisms. But which species are most at risk? Organisms in which water potential influences developmental plasticity may be one highly susceptible category.

For most vertebrates, offspring fitness is shaped by parental variation (Olsson et al. 1996) and the history of the reproducing individual (Ballinger et al. 1972). In addition, for egg laying species, the physical environment surrounding the egg plays an independent role in offspring variation and can be a major factor in determining hatchling characteristics that are important to early life stages of individuals, such as the sex (Charnier 1966), hatch time (Yntema 1978) and size of hatchlings (Finkler 1999). Water exchange between the egg and its external environment is one major determinant of hatchling characteristics and may be influenced by many factors. Temperature, water potential, thermal conductivity and degree of contact with the incubation medium all influence rates of water exchange. Additional variation arising from characteristics of the eggs will further determine water exchange such as the size, position and porosity of the egg [reviewed in Packard (1999)].

The broad-shelled turtle, *Chelodina expansa* provides a useful model to assess the influence of salinity because

previous studies have addressed the impacts of variation in the hydric environment on the development of *C. expansa* embryos (Booth 2002a). Whilst moisture does not affect mortality rates or hatching time of *C. expansa*, embryos that develop in drier conditions hatch lighter and smaller with larger residual yolk sacs (Booth 2002a), which may have consequences for fitness (Booth 2002a; Finkler et al. 2000). *Chelodina expansa* inhabits the river systems of south-east Australia (Cogger 2000) where dry land salinity is a widespread occurrence (Gell et al. 2007). In addition, *C. expansa* embryos enter secondary diapause when temperatures decline through winter (Booth 2002a, b); which extends incubation time and leaves *C. expansa* susceptible to the influence of the incubation environment for long periods.

We aimed to quantify the influence of salt on the development of *C. expansa* hatchlings in four substrate treatments varying in salinity. We compared mortality, development time, hatchling morphology and the concentration of ions in the plasma of hatchlings.

Method

Experimental design

Between the 1 March 2007 and the 1 April 2007, 6 gravid *C. expansa* were collected from Wentworth, New South Wales, at the confluence of the Murray and Darling Rivers (34° 6.884′S 141° 54.832′E; WG84). They were transported to the University of Canberra by motor car and individually housed indoors in 46 l plastic bins (597 × 362 × 266 mm), half filled with tap water, which was changed every 2–3 days. The diel light cycle was 12 h alternating light and dark. Turtles were offered raw beef periodically.

At 0900 h on the 3 April 2007, turtles were injected subcutaneously into their rear leg muscle with calcium gluconate at a dosage of 1 unit/100 g of body mass (Norton 2005) to supplement calcium lost in egg production. At 1700 h on the same day, they were injected subcutaneously into their body cavity with the synthetic hormone Oxytocin® (Troy Laboratories, Smithfield, NSW, Australia) at a dosage of 1 unit/100 g body mass (Ewert and Legler 1978) to induce egg laying. Eggs were removed from water in the 46 l plastic tub every 2 h, dried with paper towel, marked with an individual identification number on their dorsal surface using graphite pencil, weighed using a Sartorius Basic scale (BA210S, Germany) and measured using Vernier callipers.

Water solutions of 0, 15, 35 and 70 ‰ NaCl (rehydrated sea salt) were mixed with vermiculite at a ratio of 1:1 by weight. The water potential of the substrate treatments was quantified in 15 ml sample tubes in a measurement chamber using thermocouple psychrometry (WP4-T Dewpoint PotentiaMeter, Decagon Devices) and corresponded to water potentials of <-10 kPa, -703 ± 218 kPa, $-1{,}368 \pm 265$ kPa, $-4{,}900 \pm 427$ kPa. We chose these salinities to capture the possible variation of salt in natural nests. Saline water was created by mixing dissolved sea salt with tap water and verifying with a multi-parameter water analyser (model 611, Yeo-KAL, www.yeokal.com.au). Eggs were partially buried in the vermiculite to reflect natural conditions (2/3rd covered) and placed into 310 × 198 × 47 mm plastic box with 50 × 62 × 47 mm individual compartments for each egg. Boxes were placed into a clip-seal plastic bag (SC Johnson, Lane Cove, NSW) with a corner snipped to allow air transfer to the eggs and small quantify of water (5 ml). Eggs were incubated (Model i300, Axyos, Brendale, NSW, Australia) at a temperature of 14 °C from their laying date on the 7th April 2008. On the 20th May 2008, once eggs had developed white patches on the dorsal surface (Thompson 1985), eggs from each clutch were distributed evenly over the four salinity treatments to investigate for possible clutch effects and incubation continued at 14 °C. On the 10th August the incubator was increased to 26 °C to mimic spring and induce embryonic development, for the duration of the experiment. The position of boxes was changed within the incubator every few weeks to minimise any spatial bias in temperature. The mass of the vermiculite was weighed fortnightly and when weight loss occurred it was replaced.

After 250 days of incubation, eggs were checked every 24 h for hatchlings. They were considered pipped when the shell was visually broken by the hatchling and they were considered to have emerged when they had completely exited the egg shell. Once hatchlings had pipped they were placed on paper towel in a glass container and replaced in the incubator. Once hatchlings completely emerged from the eggshell, they were reweighed, measured and replaced into the incubator. Fourteen days following emergence, hatchlings were pithed and bled. We collected 0.5 ml of blood using 25 gauge needles and syringe. Coagulation was prevented by first irrigating the syringe with lithium heparin, and evacuating all but a residue so as not to appreciably dilute the blood sample. Blood was then spun at 5,000 rpm for 5 min in a Christ AVC 2-25 CD Plus centrifuge (Martin Christ Gefriertrocknungsanlagen). Plasma was extracted using a pipette, transferred to seal tubes and frozen at −80 °C for up to 6 weeks. Plasma was assayed for ionic concentration by a commercial pathology laboratory using an automated chemical analyzer (Olympus AU400, Integrated Science, Tokyo, Japan) calibrated using ISE (ion selective electrodes) serum standards for sodium, chloride and potassium and specific reagent test kits: OSR6134 for urea, OSR698 for uric acid, OSR6121 for

Table 1 The survival rate of *Chelodina expansa* incubated in four salinity treatments from April 2007

Chelodina expansa that developed in higher salinities had higher mortality

Treatment	No. of eggs from individual clutch				No. live hatching	Dead	*n*	Survival (%)
	A	B	C	D				
0 ‰	5	5	4	4	17	1	18	94.4
15 ‰	5	5	5	5	13	7	20	65.0
35 ‰	5	5	5	5	16	4	20	80.0
70 ‰	5	7	5	5	12	10	22	54.5

glucose and OSR60117 for calcium (Clinical Pathology Laboratory at the School of Veterinary Science, University of Queensland). Immediately following blood collection, the liver and yolk sac of each hatchling was dissected using scalpels, weighed using a Sartorius Basic scale (BA210S, Germany) and dried in a Thermoline Australia Dehydrating Oven, BTC-9090, Model D100 bags at 48 °C for 4 days before they were removed and reweighed.

Data analysis

A two by two Chi square contingency table was used to separately compare the proportion of survival in salinity treatments with freshwater (0 ‰). A two way ANCOVA with clutch ($n = 4$) as a random factor, salinity (0, 15, 35, 70 ‰) as a fixed factor and egg length as a covariate was used to determine if clutch, salinity and egg size influenced the time taken to hatch. This same test was used to compare variation in carapace length, and plasma ionic concentrations; however, egg size did not explain a significant source of variation in the data and was removed from the model. Carapace length was used as a covariate with response variables in hatchling mass, dry liver mass and dry yolk mass. Assumption of normality and equal variance was tested by visually examining the data in box-plots and where violated, response variables were ln transformed to normalise the distribution. All analyses were conducted in SPSS 17.0. Significance was established at 0.05. Eggs from the two clutches where the majority of eggs (>85 and 100 %) failed to develop were omitted from the analysis.

Results

Survival of developing eggs was 54.5% in 70 ‰ salinity, which was significantly lower than the 94.4 % survival of embryos incubated in the control treatment—0 ‰ salinity (Table 1; $\chi^2 = 4.94$, $df = 1$, $P < 0.05$). The intermediate salinity treatments had intermediate proportions of embryonic survival, but the relationship was not linearly related to salinity. Survival of embryos incubated in 15 ‰ salinity was 65 %, which was significantly lower than the 0 ‰ salinity ($\chi^2 = 7.9$, $df = 1$, $P < 0.005$) yet in 35 ‰ salinity, survival was not significantly different to values in any other treatment ($\chi^2 = 1.73$, $df = 1$, $P = 0.19$).

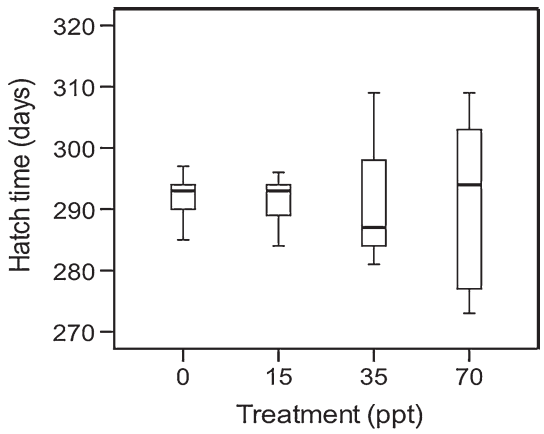

Fig. 1 The time taken until shell pipping for *Chelodina expansa* incubated in four salinity treatments in April 2007. *Chelodina expansa* in higher salinities began hatching earlier and hatched over a longer time range than those in 15 and 0 ‰

Embryos incubated in higher salinities began hatching earlier and hatched over a longer time range than in other treatments. In 70 ‰ salinity, hatching began on day 273 and continued for 39 days whereas in 0 ‰ salinity hatching began on day 285 and only continued for 13 days (Fig. 1). However, the average time of hatching did not vary among salinities (ln $F_{3,42} = 0.42$, $P = 0.738$), or clutches (ln $F_{3,42} = 1.69$, $P = 0.18$), and there was no interaction of treatments (ln $F_{9,42} = 1.69$, $P = 0.18$).

Mean carapace length of hatchlings incubated in 70 ‰ salinity was 36 ± 3 mm, this was significantly smaller than hatchings from 0 ‰, which averaged 42 ± 2 mm. Clutch of origin ($F_{3,35} = 8.13$, $P < 0.0001$) and salinity ($F_{3,35} = 17.78$, $P < 0.0001$) had a significant effect on carapace length. The influence of salinity interacted with the clutch of origin on mean carapace length of hatchlings ($F_{8,35} = 2.99$, $P < 0.01$; Fig. 2a); egg length had no effect (ln $F_{1,35} = 1.10$, $P = 0.17$).

Mass of hatchlings (yolk removed) was greatest in the lowest salinity; hatchlings from 0 ‰ salinity weighed 2.9 ± 0.1 g and decreased with increasing salinity to 1.5 ± 0.2 g in 70 ‰ salinity. Salinity interacted with clutch of origin to affect the mean yolk free mass of hatchlings

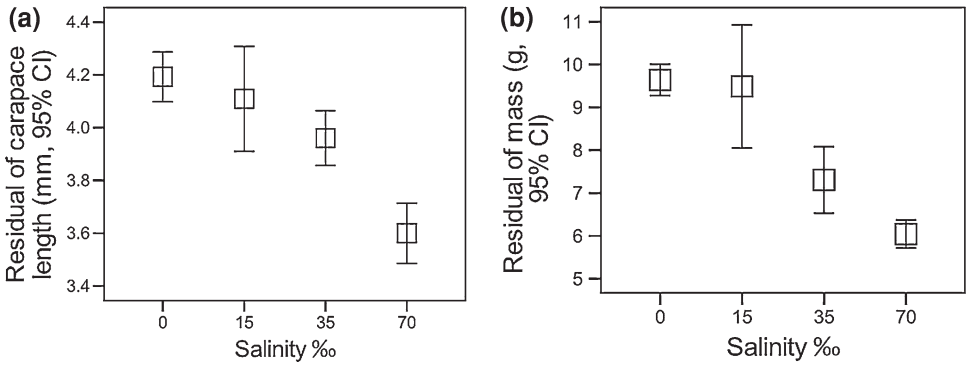

Fig. 2 Mean carapace length (**a**) and yolk free mass (**b**) of *Chelodina expansa* incubated in four substrate treatments varying in salinity. Plotted means are adjusted means from an ANCOVA in which initial egg mass was the covariate for carapace length and carapace length was the covariate for mass, salinity treatment was a fixed factor and clutch a random factor. Hatchlings incubated in saline treatments were smaller and lighter relative to their size

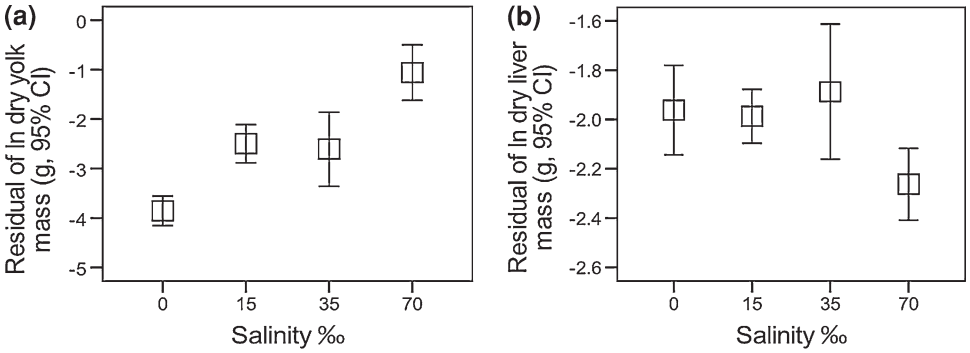

Fig. 3 Mean carapace length of dry yolk mass (**a**) and dry liver mass (**b**) of *Chelodina expansa* incubated in four substrate treatments varying in salinity. Data were ln transformed to meet assumptions of normality. Plotted means are adjusted means from an ANCOVA in which carapace size was the covariate, salinity treatment a fixed factor and clutch a random factor. Hatchlings incubated in saline treatments were smaller and lighter relative to their size

($F_{7,32} = 2.51$, $P < 0.01$; Fig. 2b), where as carapace length did not have an influence ($F_{1,32} = 0.02$, $P = 0.89$).

Mean dry yolk mass averaged 0.04 ± 0.05 g in hatchlings from 0 ‰ salinity and increased with a corresponding increase in salinity treatment to 0.49 ± 0.10 g in 70 ‰ salinity. Dry yolk mass was not affected by carapace length (ln $F_{1,34} = 2.84$, $P < 0.101$). However, clutch of origin (ln $F_{3,34} = 5.57$, $P < 0.01$) and salinity (ln $F_{3,34} = 3.27$, $P < 0.05$) had a significant effect. The influence of salinity did not interact with clutch of origin on mean dry mass of yolk (ln $F_{8,34} = 1.11$, $P < 0.382$; Fig. 3a). Dry liver mass averaged 0.16 ± 0.04 and did not vary among treatments; there was a significant effect of clutch (ln $F_{3,35} = 4.04$, $P < 0.5$) but no effect of salinity (ln $F_{3,35} = 0.216$, $P = 0.885$) or an interaction (ln $F_{8,35} = 0.1.69$, $P = 0.14$; Fig. 3b) and carapace length did not have an effect (ln $F_{1,35} = 1.13$, $P = 0.296$).

Mean concentrations of plasma sodium, chloride, urea and potassium were higher in hatchlings that were incubated in higher salinities, whereas differences in mean calcium, glucose and urate were not consistent with the increase in salinity (Table 2). Clutch of origin had no effect on these variables; egg length was only significant in determining calcium (statistics presented Table 2).

Discussion

The salinity of the substrate surrounding embryonic development had marked effects on the development of *C. expansa*. The larger residual yolk sac of hatchings, smaller carapace size and smaller yolk free mass of hatchlings emerging from eggs incubated in vermiculite in higher salinities mirrors the plastic response of *C. expansa* incubated in drier hygric environments (Booth 2002a). However, the higher mortality of *C. expansa* in the salinity treatments in our study was not reported previously for *C. expansa* (Booth 2002a). This may be attributed to the more negative water potential created by variation in the osmotic potential (−4,900 kPa) rather than in studies with differences in matrix potential (−750 kPa Booth 2002a). Our study incubated eggs in water potentials much lower (−4,900 kPa) than the water potential inside a turtle egg

Table 2 The mean plasma concentration and analysis of covariance results for *Chelodina expansa* incubated in four different salinity treatments from April 2007

	Mean plasma concentrations (mmol/L) with standard error				Treatment			Clutch			Treatment*Clutch			Egg length		
	0 ‰	15 ‰	35 ‰	70 ‰	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Na	149.8 (4.51)	158.4 (5.58)	177.8 (4.83)	198.3 (7.59)	12.1	3.34	<0.001*	0.96	3.34	0.43	1.56	9.34	0.17	0.18	1.34	0.68
Cl	119.8 (4.55)	129.6 (5.64)	156.5 (4.83)	184.6 (7.67)	21.4	3.34	<0.0001*	1.2	3.34	0.32	1.86	9.34	0.09	0.23	1.34	0.63
UE	21.6 (4.18)	41.4 (5.18)	68.1 (4.48)	78.5 (7.24)	24.8	3.33	<0.0001*	0.97	3.33	0.42	1.53	9.33	0.18	0.56	1.33	0.46
UA	233.9 (32.20)	258.7 (38.31)	223.5 (33.89)	99.2 (53.58)	2.06	3.31	0.13	2.56	3.31	0.07	1.33	9.31	0.26	0.08	1.31	0.78
Ca	1.8 (0.08)	1.8 (0.10)	2.3 (0.13)	1.9 (0.34)	4.51	3.24	<0.05*	2.84	3.24	0.06	0.62	9.24	0.71	5.69	1.24	<0.05*
Glc	5.2 (0.35)	4.4 (0.42)	6.9 (0.51)	5.4 (1.35)	5.02	3.24	<0.01*	0.67	3.24	0.58	1.73	9.24	0.16	0.01	1.24	0.94
K	6.9 (0.58)	6.7 (0.71)	7.9 (0.62)	10.2 (0.97)	3.12	3.34	<0.05*	1.98	3.34	0.14	2.05	9.34	0.06	0.05	1.34	0.82

Higher salinity treatment during incubation resulted in higher plasma concentrations of sodium, chloride, urea and potassium but not uric acid, calcium or glucose in *Chelodina expansa* hatchlings while the clutch of origin did not affect the parameters

* Denotes significance

(−800 kPa) and therefore exposed the eggs to a more negative pressure in the surrounding environment. Concentration of NaCl affects hatching success, hatchling mass and yolk mass of snapping turtles in a similar way to *C. expansa* (Rimkus et al. 2002).

Water exchange in eggs occurs through two pathways. Water vapour exchange occurs when there are vapour pressure differences between the egg and its environment, whereas liquid water exchange occurs when the chorioallantois in the blood of the embryo is a different water potential to the incubation medium (Packard 1999; Thompson 1987). Although the relative contribution from each of the processes of water exchange is disputed (Ackerman 1991; Ackerman et al. 1985 vs. Packard and Packard 1988), rigid-shelled eggs lose water when incubated partially buried in water potentials greater than −350 kPa (Booth and Chung 1998). Therefore, all salinity treatments in our study (15–70 ‰) would have lost water during incubation, while the 0 ‰ treatment would have gained water. This was reflected by the larger mass of hatchlings incubated in higher water potentials which was independent of carapace length suggesting that they also had higher water contents. However, this could not be verified with dry mass measurements because blood was taken from hatchlings.

A wide variety of factors affect development of embryos and confound inter-study comparisons (reviewed in Packard 1999), but general trends are evident. In natural situations, nests are not subject to constant water potentials; they are exposed to differing environmental conditions (Booth 2002a; Thompson et al. 1996). Eggs of *C. expansa* are able to absorb water during wet periods which may then provide a reserve to tolerate dry conditions (Booth 2002a). In species with parchment-shelled eggs in North America, lethal effects are evident in comparatively high water potentials (−500 kPa; (Packard et al. 1987) because eggs are permeable and water exchange occurs readily. In contrast, studies incubating buried *C. expansa* eggs in media as low as −750 kPa did not reduce survival (Booth 2002a). Here we found increased mortality (54 %) in partially buried eggs in −4,900 kPa, a consequence of the lower osmotic water potential created by the saline environment.

The amount of yolk converted to tissue is correlated with the amount of water absorbed by the egg (Booth 2002a). Consequently, we found larger yolk sacs in hatchlings of eggs incubated on lower osmotic water potentials. This may be disadvantageous because the yolk sac is only adequate for maintenance metabolism and does not provide the calcium necessary for growth (Packard and Packard 1986) and may reduce post hatchling growth and speed (Packard 1991; Packard 1999). It has been hypothesised that reduced growth of embryos occurs from regulation of embryo water content, which could explain the smaller size and larger yolks in hatchlings in lower water potentials (Rimkus et al. 2002).

Despite large differences in hatchling morphology and survival, which mirror studies of parchment-shelled eggs, we found that incubation time did not follow the same trend. We did not observe a difference in incubation time with variation in osmotic water potential, which supports

findings of other turtles incubated in saline incubation media (Rimkus et al. 2002) and supports the hypothesis that incubation time is independent of egg water exchange and instead affected by a different cue associated with wetness (Rimkus et al. 2002). Conversely, clutch of origin influenced hatchling dimensions, a finding consistent with other studies (Packard 1999) and reflects variation in maternal contribution to hatchling quality, as is expected through genetic differences and those arising from history of the reproducing females (Ballinger et al. 1972).

The physiological and morphological changes in hatchings may have consequences for fitness of turtles incubated in saline environments; larger hatchlings and individuals with greater water content may survive longer, grow faster and move faster (Finkler 1999; Finkler et al. 2000; Miller 1993; Miller et al. 1987), although these findings are not consistent among studies (Brooks et al. 1991; Congdon et al. 1999). Increased ionic concentrations in the plasma of hatchlings incubated in higher salinities is similar to the effects on development of other turtle species incubated in low water potentials created by using a dry substrate medium (Packard and Packard 1989; Rimkus et al. 2002). Turtles incubated in low water potentials have higher osmolality and urea concentration (Packard and Packard 1989) than those incubated in high water potential. The lower availability of water may cause concentration of plasma osmolytes. The increase in plasma potassium of *C. expansa* hatchlings may indicate that ionic concentrations cannot be regulated effectively because, even under high levels of osmotic stress in adult turtles, potassium is highly regulated (Gilles-Baillen 1970).

The effect of salinity on the mortality and physiology of developing embryos highlights the potential effects of salinisation on nesting species of reptiles. The presence of salt in the incubation medium causes a reduction in water potential, which in turn has physiological effects on developing embryos. Mortality caused by high salinities will restrict recruitment of populations, at a moisture threshold specific to the species. In intermediate salinities, the consequences to fitness of *C. expansa* that hatch smaller with larger residual yolks is unclear and requires further exploration to identify the post hatching impact of development in a low water potential. In addition, not all eggs in a natural clutch incubate in direct contact with the soil, which may reduce the significance of low water potential in a nest (Thompson 1987). The influence of salt on development of nesting species is likely to interact with temperature, moisture and medium because these variables all influence embryonic uptake of water (Packard 1999). Our study further demonstrates the complexity of predicting impacts of landscape change; uncertainty which should be incorporated into predictive models in future.

Acknowledgments This study was funded by a Linnaean Society of New South Wales research grant and an ARC Linkage Grant (LP0560985) awarded to Arthur Georges, Stephen Donnellan and Mark Hutchinson, the South Australian Museum, the Department of Environment and Heritage, the Department of Sustainable Environment, the Foundation for Australia's Most Rare Species, the Nature Foundation SA, and the Murray-Darling Basin Natural Resource Management Board. J. Richardson kindly completed part of the laboratory work. Two anonymous reviewers and M. Thompson greatly improved the manuscript through constructive comments. The publication also benefited from the input of J. Clulow, D. Booth, N. Pezaro and C. Eisemberg. S. Foster, B. Maherand A. Taylor offered advice, donated material and lent equipment for the experiment. M. Thompson and J. Herbert kindly gave their time, equipment and expertise to assist in data collection. P. McGee kindly lent his laboratory at short notice. These contributors are gratefully acknowledged.

References

Ackerman RA (1991) Physical factors affecting the water exchange of buried reptile eggs. In: Deeming DC, Ferguson MWJ (eds) Egg incubation: its effects on embryonic development in birds and reptiles. Cambridge University Press, New York, pp 213–228

Ackerman RA, Seagrave RC, Dmi'el R, Ar A (1985) Water and heat exchange between parchment-shelled reptile eggs and their surroundings. Copeia 1985:703–711

Ballinger RE, Tyler ED, Tinkle DW (1972) Reproductive ecology of a west Texas population of the greater earless lizard, *Cophosaurus texanus*. Am Midl Nat 88:419–428

Booth D (2002a) Incubation of rigid-shelled turtle eggs: do hydric conditions matter? J Comp Physiol B Biochem Syst Environ Physiol 172:627–633

Booth DT (2002b) The breaking of diapause in embroyonic broad-shelled river turtles (*Chelodina expansa*). J Herpetol 36:304–307

Booth DT, Chung YY (1998) Effects of incubation temperature on the energetics of embryonic development and hatchling morphology in the Brisbane river turtle *Emydura signata*. J Comp Physiol B Biochem Syst Environ Physiol 168:399–404

Brooks RJ, Bobyn ML, Galbraith DA, Layfield JA, Nancekivell EG (1991) Maternal and environmental influences on growth and survival of embryonic and hatchling snapping turtles (*Chelydra serpentina*). Can J Zool 69:2667–2676

Charnier M (1966) Action de la temperature sur la sex-ratio chez l'embryon d'*Agama agama* (Agamidae, Lacertilien). Societe de Biologie de l'Ouest Africain 160:620–622

Cogger H (2000) Reptiles and amphibians of Australia. Reed New Holland, Sydney

Congdon JD, Nagle RD, Dunham AE, Beck CW, Kinney OM, Yeomans SR (1999) The relationship of body size to survivorship of hatchling snapping turtles (*Chelydra serpentina*): an evaluation of the "bigger is better" hypothesis. Oecologia 121:224–235

Ewert MA, Legler JM (1978) Hormonal induction of oviposition in turtles. Herpetologica 34:314–318

Finkler MS (1999) Influence of water availability during incubation on hatchling size, body composition, desiccation tolerance, and terrestrial locomotor performance in the snapping turtle *Chelydra serpentina*. Physiol Biochem Zool 72:714–722

Finkler MS, Knickerbocker DL, Claussen DL (2000) Influence of hydric conditions during incubation and population on overland movement of neonatal snapping turtles. J Herpetol 34:452–455

Gell P, Tibby J, Fiona L, Baldwin D, Hancock G (2007) The impact of regulation and salinisation on floodplain lakes: the lower River Murray, Australia. Hydrobiologica 591:135–146

Gilles-Baillen M (1970) Urea and osmoregulation in the diamondback terrapin (*Malaclemys centrata centrata* (Latreille). J Exp Biol 52:691–697

Hart BT, Bailey P, Edwards R, Hortle K, James K, McMahon A, Meredith C, Swadling K (1991) A review of the salt sensitivity of the Australian freshwater biota. Hydrobiologica 210:105–144

Miller K (1993) The improved performance of snapping turtles (*Chelydra serpentina*) hatched from eggs incubated on a wet substrate persists through the neonatal period. J Herpetol 27:228–233

Miller K, Packard GC, Packard MJ (1987) Hydric conditions during incubation influence locomotor performance of hatchling snapping turtles. J Exp Biol 127:401–412

Norton TM (2005) Chelonian emergency and critical care. Seminars in Avian and Exotic Pet Medicine 14:106–130

Olsson M, Gullberg A, Shine R, Madsen T, Tegelstrom H (1996) Paternal genotype influences incubation period, offspring size, and offspring shape in an oviparous reptile. Evolution 50:1328–1333

Packard GC (1991) Physiological and ecological importance of water to embryos of oviparous reptiles. In: Deeming DC, Ferguson MWJ (eds) Egg incubation: its effects on embryonic development in birds and reptiles. Cambridge University Press, New York, pp 213–228

Packard GC (1999) Water relations of chelonian eggs and embryos: is wetter better? Am Zool 39:289

Packard MJ, Packard GC (1986) Effect of water balance on growth and calcium mobilization of embryonic painted turtles (*Chrysemys picta*). Physiol Zool 59:398–405

Packard GC, Packard MJ (1988) Water relations of embryonic snapping turtles (*Chelydra serpentina*) exposed to wet or dry environments at different times in incubation. Physiol Zool 61:95–106

Packard GC, Packard MJ (1989) Control of metabolism and growth in embryonic turtles: a test of the urea hypothesis. J Exp Biol 147:203–216

Packard GC, Packard MJ, Miller K, Boardman TJ (1987) Influence of moisture, temperature, and substrate on snapping turtle eggs and embryos. Ecology 68:983–993

Pannell DJ, Ewing MA (2006) Managing secondary dryland salinity: options and challenges. Agric Water Manag 80:41–56

Rengasamy P (2006) World salinization with emphasis on Australia. J Exp Bot 57:1017–1023

Rimkus TA, Hruska N, Ackerman RA (2002) Separating the effects of vapour pressure and heat exchange on water exchange by snapping turtle (*Chelydra serpentina*) eggs. Copeia 2002:706–715

Thompson MB (1985) Functional significance of the opaque white patch in eggs of *Emydura macquarii*. In: Grigg G, Shine R, Ehmann H (eds) The biology of Australasian frogs and reptiles. Surrey Beatty & Sons, Sydney, pp 387–395

Thompson MB (1987) Water exchange in reptilian eggs. Physiol Zool 60:1–8

Thompson MB, Packard GC, Packard MJ, Rose B (1996) Analysis of the nest environment of tuatara, *Sphenodon punctatus*. J Zool Lond 238:239–251

Yntema CL (1978) Times for eggs of the turtle *Chelydra serpentina* (Testudines: Chelydridae) at various temperatures. Herpetologica 34:227–274