

Reproduction of two species of freshwater turtle, *Chelodina rugosa* and *Elseya dentata*, from the wet–dry tropics of northern Australia

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(Accepted 13 July 1998)

Abstract

Chelodina rugosa is a fast-growing, carnivorous turtle that occupies seasonally ephemeral habitats, whereas *Elseya dentata* is relatively slow-growing, largely herbivorous and confined to permanent water. Ovarian cycles followed an annual cycle of enlargement beginning in December (*E. dentata*) and January (*C. rugosa*), followed by ovulation and then follicular regression. Nesting season was determined from presence of gravid females, and for *C. rugosa* spans some 8 months from the late wet season until mid dry season, during which the turtle lays multiple clutches of eggs in underwater nests. Nesting may continue for longer when waterholes dry later following prolonged wet season rains. Nesting by *E. dentata* also begins in the late wet season but is complete by May and only one clutch is laid. Females of both species reproduce each year and clutch size and mass are related to female size. Annual spermatogenic cycles are similar in timing to those reported for temperate-zone chelids but would be classified as pre-nuptial because spermatogenesis begins before breeding. Gonial proliferation begins at the start of the wet season and spermiation coincides with the onset of ovulation. Mating was not observed but in *C. rugosa* probably occurs during December and January soon after turtles emerge from aestivation. Post-mating storage of sperm in the epididymes did not occur in *E. dentata* but did appear to occur in some *C. rugosa*. The high reproductive output and extended nesting season of *C. rugosa* reflects its occupation of highly productive but unpredictable habitats and a reproductive strategy which ensures that some hatchlings meet optimal conditions for emergence. In contrast, the comparatively low fecundity and short nesting season of *E. dentata*, despite year-round warm temperatures and access to permanent water, suggests that its reproductive output may be energetically limited by a largely herbivorous protein-poor diet.

Key words: Testudines, *Chelodina rugosa*, *Elseya dentata*, reproduction, gonadal cycles

INTRODUCTION

Reproduction of freshwater turtles in temperate latitudes is largely constrained by seasonal variation in temperature, with nesting and hatchling emergence confined to warmer months. Low winter temperatures, sometimes with freezing of waterbodies, may halt growth and activity entirely (see Moll, 1979). In contrast, freshwater turtles in tropical climates generally experience warm temperatures year round. Rainfall, especially in regions where it is highly seasonal, may become more important than temperature in determining the seasonality of reproduction.

Many tropical chelonians nest in the dry season,

ensuring dry nest sites and minimizing the risk of flooding (e.g. *Carettochelys insculpta*, Webb, Choquenot & Whitehead, 1986; *Pseudemys* spp., Moll & Legler, 1971; Moll & Moll, 1990; *Podocnemis expansa*, Ahlo & Padua, 1982). Others nest during the rainy season (e.g. *Dermatemys mawii*, Polisar, 1992, 1996) and one species is reported to nest throughout the year (*Kinosternon leucostomum*, Moll & Legler, 1971). Hatchling emergence often coincides with the arrival of wet season rains and flooding (Moll & Legler, 1971; Webb *et al.*, 1986; Kennett, Christian & Pritchard, 1993a).

Marked seasonality of rainfall in some regions, such as the wet–dry tropics of northern Australia, results in large, often unpredictable annual fluctuations in the availability of aquatic habitats. As water levels recede, turtles may become concentrated into remaining permanent waterbodies (e.g. *C. insculpta*, Georges & Kennett, 1989) or aestivate in terrestrial refugia (Ernst &

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Barbour, 1989). The resulting reduction in the time available for activity, feeding and growth, as well as the metabolic costs of accruing and maintaining energy reserves necessary to survive aestivation, will all have consequences for reproduction. For these reasons, studies of the reproduction of tropical species are likely to reveal a diversity of reproductive patterns or strategies not encountered in temperate species.

The reproductive biology of tropical freshwater turtles throughout the world has received little attention in comparison with that of temperate species. This situation holds true for the 16 species of Australian freshwater turtles currently recognized (Cogger, 1992). The reproductive biology of only one tropical species, *C. insculpta* (Webb *et al.*, 1986; Georges & Kennett, 1989; Georges, 1992), and one sub-tropical species, *Emydura krefftii* on Fraser Island, have been studied in detail (Georges, 1982, 1983). For most tropical species, including the widespread *Chelodina rugosa* and *Elseya dentata*, even basic reproductive data, such as egg and clutch size, are scant. The unpublished reports of Legler (1980, 1982), and a comparative biogeographic study by Legler (1985), describe *C. rugosa* and *E. dentata* as wet season breeders. A short note by Cann (1980) described the artificial incubation of eggs obtained from a gravid *C. rugosa*. Legler & Cann (1980) provide limited data on the eggs and hatchlings of *Rheodytes leukops*, *Emydura krefftii*, *Chelodina longicollis*, *C. expansa* and *E. dentata* in the Fitzroy River near Rockhampton, Queensland.

Chelodina rugosa and *E. dentata* occur widely throughout tropical northern Australia. Recent genetic studies indicate that *E. dentata* is actually a complex of four species with poorly known but probably allopatric distributions (Georges & Adams, 1992; 1996). New species descriptions are being prepared (Scott Thomson, pers. comm.) and the species referred to in the present study (Daly River drainage) will retain the name *E. dentata* (Scott Thomson, pers. comm.).

The climate throughout much of the range of *C. rugosa* and *E. dentata* is seasonal, with the monsoonal 'wet' season lasting from about December to April, and little or no rain in the intervening 'dry' season. The two species differ markedly in both habitat requirements and diet. *Elseya dentata* is largely herbivorous and is limited to riverine habitats with permanent water, whereas *C. rugosa* is carnivorous and is most abundant in ephemeral swamps and waterholes on the coastal floodplains (Kennett, 1994; Kennett & Tory, 1996). These floodplains have a pronounced seasonal cycle of inundation with extensive flooding over thousands of square km during the wet season, followed by drying of the floodplain surface and many of the floodplain waterbodies during the subsequent seasonal drought (Finlayson *et al.*, 1988). *Chelodina rugosa* responds to this dramatic hydrological cycle by nesting underwater when the ground is flooded (Kennett, Christian *et al.*, 1993a; Kennett, Georges & Palmer-Allen, 1993b), and aestivating underground as waters recede (Grigg *et al.*, 1986; Kennett & Christian, 1994).

This study reports the reproduction of *C. rugosa* and

E. dentata and discusses the differences in their respective reproductive strategies in relation to their different habitat requirements and diet

MATERIALS AND METHODS

Capture and measurement of turtles

Turtles were captured from mid-1989 to mid-1992 in baited traps (Kennett, 1992), as part of a broader mark-recapture study (Kennett, 1994). *Chelodina rugosa* were captured from 3 seasonally ephemeral waterholes near Darwin, Northern Territory, Australia (12°26' S, 130°57' E). *Elseya dentata* were captured in a permanent waterhole in the Douglas River (13°49' S, 131°12' E) c. 300 km south of Darwin.

Body mass (± 0.1 g) was recorded for both species. Midline straight-line carapace length (CL) was recorded for *C. rugosa* (± 0.1 mm), whereas maximum straight-line carapace length (CL) was recorded for *E. dentata* (± 0.1 mm). Turtles were individually marked by a unique combination of filed notches in marginal scutes of the carapace. Eggs in gravid turtles were detected by palpating the abdomen through the inguinal pocket. All gravid females except some individuals retained for dissection were x-rayed to obtain a complete egg count and data on egg widths (Gibbons & Greene, 1979). Turtles were killed by intravenous injection of a lethal dose of ketamine hydrochloride according to recommended procedures of the Northern Territory University Animal Experimentation Ethics Committee.

Reproductive condition and gonadal cycle

A total of 20 male and 59 female *C. rugosa* (Appendix 1) were dissected between March 1989 and July 1991. Of these turtles, 70 were collected from the mark-recapture locations around Darwin and 9 were collected for food by Aboriginal people at the Reynolds and Adelaide Rivers. Ovaries from 7 females were removed when the turtles were killed at the site of capture, and the 2 males were retained for later dissection (< 48 h). A total of 18 male and 23 female *E. dentata* (Appendix 2) were dissected between March 1991 and March 1992. Gravid females were held for 10 days to ensure that the formation of the eggshell was complete, otherwise turtles were killed and immediately examined as soon as possible after capture, usually within 1 week.

Testes and epididymes were removed and fluid from them was examined under a light microscope to determine the abundance of spermatozoa (recorded as absent, scarce, common or abundant). The testes were weighed (± 0.1 g) and placed in Bouin's fixative. An index of testes size was calculated for each animal as the combined mass of both testes expressed as a percentage of total body mass. The tissue was embedded in wax, sectioned (nominally 6 μ m) and stained with haematoxylin and eosin. The sections were examined under a light

microscope and the relative abundance of each spermatogenic stage was qualitatively assessed. The spermatogenic cell types were identified and classified using published photomicrographs of turtle testes (Georges, 1983) and alligator testes (Lance, 1989). The diameters of 30 seminiferous tubules from each pair of testes were measured using a calibrated eyepiece and a mean tubule diameter was calculated for each pair.

Ovaries were removed and weighed (± 0.1 g). An ovarian index was calculated for each female as the combined mass of both ovaries expressed as a percentage of body mass. A similar index was calculated for inguinal fat mass. Any eggs present were measured as outlined below. Corpora lutea were distinguished from other ovarian structures by their cup-like shape and translucent white colour and were counted fresh before the ovaries were preserved in 10% formalin. Once the tissue hardened, oocytes (diameter > 4 mm) were removed and measured (± 1 mm) using a gauge containing a graduated series of holes. Atretic follicles were distinguished from the yellow developing follicles by their pink or reddish-brown colouration and pitted surface (Georges, 1983). Corpora lutea were removed and their maximum diameters were measured (± 0.1 mm).

Additional unpublished data on the body size and gonad mass of 10 male and 7 female *C. rugosa* caught and killed for food by Aboriginal people at the Reynolds River in November 1979 were supplied by R. Pengilley.

Attainment of sexual maturity

The size at onset of sexual maturity for males was determined by histological examination of the testes, the presence of sperm in the epididymes and the size at which basal thickening and elongation of the tail of the male turtle was largely complete (see Kennett, 1996 for methods). Mature male freshwater turtles commonly have longer, thicker tails than mature females (Pritchard, 1979) and the onset of sexual maturity coincides with tail elongation and thickening in the Australian chelid turtle *Emydura krefftii* (Georges, 1982). A similar relationship was assumed for *C. rugosa* and *E. dentata*. Total tail length and the length of the section of tail anterior to the cloaca were measured on a sub-sample of individuals, including turtles whose sex was determined definitively by either dissection or the presence of oviducal eggs (see Kennett, 1996 for methods).

Size at maturity for females was determined from the mean size of the 10 smallest gravid females. A total of 166 gravid *C. rugosa* and 45 gravid *E. dentata* was examined.

Incubation of eggs

Eggs from both species were obtained either by dissection, or egg laying was induced by intracoelomic injection of synthetic oxytocin at a rate of 1 unit per

100 g body mass (Ewert & Legler, 1978), with a further dose of 0.5 units per 100 g if no eggs were laid within 3 h. Eggs were weighed (± 0.1 g) and their maximum lengths and widths were measured (± 0.1 mm). Eggs were incubated to yield data on hatchling sizes and incubation period and success. *Chelodina rugosa* eggs were used in a number of experiments that are reported elsewhere (Kennett, Christian *et al.*, 1993a; Kennett, Georges & Palmer-Allen, 1993b). Eggs were incubated at a constant 30°C (range $\pm 0.2^\circ\text{C}$) on a bed of moistened vermiculite in plastic containers enclosed in sealable plastic bags to maintain a high but unmeasured humidity. One corner of each bag was snipped off to allow free gas exchange while holding water loss to a minimum. Containers were weighed weekly and water added as necessary to maintain a constant ratio of 3 parts vermiculite:4 parts water by mass. Temperatures were monitored daily using a mercury thermometer calibrated against a NATA certified thermometer ($\pm 0.1^\circ\text{C}$), with the bulb placed amongst the eggs of one container.

All statistical analyses were performed with the statistical package SAS (SAS Institute 1986) following the recommended procedures of Sokal & Rohlf (1981) or Zar (1984). All means are given with their standard errors, unless otherwise specified.

RESULTS

Testicular cycles and size at maturity

Chelodina rugosa Both testicular index and mean seminiferous tubule diameter underwent a seasonal cycle congruent with an annual cycle of sperm production (Fig. 1a, b). Throughout most of the dry season, from June to September, the testes of *C. rugosa* are quiescent and the germinal cells show no sign of proliferating. The seminiferous tubule lumen is largely occluded by Sertoli cell cytoplasm and contains no spermatozoa. The testes and their tubules were both smallest during this quiescent phase. Spermatogonia begin to proliferate in October and by late November primary spermatocytes are abundant. This increase in spermatogenic activity is accompanied by increases in both testicular size and tubule diameter. The testes of two individuals collected from aestivation in October were also enlarged and gonial proliferation had begun. Spermiogenesis peaks in January as spermatids mature into spermatozoa and accumulate in bunches in the lumen. Testes size and tubule diameter are at their maximum. Spermiation occurs through February and March as mature sperm are released from the Sertoli cells, accumulate in the lumen and move into the epididymes. Epididymes containing sperm were thicker and whiter than epididymes without sperm. Testes size and tubule diameter decline in size from January through to June when the testes become quiescent.

There was no clear seasonal pattern in presence of sperm in the epididymes. Four males of mature size

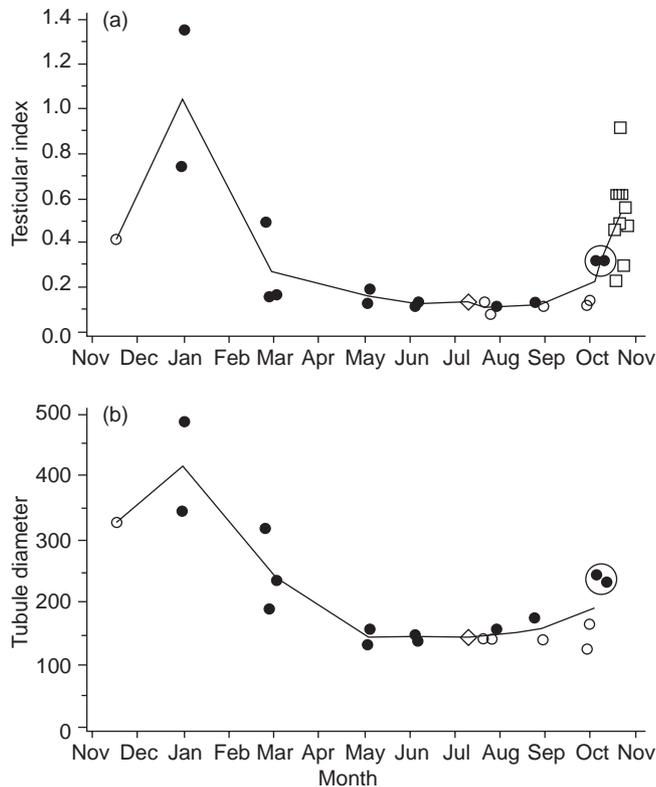


Fig. 1. Seasonal variation in (a) testicular index (combined mass of both testes expressed as a percentage of body mass) and (b) mean seminiferous tubule diameter of 20 male *Chelodina rugosa*. Individuals are characterized as either with sperm in their epididymes (●), without sperm (○) or sub-adult (◇). The individuals collected from aestivation are circled. Additional data supplied by R. Pengilley on testes size in 10 aestivating turtles (see text) are represented by (□). The fitted line joins the mean values for each monthly sample.

examined between July and November contained sperm while six males did not. There was no apparent correlation between lack of sperm and body size; both small and large males had empty epididymes. Nor was there a consistent seasonal pattern in abundance of sperm in the epididymes. Sperm was common in two individuals dissected in May and September, scarce in one individual dissected in October and abundant in all other individuals.

The three smallest dissected male *C. rugosa* had carapace lengths (CL) of 187.7 mm, 185.8 mm and 155.1 mm and were examined in March, June and July respectively. The two larger individuals contained abundant sperm in their epididymes but the smallest male did not. The absence of sperm in this male cannot be used to define immaturity, because six out of eight large turtles that were clearly of mature size (CL: 207.0–255.4 mm) dissected between July and mid-October also did not contain sperm. This period corresponded to a quiescent stage in the spermatogenic cycle when testicular size and seminiferous tubule diameter were smallest (Fig. 1a, b).

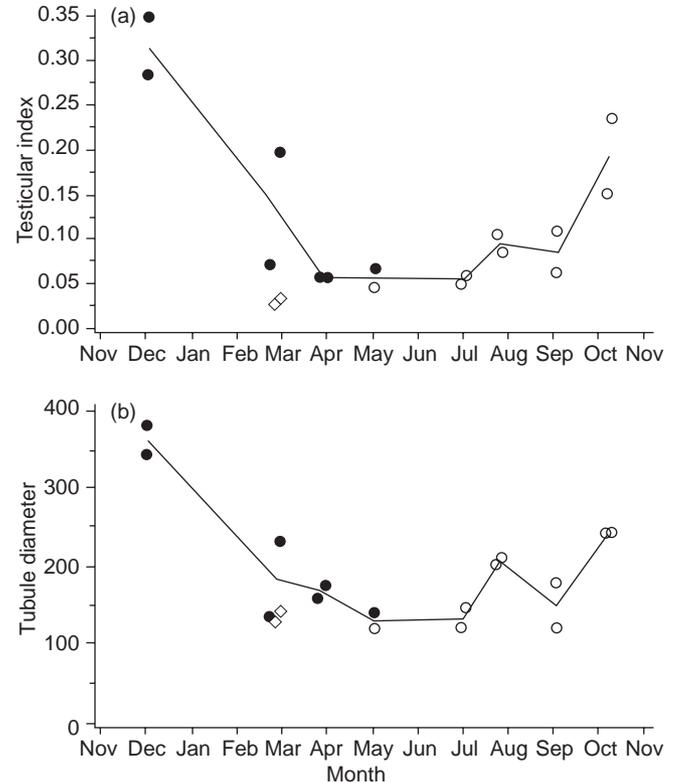


Fig. 2. Seasonal variation in (a) testicular index (combined mass of both testes expressed as a percentage of body mass) and (b) mean seminiferous tubule diameter of 18 male *Elseya dentata*. Individuals are characterized as either with sperm in their epididymes (●), without sperm (○) or immature (◇). The fitted line joins the mean values for each monthly sample.

Instead, the presence of an elongated tail, a well developed penis and some spermatogenic activity in the testis suggested that the smallest male was either mature or very close to the onset of maturity. The data from tail sizes (see Kennett, 1996) and histological examination of testes suggest that onset of maturity for male *C. rugosa* occurs at a CL of about 165 mm with lower and upper limits of 155 mm and 180 mm respectively. The age at maturity based on a von Bertalanffy growth curve derived from recapture data is 3.9 years (see Kennett, 1996 for methods).

Elseya dentata The spermatogenic cycle and seasonal variation in testicular index and mean seminiferous tubule diameter of *E. dentata* is similar to that of *C. rugosa* (Fig. 2a, b). Clear definition of the differences between the species is hampered by the small sample sizes, but it appears that gonial proliferation and germinal quiescence may occur about a month sooner in *E. dentata*. Ovulation and nesting by *E. dentata* are largely complete by the end of April compared to August for *C. rugosa* which may explain why spermatogenic activity ends sooner in *E. dentata*.

The occurrence of sperm in the epididymes of *E. dentata* was markedly seasonal. One of the males

captured in May and all of the turtles caught between June and October did not contain sperm in their epididymes. Sperm was present in all the mature males examined between December and May. Sperm was considered to be abundant in all these individuals except two examined in April where it was judged to be scarce in one and common in the other.

Sperm occurred in the epididymes of male *E. dentata* from December to May (Fig. 2a, b), hence the absence of sperm can be used to determine immaturity only during these months. The two smallest males (CL: 201.4 mm and 216.7 mm) examined were considered to be immature because they did not contain sperm in their epididymes in March and their testes were small in comparison with other males (Fig. 2a). The three next largest males examined in this period (CL: 244.3 mm, 246.3 mm and 249.0 mm) all contained sperm.

Pre-cloacal tail length in male *E. dentata* increased substantially after they achieved carapace lengths greater than 220 mm (see Kennett, 1996). These data from tail growth rates and histological examination of testes suggest that a carapace length of 220 mm is a reasonable estimate of the size at onset of maturity for male *E. dentata*. Age at maturity based on a von Bertalanffy growth curve derived from from recapture data is 8.6 years (see Kennett, 1996 for methods).

Ovarian cycles and size at maturity

Chelodina rugosa Ovarian follicles enlarge rapidly in December and January, coincident with the start of the wet season, and reach pre-ovulatory size by the end of January (Appendix 1). This rapid enlargement of follicles is illustrated by the sharp increase in the ovarian index between November and January (Fig. 3). Ovarian mass and the sizes and numbers of enlarged follicles varied substantially between individuals between March and July, reflecting the variability in clutch size. Ovarian masses are in decline by July and are at a minimum during the late dry season from September to November. Most of the follicles are small (diameter < 7 mm) and many of the larger follicles are undergoing atresia. The absence of enlarged follicles during this period and high rate of follicular atresia indicates that follicles that develop in one year are not retained into the next.

Most nesting activity occurs from February to July based on the presence of oviducal eggs and the capture rates of gravid females (Appendix 1, Fig. 4). There was some evidence that ovulation and nesting may continue longer in seasons when seasonal waterholes retain water for longer following high wet season rainfall. In July, August and September 1989, following above average rainfall in the preceding wet season (2100 mm, Bureau of Meterology, Darwin), 13% (*n* = 90), 6% (*n* = 50) and 5% (*n* = 18) respectively of captured females were gravid. Wet season rainfall in the following year was much lower (1522 mm, Bureau of Meterology, Darwin), only 2% (*n* = 55) of females captured in July were gravid

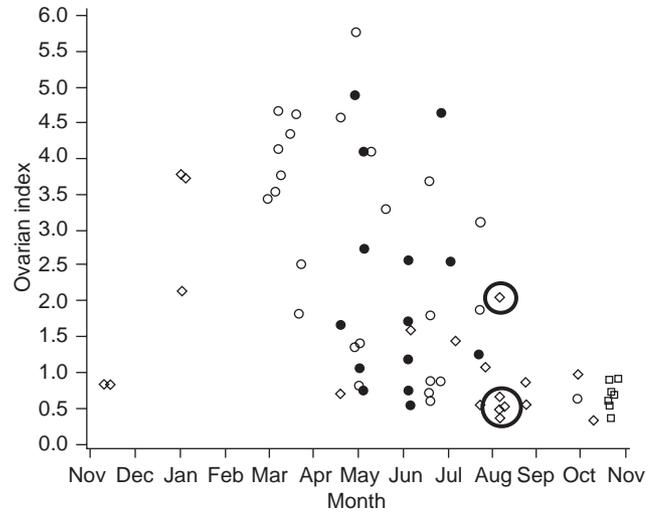


Fig. 3. Seasonal variation in ovarian index (mass of both ovaries expressed as a percentage of body mass) for 58 mature female *Chelodina rugosa*. Individuals with two sets of corpora lutea on their ovaries (●), individuals with one set of corpora lutea (○) and with no visible corpora lutea (◇). Individuals that were in aestivation at time of collection are circled. Additional data supplied by R. Pengilly on ovary size in seven aestivating turtles (see text) are represented by (□).

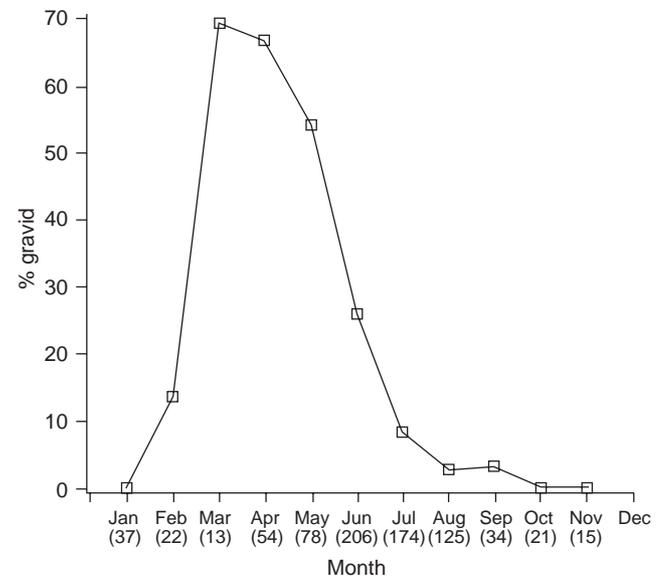


Fig. 4. Percentage of each monthly sample of mature female *Chelodina rugosa* that were gravid at time of capture. All locations are included and the number of mature-sized females examined per month is given in parentheses.

and no females captured in August (*n* = 56) or September (*n* = 16) were gravid.

Corpora lutea from a single female often varied in size but could usually be grouped into one or two distinct classes. With few exceptions the number of corpora lutea in the larger class (Class I) equalled the number of oviducal eggs in gravid females. Hence the

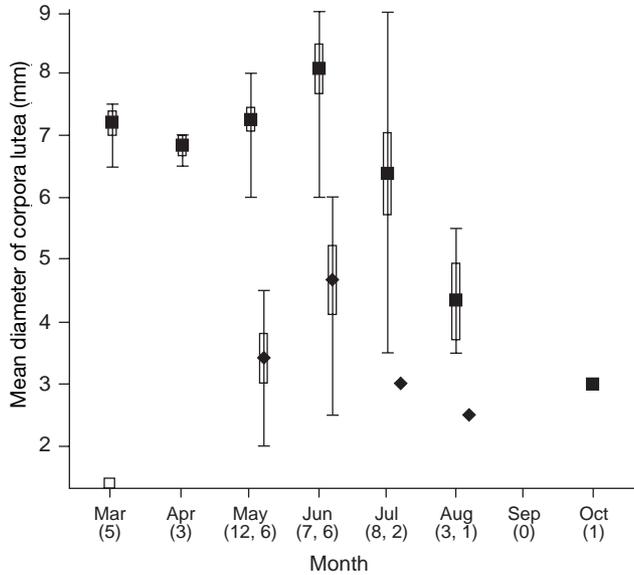


Fig. 5. Mean diameter of Class I corpora lutea (■) and Class II corpora lutea (◆) on the ovaries of *Chelodina rugosa* for each monthly sample. Mean, 1 SE error and range are plotted, and sample sizes are given in parentheses.

presence of a second smaller class of corpora lutea (Class II) in many individuals was taken to indicate the production of an earlier clutch (Appendix 1). The mean diameter of corpora lutea declined over the season (Fig. 5) and none were observed on the ovaries of females between November and January, indicating that corpora lutea from one season are not retained into the next.

Carapace length at onset of maturity was 210.5 ± 3.4 mm (range 204.4–213.5 mm). The corresponding ages predicted by a von Bertalanffy growth curve based on recapture data are 6.2 years with upper and lower limits of 5.6 years and 6.5 years (see Kennett, 1996 for method).

Elseya dentata Ovarian follicles began to enlarge after September and reached pre-ovulatory size by December (Appendix 2). The increase in follicle size and hence ovarian mass is reflected in the increase in the ovarian index between October and December (Fig. 6). Groups of pre-ovulatory follicles occurred in both the December individuals but the first evidence of ovulation is visible in one of the individuals examined in early February. This individual had recently ovulated 11 follicles to form a clutch of 11 partially-shelled oviducal eggs, which accounts for its substantially lower ovarian index. The second February individual contained 11 enlarged follicles that would have been ovulated shortly to form its first clutch of the season. These data suggest that the nesting season begins in February but the possibility of gravid females occurring in January cannot be ruled out because no turtles were captured or dissected.

Oviducal eggs occurred in dissected animals in February and March when 50% and 60% respectively

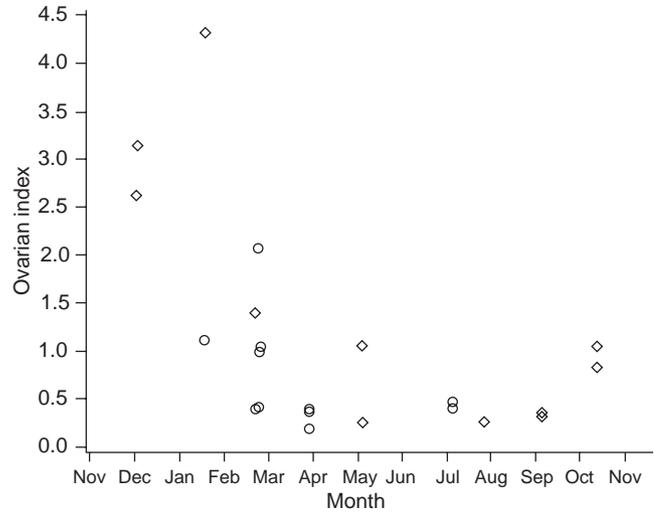


Fig. 6. Seasonal variation in ovarian index (mass of both ovaries expressed as a percentage of body mass) for 23 mature female *Elseya dentata*. Individuals with corpora lutea on their ovaries (○) and with no visible corpora lutea (◇).

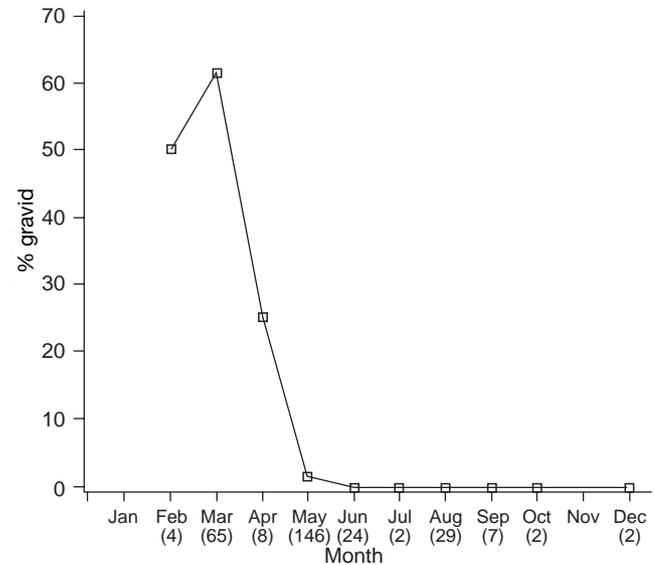


Fig. 7. Percentage of each monthly sample of mature female *Elseya dentata* that were gravid at time of capture. The number of mature-sized females examined per month is given in parentheses.

of captured females were gravid (Fig. 7). Twenty-five percent of females were gravid in April but the sample size was small ($n = 8$) and by May only two out of 146 turtles (1.4%) were gravid, indicating that nesting was largely complete. The ovarian index declined rapidly between February and April and follicular enlargement had ceased by April. The ovaries remain quiescent between April and September when most of the follicles are small (diameter < 7 mm) and many of the larger follicles are undergoing atresia (Appendix 2). The absence of enlarged follicles during this period and high rate of follicular atresia indicates that follicles

Table 1. Results of regression analyses of female carapace length against various clutch parameters of *Chelodina rugosa* and *Eelseya dentata*

Regression of female carapace length against	<i>Chelodina rugosa</i>	<i>Eelseya dentata</i>
Mean egg width per clutch	$r^2 = 0.15, n = 69, P < 0.001$	$r^2 = 0.23, n = 21, P < 0.05$
Mean egg mass per clutch	$r^2 = 0.10, n = 69, P < 0.01$	$r^2 = 0.06, n = 21, P = 0.14$
Mean egg length per clutch	$r^2 = 0.03, n = 69, P = 0.08$	$r^2 = 0.01, n = 21, P = 0.5$
Clutch size	$r^2 = 0.21, n = 140, P < 0.001$	$r^2 = 0.40, n = 41, P < 0.001$
Clutch mass	$r^2 = 0.37, n = 69, P < 0.001$	$r^2 = 0.64, n = 41, P < 0.001$

that develop in one year are not retained into the next.

There was never more than one size class of corpora lutea on the ovaries of *E. dentata* nor were there more corpora lutea than would be expected from the ovulation of a single clutch (Appendix 2), hence there is no clear evidence that *E. dentata* produce multiple clutches per year. The mean diameter of corpora lutea declined over the season from 12.5 mm ($n = 1$ turtle) in February, 8.5 ± 1.5 mm ($n = 5$) in March, 5.1 ± 1.6 mm ($n = 3$) in April and 2.0 mm ($n = 1$) in July. None were observed on the ovaries of females between August and December indicating that corpora lutea from one season are not retained into the next.

Carapace length at onset of maturity was 276.5 ± 6.3 mm (range 264.1–284.4 mm). Age at maturity based on a von Bertalanffy growth curve derived from recapture data are 13.5 years with upper and lower limits of 11.4 years and 15.4 years (see Kennett, 1996 for methods).

Clutch size, egg size and hatchling size

Chelodina rugosa Larger females produced larger clutches of larger eggs but the relationships between

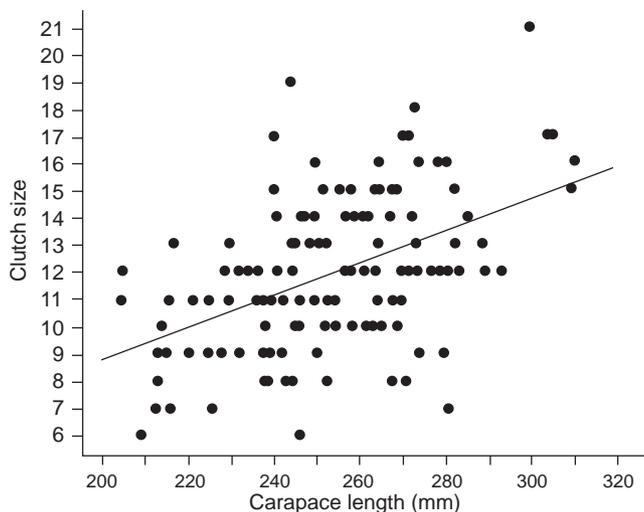


Fig. 8. Clutch size plotted against carapace length (CL) of *Chelodina rugosa*. The solid line shows the predicted clutch size from the equation; Clutch size = $0.06(\text{CL}) - 2.6$, ($r^2 = 0.21, n = 140, P < 0.001$).

Table 2. Mean dimensions (\pm SE and range) of the eggs and hatchlings of *Chelodina rugosa* and *Eelseya dentata*

	Eggs	Hatchlings
<i>Chelodina rugosa</i>		
	($n = 745$ eggs from 69 clutches)	($n = 75$)
Length	34.6 ± 0.07 mm (29.0–41.7)	Carapace length 32.2 ± 0.31 mm (27.3–36.9)
Width	26.9 ± 0.05 mm (24.0–30.2)	Head width 11.4 ± 0.07 mm (10.6–12.2)
Mass	14.9 ± 0.07 g (10.6–20.0)	Body mass 7.9 ± 0.17 g (5.1–11.2)
<i>Eelseya dentata</i>		
	($n = 232$ eggs from 21 clutches)	($n = 7$)
Length	51.5 ± 0.2 mm (41.5–59.4)	Carapace length 44.4 ± 1.44 mm (39.7–50.7)
Width	29.6 ± 0.07 mm (27.1–32.1)	Head width 12.7 ± 0.48 mm (11.3–14.8)
Mass	27.3 ± 0.02 g (19.9–34.8)	Body mass 12.2 ± 0.93 g (10.3–16.4)

female body size and clutch and egg parameters were highly variable (Fig. 8 & Table 1). Egg size was unrelated to clutch size ($r^2 < 0.01$ and $P > 0.3$ in each case). Clutch size is related to carapace length (CL) by the formula:

$$\text{Clutch size} = 0.06 (\text{CL (mm)}) - 2.6$$

$(r^2 = 0.21, n = 140, P < 0.001)$ (Fig. 8)

Clutch mass is related to CL by the formula:
Clutch mass (g) = $1.31 (\text{CL (mm)}) - 163$
 $(r^2 = 0.37, n = 69, P < 0.001)$

The eggs of *C. rugosa* are hard-shelled (calcareous) and ellipsoid in shape and varied substantially in size (Table 2). Heavier eggs produced heavier hatchlings ($r^2 = 0.66, n = 65, P < 0.0001$); hatchling dimensions are presented in Table 2.

The sizes of clutches and mean mass of eggs produced consecutively by the same female within a season (based on corpora lutea and/or oviducal eggs) or between years did not vary substantially (Table 3). Mean egg mass in two clutches produced by the one female in the same year differed by only 0.1 g, mean egg width by only 0.5 mm and mean egg length by only 0.3 mm. The maximum difference in clutch size was five eggs for one individual but was usually only one or two eggs. Variation in clutch size within a season was further examined

Table 3. Number of eggs and mean egg mass (g) in clutches of *Chelodina rugosa* eggs from the same individual

Carapace length at initial capture (mm)	Year of capture		
	1990	1991	1992
212.2	7 16.99	9 14.3	11 ^a –
224.4		9 15.0	8 14.0
238.1 ^b	8	7	
240.0 ^a		15	13
245.2 ^a		13	14
246.1	10 16.3	11 ^a –	
249.0 ^a		14	13
261.6		10	12
262.5		10	12 ^a
264.1 ^b		17.9	–
264.9 ^a	11	11	
268.6	10	10	
269.7 ^a	15.4	15.3	
272.2 ^b	14	14	
278.7 ^b	13	12	
282.4 ^b	16	11	
293.0 ^b		13	11
303.9	17 14.6		17 ^a

^a Number of eggs estimated from x-ray.

^b Number of eggs estimated from counts of corpora lutea from dissected ovaries.

by combining data from all years and dividing it into clutches laid early (before 1 May) in the season and clutches laid late in the season (after 1 May). In ANCOVA using season as a factor (early or late) and carapace length as a covariate to correct for differences in body size, season was not significant ($F=0.01$, d.f. = 2, 136, $P=0.98$), indicating that there was no significant difference in mean clutch size adjusted for body size between the first and second half of the season.

Elseya dentata Larger females produced larger clutches of larger eggs but the relationship between female size and clutch and egg parameters were highly variable and often non-significant (Table 1 & Fig. 9). Egg dimensions were unrelated to clutch size ($r^2 < 0.1$ and $P > 0.1$ in each case). Clutch size is related to carapace length (CL) by the formula:

$$\text{Clutch size} = 0.07 (\text{CL (mm)}) - 11.4$$

$$(r^2 = 0.50, n = 41, P < 0.001) \text{ (Fig. 9)}$$

Clutch mass is related to CL by the formula:

$$\text{Clutch mass (g)} = 2.98 (\text{CL (mm)}) - 615$$

$$(r^2 = 0.64, n = 41, P < 0.001)$$

The eggs of *E. dentata* are hard-shelled (calcareous) like those of *C. rugosa*, but are larger and more elongated in shape and yield larger hatchlings (Table 1).

Table 4. Number of eggs and mean egg mass (g) in clutches of *Elseya dentata* eggs from the same individual

Carapace length at initial capture (mm)	Year of capture		
	1990	1991	1992
277.0	8 25.89		9 25.1
283.4		9 25.69	9 ^a –
284.4		7 29.1	8 ^a –
300.5	10 23.5		10 27.9
304.0		16	13
320.2 ^a		22.5	21.1
		12	9 ^a

^a Number of eggs estimated from x-ray.

The sizes of clutches and of mean mass of eggs produced by the same female in successive years did not vary substantially (Table 4). Clutch size decreased by three eggs in two individuals, increased by one egg in one individual and did not change in a fourth individual.

Incubation of *E. dentata* eggs was largely unsuccessful. The majority of eggs developed an opaque white patch indicating that embryonic development had begun (Thompson, 1985) but only seven out of 205 eggs (19 clutches) completed incubation and hatched after 150 to 188 days (mean = 162 ± 7 days) to yield apparently normal hatchlings. For all other eggs the size of the patch did not increase and embryonic development appeared to remain arrested at this point. No embryo could be seen when eggs were candled nor could any be found when eggs were opened after about 190 days of incubation. Many eggs were mouldy or discoloured by this stage.

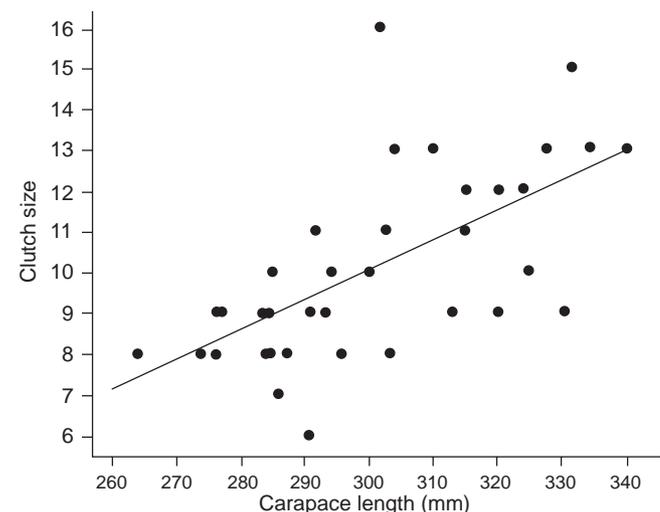


Fig. 9. Clutch size plotted against carapace length (CL) of *Elseya dentata*. The solid line shows the predicted clutch size from the equation; Clutch size = $0.07(\text{CL}) - 11.4$, ($r^2 = 0.5$, $n = 41$, $P < 0.001$).

Number of clutches per year and proportion of females reproducing each year

Chelodina rugosa The presence of more than one size class of corpora lutea and enlarged ovarian follicles regarded as being close to pre-ovulatory size on the ovaries of many individuals indicates that the production of multiple clutches by *C. rugosa* is common (Appendix 1). Females may lay up to three and possibly four clutches per year. One individual was captured twice at an interval of 1 month and induced to release a clutch of fully-shelled eggs on each occasion, giving an interval between clutches of approx. 1 month. The peak nesting period spans 4 months (Fig. 4) and if females can produce clutches as close as 1 month apart then four clutches per year is possible.

However, accurate estimates of the total number of clutches produced by an individual may be obscured by the length of the nesting season. Individuals examined near the start of the season may have gone on to enlarge and ovulate follicles that were overlooked at the time of dissection, leading to underestimates of total clutches. Moreover, corpora lutea from earlier clutches on the ovaries of females examined later in the season may have regressed completely also leading to underestimates of total clutch production. The possibility that evidence of earlier clutches was not visible in females examined late in the season is supported by the low estimates of total clutch production in July (1.5 ± 0.2 clutches, $n=8$ turtles) compared with estimates from earlier months (March 2.8 ± 0.2 , $n=6$; April 2.3 ± 0.33 , $n=3$; May 2.2 ± 0.2 , $n=11$; June 2.4 ± 0.2 , $n=7$). Discriminant analysis indicated that month was significant in determining the estimate of total clutch production ($F=4.749$, d.f. = 2, 33, $P<0.02$) but that carapace length was not ($F=0.076$, d.f. = 2, 32, $P=0.29$). Thus, although there was a slight trend for larger females to produce more clutches per season (one clutch, mean CL = 253.5 ± 8.1 mm, $n=8$ turtles; two clutches, mean CL = 257.1 ± 6.2 mm, $n=12$; three clutches, mean CL = 267.5 ± 4.5 mm, $n=15$) it was not significant and was possibly obscured by the effect of the month in which the female was examined.

Three lines of evidence indicate that all mature females lay eggs each year. Firstly, all females dissected during the peak breeding months of January to July ($n=42$) showed evidence of reproductive activity (i.e. oviducal eggs, corpora lutea or enlarged follicles) (Appendix 1).

Secondly, the high proportion of gravid females per capture sample in 3 successive months (Fig. 4) suggests that a high proportion of females in the population are producing eggs. The possibility that reproductively active females are more trap-prone than inactive females, thereby artificially inflating the proportion gravid, was tested by comparing sex ratios of adult turtles caught during the breeding season with the sex ratios of turtles caught outside the breeding season. The sex ratios did not differ from 1:1 in any of the three study populations ($\chi^2=1.3$; $\chi^2=1.0$; $\chi^2=0.2$, d.f. = 1,

$P>0.1$ in each case) indicating that capture rates of adult females and by inference, reproductively active females, were not greater during the breeding season.

Thirdly, one individual was captured with eggs in 3 consecutive years and eight individuals were captured with eggs in 2 consecutive years (Table 3).

Elseya dentata Unlike *C. rugosa*, the production of multiple clutches by *E. dentata* occurs rarely, if at all. Only one size class of corpora lutea was ever observed on ovaries (Appendix 2), and no individuals were found gravid twice in the same season. Circumstantial evidence suggests that some individuals may produce a second clutch. One female dissected in early March contained 10 oviducal eggs in addition to 11 enlarged ovarian follicles (18–21 mm) that may have been ovulated to form a second clutch. Three other turtles (one in February and two in March) with corpora lutea on their ovaries also had groups of enlarged follicles that may have gone on to form a second clutch. However, no data are available on the rate of follicular enlargement by *E. dentata* or the period between clutches, so multiple clutching by *E. dentata* remains unconfirmed.

Three lines of evidence indicate that all mature female *E. dentata*, like *C. rugosa*, lay eggs each year. Firstly, all females dissected between December and April showed evidence of reproductive activity: either oviducal eggs, corpora lutea or enlarged follicles (Appendix 2). Secondly, a high proportion of captured females were gravid in 3 successive months (Fig. 7), suggesting that a high proportion of females in the population are producing eggs. The sex ratio of captured adult turtles did not differ between breeding and non-breeding seasons ($\chi^2=2.2$, d.f. = 1, $P>0.1$) indicating that, as for *C. rugosa*, the high proportion of gravid females was not the result of a trap bias towards reproductively active females. Thirdly, four individuals were captured with eggs in 2 consecutive years (Table 4).

DISCUSSION

Reproductive strategies

Chelodina rugosa exhibits a suite of adaptations for life in productive but unpredictable seasonally ephemeral habitats. Most notable are the habit of nesting underwater (Kennett, Georges & Palmer-Allen, 1993b), and the ability to depress metabolic rate and thus conserve energy reserves during long periods of underground aestivation (Kennett & Christian, 1994).

Its reproductive strategy is also well suited to an unpredictable environment and can best be described as a 'scatter gun' approach in which many clutches and eggs with a variety of developmental 'trajectories' are produced over an extended nesting season. This diversity of developmental trajectories, generated by large intra- and inter-clutch variation in embryological development rate (Beynon, 1991) in multiple clutches laid over several months, ensures that some offspring hit the 'moving

target' of optimum conditions for hatchling emergence. Hatchlings presumably emerge at onset of wet season rains (Kennett, Georges & Palmer-Allen, 1993b), when dried waterholes adjacent to nesting areas refill and the hard mud is softened so hatchlings can escape the nest. However, the onset and duration of wet season rains are highly unpredictable, and can vary substantially from year to year (Taylor & Tulloch, 1985).

Delayed rains will extend the period that *C. rugosa* hatchlings must remain in nest. Late-term embryos may prolong their stay in their egg by entering a form of aestivation (Ewert, 1985, 1991; Beynon, 1991), but the costs of maintaining a fully developed embryo may be high and cannot be maintained indefinitely. In addition, a prolonged stay in the nest will consume yolk reserves that may be important for survival after hatching. Conversely, an early wet season may drown nests laid later in the season in which embryos have had insufficient time to complete development. By producing several clutches spaced at intervals over the season, with individual embryos developing at different rates, females can have offspring that are ready to emerge at different times, an adaptation against unpredictable timing in the start of the wet season.

Duration and quantity of wet season rainfall, and the period that habitats retain water may influence the length of the nesting season of *C. rugosa*. For example, gravid turtles were captured as late as August and September in 1989, following above average wet season rains. A longer nesting season potentially allows females to produce more clutches, but egg-laying must be completed in time to allow adults to accrue energy reserves as stored fat should a period of aestivation be necessary. There must also be sufficient time for embryos to complete development before the nest is flooded. Artificial incubation at 30°C takes between 81 and 140 days (Beynon, 1991), but the incubation period in natural nests is probably longer because temperatures remain low until the ground dries and may not reach 30°C until after 2 months of incubation (Kennett, Georges & Palmer-Allen, 1993b). Hence, eggs laid in August or September may not complete incubation if early wet season rains in November lead to early flooding. Nesting in sites that are still inundated late in the season could pose additional risks because such low-lying areas would also be the first to flood. There was no evidence from visual searches of nesting banks that turtles switched to terrestrial nesting late in the season.

Multiple clutching will also enhance reproductive success when nest and adult survivorship are low. Data on predation on *C. rugosa* nests are unavailable but predation on freshwater turtle nests is typically high, often in excess of 95% of nests being destroyed (Petokas & Alexander, 1980; Thompson, 1983; Congdon *et al.*, 1987; Robinson & Bider, 1988), and this is likely to be the case for *C. rugosa*. Underwater nesting may reduce the chances of terrestrial predators such as sand goannas (*V. panoptes* and *V. gouldii*) finding the eggs (Kennett, Georges & Palmer-Allen, 1993b), but water monitors (*V. mertensi*) can locate underwater nests (A. Dudley,

pers. comm.; G. Wallace, pers. comm.) and the remains of *C. rugosa* eggs were often found in their scats. Survivorship of adult *C. rugosa* is relatively low (0.59–0.63; Kennett, 1994) compared with most freshwater turtles (mean survivorship across eight species 0.88 ± 0.084 SD, Iverson, 1991), hence total longevity and reproductive longevity would also be expected to be correspondingly shorter. Laying multiple clutches each year will increase the probability of a successful reproductive event.

Eseya dentata occupies riverine habitats with permanent water, and thus it is buffered to some extent from the extremes of the tropical wet–dry cycle. Water level, turbidity and river flow in the Douglas River all increase during the wet and water levels drop again in the dry, but *E. dentata* rarely faces the complete loss of aquatic habitat experienced by *C. rugosa*. These influences have resulted in an ovarian cycle that is broadly similar in its timing to that of *C. rugosa* (Figs 3 & 6).

Like *C. rugosa*, *E. dentata* begin nesting at a time when wet season rains can lead to extensive flooding but flooding of high ground along the elevated levee banks of the Douglas River would be a relatively rare event (P. Caley, pers. comm.). Other high and dry ground would also usually be available within 100–200 m of the river (pers. obs.) and travelling 200 m overland to nest is well within the capabilities of most turtles. It seems likely, therefore, that conventional terrestrial nesting behaviour would be successful for *E. dentata*. Unfortunately there have been no observations on nest site selection by *E. dentata* or the capacity of *E. dentata* eggs to withstand immersion like *C. rugosa* eggs. Information from Aboriginal people (J. Roberts, pers. comm.) suggests that *E. dentata* nest on steeply sloping banks within 1–2 m of the water. Such sites would be inundated by relatively small rises in water level and the substrate in such close proximity to the river is often very damp. Given that Aboriginal knowledge regarding the apparently improbable nesting habits of *C. rugosa* proved correct (Kennett, Georges & Palmer-Allen, 1993b), an investigation of the nesting behaviour of *E. dentata* could be rewarding.

The timing of hatchling emergence to coincide with the start of the wet season is advantageous for *C. rugosa* as it would prevent hatchlings emerging at times or places where no water is available. Emergence of hatchlings of the tropical *Carettochelys insculpta* is also timed to coincide with wet season flooding (Webb *et al.*, 1986; Georges, 1987), and this may enhance hatchling survivorship in several ways. It would ensure that hatchlings do not emerge into dry pools and increase the chances of hatchlings entering turbid water where predators that rely on visual cues would have reduced success. Wet season flooding could also reduce effective predator density and enable hatchlings to disperse widely. These advantages would apply equally to *E. dentata*.

The rate of embryonic development in natural nests of *E. dentata* is unlikely to be faster than the 150–188 days incubation at 30°C in the present study, or the 160 days recorded by Legler (1980). For example, the maximum nest temperature recorded in November in fully exposed

C. rugosa nests was 32.4°C (Kennett, Georges & Palmer-Allen, 1993b), and it is unlikely that substrate temperatures in the riparian forest at the Douglas River, where *E. dentata* is likely to nest, would exceed this temperature. If so, then *E. dentata* must complete nesting no later than June or July for incubation to be completed before the onset of an early wet season in November. Nesting by *E. dentata* in the Douglas River is largely completed by the end of April but may possibly continue into June and July elsewhere in the Northern Territory (Legler, 1980).

The requirement of a lengthy incubation period may explain why *E. dentata* does not continue to nest further into the year like *C. rugosa* or to begin nesting in the late dry season like tropical *Emydura* species (Legler, 1985). Alternatively, if nesting by *E. dentata* is limited to the early dry season by other unknown factors, then the embryonic diapause (Ewert, 1985) that appears to occur in artificially incubated *E. dentata* eggs may be a mechanism to prolong incubation and ensure that hatchlings emerge at the start of the wet. The high egg mortality in the present study presumably occurred because eggs did not receive the appropriate environmental stimulus to restart development (Ewert, 1985, 1991). Arresting development at an early stage may be less costly energetically than late-term embryonic aestivation (Webb *et al.*, 1986; Beynon, 1991) because maintenance costs would be lower for the smaller embryo. A combination of early developmental arrest to slow development and late term aestivation (Ewert, 1985, 1991) in order to 'fine tune' the emergence period is also possible.

Gonadal cycles

The spermatogenic cycles of male freshwater turtles are presumably timed to maximize the availability of viable sperm at a time when their female conspecifics are ovulating. Amongst temperate species, ovulation and mating occurs in the spring (Australian chelids: Georges, 1983; Parmenter, 1985; Kuchling, 1988; other species: see Moll, 1979), but the timing of spermiation to coincide with spring ovulation is presumably prevented by low ambient winter temperatures that inhibit spermatogenesis (Ganzhorn & Licht, 1983). Instead spermatogenic activity is largely confined to the warmer months, beginning in spring, peaking in late summer, and sperm are released into the epididymes in late summer and autumn where they overwinter in preparation for a spring mating period.

In the tropical environment occupied by *C. rugosa* and *E. dentata*, rainfall rather than temperature is markedly seasonal but testicular cycles are remarkably similar to those of chelid turtles in temperate Australia. For *C. rugosa* and *E. dentata* peak testicular activity occurs in the wet season, equivalent to the temperate summer, and declines towards the end of the wet season, equivalent to the temperate autumn. Testicular activity ceases and testes remain regressed through the dry

season equivalent to the winter. Both testis size and seminiferous tubule diameter have been shown to be good indicators of spermatogenic activity in many species (Georges, 1983) and this is also the case for *C. rugosa* and *E. dentata*. Testes were smallest during periods of no activity, became enlarged as spermatogenic activity increased and regressed in size following spermiation (Figs 1 & 2).

The spermatogenic cycle typical of temperate species is described by Lofts (1978) as post-nuptial because spermatogenesis begins immediately after breeding. However, defining testicular cycles on the basis of their temporal relationship with the ovarian cycle may be too simplistic. A comparison of the reproduction of *Pseudemys* (= *Trachemys*) *scripta* in Panama and North America is a case in point. In both Panama and North America, male *P. scripta* follow a typical temperate zone testicular cycle, but in North America females ovulate in late April and into July, while in Panama ovulation begins in late December and continues to May (Moll & Legler, 1971). This shift in the female cycle without any change in the male cycle effectively alters the temporal relationship between spermiation and ovulation, such that what was classified as post-nuptial breeding in North America becomes pre-nuptial breeding in Panama.

The reproductive cycles of *C. rugosa* and *E. dentata* pose a similar problem. The annual timing of their spermatogenic cycles mirrors those of temperate-zone chelids but would be classified as pre-nuptial because spermatogenesis begins before breeding. Gonial proliferation in the testes and enlargement of ovarian follicles in the tropical species both begin at the start of the wet season and spermiation coincides with the start of ovulation. Mating was not observed in either species but presumably commences around the time of spermiation (January to March) and may continue longer in *C. rugosa* because ovulation and nesting continue further into the year. In many chelonians sperm occurs in the epididymes throughout the year (Moll, 1979; Georges, 1983) but the occurrence of spermiation before the next mating season (pre-nuptial pattern) in *C. rugosa* and *E. dentata* negates the need for long-term epididymal sperm storage. Hence no *E. dentata* and few *C. rugosa* contained sperm in their epididymes after July.

The ovarian cycles of *C. rugosa* and *E. dentata* follow a pattern typical of that described for temperate species in that they exhibit distinct phases of follicular enlargement, ovulation, oviducal period and quiescence (Figs 3 & 6). However, the timing of their ovarian cycles, with ovulation and nesting commencing in January and February, differs markedly from the spring ovulation and nesting that is typical of turtles in temperate Australia (Chessman, 1978; Georges, 1983; Parmenter, 1985; Kuchling, 1988). The rate of follicular enlargement in the tropical species is also faster, presumably aided by warm temperatures. In temperate species follicular enlargement is slowed by cold winter temperatures which lower metabolic rates and digestive efficiency (Georges, 1985).

This apparent dichotomy in the reproductive patterns

Table 5. Clutch sizes and frequency of reproduction of Australian chelid turtles

Species (location)	Zone	Clutch size	Number of clutches per year	Mean annual reproductive potential ^a	Source
<i>Rheodytes leukops</i> (Fitzroy River, Qld)	Tropical	18.8 (188 eggs in 10 clutches)	2–3	38–56	Legler & Cann, 1980
<i>Emydura krefftii</i> (Fitzroy River, Qld)	Tropical	16.4 (82 eggs in 5 clutches)	2–3	33–49	Legler and Cann, 1980
<i>Chelodina rugosa</i> (Darwin, NT)	Tropical	10 (at maturity) 15 (at full size) ^b	2–4	20–40 30–60	This study
<i>Elseya dentata</i> (Douglas River, NT)	Tropical	8 (at maturity) 12 (at full size) ^b	1, rarely 2 12	8	This study
<i>Emydura krefftii</i> (Fraser Island, Qld)	Sub-tropical	4 (at maturity) 10 (at full size) ^b	3	12 30	Georges, 1983
<i>Pseudemydura umbrina</i> (Perth, WA)	Temperate	3–5	1	3–5	Kuchling & Bradshaw, 1993
<i>Emydura macquarii</i> (Murray River, NSW)	Temperate	18.2 ± 6.3 SD ^c (n = 11, range 10–28)	2–3	36–55	Chessman, 1978
<i>Chelodina oblonga</i> (Perth, WA)	Temperate	8.2 ± 1.2 ^d (spring nests, n = 15) 4.0 ± 0.2 ^d (summer nests, n = 10)	possibly 2, 1 per nesting season	12 ^e	Clay, 1981
<i>Chelodina oblonga</i> (south-east WA)	Temperate	13–16 (n = 3)	2–3	25–40 ^f	Kuchling, 1988
<i>Chelodina oblonga</i> (Lake Joondalup, WA)	Temperate	7.3 ± 3.5 SD (n = 3, range 2–15)	NS	NC	Nicholson, 1975
<i>Chelodina steindachneri</i> (WA)	Temperate	7–8 (n = 4)	1	7–8	Kuchling, 1988
<i>Chelodina longicollis</i> (Armidale, NSW)	Temperate	14.0 ± 0.5 SE (n = 74, range 6–23)	1, occasionally 2	14–28	Parmenter, 1985
<i>Chelodina longicollis</i> (Murray River, NSW)	Temperate	13.9 ± 3.4 SD ^c (n = 14, range 7–18)	1–2	14–28	Chessman, 1978
<i>Chelodina longicollis</i> (Canberra, ACT)	Temperate	19 ^g (n = 15, range 13–24)	1	NC	Vestjens, 1969
<i>Chelodina longicollis</i> (Patho, Vic.)	Temperate	10 (n = 10, range 6–15)	NS	NC	Goode & Russell, 1968
<i>Chelodina longicollis</i> (Gippsland, Vic.)	Temperate	9.6 ± 1.4 SD ^c (n = 26, range 6–12)	1–2	10–20	Chessman, 1978
<i>Chelodina expansa</i> (south-east Qld)	Temperate	12.4 ± 3.2 SD ^c (n = 5, range 9–17)	NS	NC	Georges, 1984

^a Calculated as mean clutch size x estimated annual clutch production. Note: this may differ from original calculation by author.

^b Based on regression relationship between clutch size and body size.

^c Based on counts of oviducal eggs, enlarged follicles and corpora lutea.

^d Not stated if SD or SE.

^e Calculated as summer mean plus spring mean clutch size.

^f Based on counts of eggs and enlarged follicles for medium sized females.

^g Mode.

of Australian chelids has led Legler (1981, 1985) to classify them into two broad categories: a temperate pattern of spring ovulation and nesting, small eggs and short incubation times; and a tropical pattern of dry season ovulation and nesting, large eggs and long incubation times as exhibited by *C. rugosa* and *E. dentata*. The two patterns are said to reflect the regions in which the species first evolved. *Eelseya dentata* is postulated to have retained the tropical pattern as it moved into southern Australia and tropical *Emydura* species are thought to have retained their temperate ovarian cycle. *Chelodina expansa*, a species that is similar morphologically to *C. rugosa*, is believed to be a recent arrival to the temperate zone which has retained its tropical reproductive cycle (Legler, 1985). This phylogenetic interpretation of reproductive patterns has some attraction and may help to explain why *C. expansa*, in the Murray River in New South Wales, nests at the seemingly inappropriate time of autumn and winter (Chessman, 1978; Georges, 1984). Developing embryos must survive long periods of low temperature in winter before development can recommence in the spring, and incubation normally exceeds 324 days (Goode & Russell, 1968).

However the reproductive cycles of many Australian turtles are poorly known, and the generalization of two reproductive patterns may overlook the importance of local environmental conditions in shaping reproductive strategy. For example *C. novaeguineae*, occupying semi-permanent waterholes near Daly Waters in the Northern Territory, lays the relatively small eggs typical of the temperate pattern but has the nesting season and long incubation period typical of the tropical pattern (Kennett *et al.*, 1992). These data suggest that the reproductive pattern of *C. novaeguineae* may have changed as it moved north from its hypothesized evolution in southern Australia (Legler, 1981, 1985) and that phylogenetic conclusions based on reproductive patterns may be unsound.

Comparisons with other Australian chelids

The reproductive biology of many Australian chelids, especially tropical species, is poorly known. Generally, tropical species lay more and bigger clutches and *C. rugosa* fits this pattern, reproducing every year and laying multiple clutches (Table 5). Its nesting season may span up to 8 months, which is considerably longer than those of other chelids (see Legler, 1985 for a review), and its mean annual reproductive potential of 40 eggs for small females and 60 eggs for large females is amongst the highest recorded for Australian chelids (Table 5). *Chelodina rugosa* also grows faster and matures at an earlier age than temperate chelids (Table 4; Kennett, 1996) but comparable data from tropical species are limited to *E. dentata*. The rapid growth and high fecundity of *C. rugosa* are presumably fuelled by the high productivity and high food availability of its seasonal floodplain habitat. Similarly, habitat unpre-

dictability and comparatively high mortality rates would offer strong selection for rapid growth.

In contrast, the annual reproductive output of *E. dentata* is substantially lower and it matures at almost twice the age of *C. rugosa* (Kennett 1996; Table 4). Its eggs and hatchlings are larger (Table 2) but it produces smaller clutches and only one clutch per year giving it an annual reproductive potential that is one-third to one-fifth that of *C. rugosa* (Table 5). Legler (1980) also reported that *E. dentata* in the Northern Territory rarely produce a second clutch. The slower growth and lesser fecundity of *E. dentata* may reflect a low quality diet that consists primarily of leaves, algae and windfall fruits (Kennett & Tory, 1996). It relies largely on allochthonous input for food, suggesting it occurs in environments that are not very productive, certainly less so than the ephemeral swamps occupied by *C. rugosa*. Because of its low protein diet *E. dentata* may be unable to accumulate sufficient energy reserves within the space of one season to produce two clutches. Enlarged follicles remaining at the end of one breeding season could not be used to form an additional clutch in the following season because they became atretic and were resorbed.

The slow growth and low reproductive effort of *E. dentata* are even more remarkable when compared with temperate chelids. Its activity, feeding and digestion and presumably growth are not constrained by cold winter temperatures yet it matures at the same age as chelid turtles of similar size in the Murray River (Kennett, 1996). It only produces one clutch per year whereas many temperate zone species produce multiple clutches (Table 5).

Acknowledgements

This study was part of my doctoral research at the University of Queensland. I thank Arthur Georges and Keith Christian for guidance and advice during this study. Rick Shine, John Iverson, J. Whit Gibbons and Peter Whitehead provided comments on the manuscript. I thank Roger Haensel for x-raying hundreds of turtles and Dave Pritchard and Cheryl Hay for sectioning the gonad samples. Helmut and Hardy Schimmel and Brian McWilliam provided local advice and access to waterholes. Many volunteers helped catch many turtles, especially Kristin Tidemann and Meredith Anderson. The Northern Territory University Animal Experimentation Ethics Committee and the Conservation Commission of the Northern Territory provided permits. CCNT, The Peter Rankin Trust Fund for Herpetology and the Northern Territory University provided financial and material support for the study.

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Appendix 1. Seasonal occurrence of ovarian follicles, corpora lutea and oviducal eggs in *Chelodina rugosa*. Rectangles enclose follicles or corpora lutea believed to be associated with a clutch of eggs that was past or pending at time of dissection.

Month	Size classes of ovarian follicles (mm)									Corpora lutea		Eggs	Clutches per year ^b
	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	20-21	I (n, x) ^a	II (n, x) ^a		
Jan	12 (2)	9	6	8	7	2	15	6					
Jan	5 (1)	8 (1)	6	8	6	10	1						
Jan	13 (1)	9	6	8 (2)	8	4	11	1					
Mar	20 (1)	9 (1)	7	4	4	5	2	11		10, 7.5		10	3
Mar	15	2	9	1	2	5	3	9		11, 7.5		11	3
Mar	6	5	4	5	7	8	11			9, 6.5		9	3-4
Mar	15	8	2	2	3		11			2, 7.5		12	2
Mar	2	2 (1)		(1)	1	10 (1)	13	8		15, 7.0		15	3-4
Mar	6	6	2		5	5	6	6		7		7	3-4
Apr	7	4		1		1	9			9, 7.0		9	2
Apr	14	8	12	1	4	9	6			18, 7.0		12	2
Apr	23	17	10 (1)	2		18	13	(1)		15, 6.5		14	3
May	4	5	3		1				13	13, 7.0	+, 2.5	13	3
May	6	1	6	1		1				13, 7.5	12, 4.0	12	2
May	12	2		2 (1)		3	1	8		14, 8.0	14, 4.0	14	3
May	30	12	11	10 (1)	10	9	5	20		17, 8.0		16	3
May	11	10	4	5		3	1	9	7	12, 7.5	+, 3.5	12	3
May		2		4	3	1							
May ^c	3	2	3	1	4	3	1			13, 7.5		7	1
May	17 (1)	6	2	3	1	6		9 (1)	3	12, 7.5		12	2
May		(2)	(1)	1	2	1				16, 7.0		16	1
May	12		1	2	1	3	1			16, 7.5	11, 4.5	13	2
May	(2)	(2)	2	1 (2)	2 (1)	5	1 (1)			11, 6.0		10	1
May	19 (1)	8 (1)	6	11	4	3	3	18		12, 7.5		12	2
May	7	3	6		1	16				12, 6.0	+, 2.0	12	3
Jun	1	1 (1)	2	1	2	8	1			10, 8.5	12, 5.0	10	3
Jun	3		1	4	3	2	1			11, 9.0	11, 6.0	11	2
Jun	6				3		2	9		10, 7.5	4+, 6.0		3
Jun	11 (2)					3	5			13, 9.0	11, 4.0		3
Jun	17	6	3	2 (1)	4 (2)	2	11			9, 8.0		9	2
Jun	1	(1)	(1)		(1)					10, 6.0	+, 2.5		2
Jun		2	1 (1)	(1)						8, 8.5	7, 4.5	8	2
Jun ^d	6	8	11			14	2						
Jul	21	(3)	2			6							
Jul	2	3	4			1				15, 7.0		15	1
Jul	31	7	4			2	8			13, 9.0	+, 3.0	13	2
Jul	21	8	5	2	1	2		21		15, 8.0	+, 3.0	15	3
Jul	16 (6)	(2)				3			10	11, 6.0		11	2
Jul	1						1			9, 7.5		9	1
Jul	29 (1)	2			1		8			9, 4.5			1
Jul	7 (3)	2	2	2 (1)	4	(1)				+, 3.5			1
Jul	1	1	1	1	1				(1)	8, 5.5			1
Aug	6	2		1	1	1	9 (3)			+, 3.5			1
Aug	(1)	(2)	1 (8)				8	4		5+, 4.0			1
Aug			4		3	2	2			10, 5.5	+, 2.5		2
Aug	8 (2)	5 (4)	5 (5)	9 (2)	4								
Aug ^e	3	6 (3)											
Aug	(2)	2											
Aug	1	1	6	7									
Aug	1 (2)	1 (3)	3	3 (2)									
Aug ^f		5				7	9						
Aug	12 (4)												
Sep	5 (3)	8								+, 3.0			
Sep			(2)	(12)									
Oct	2	2	3										
Oct	(4)	(3)	(1)	(1)			(4)						
Oct	(9)		(1)										
Nov	9 (3)	8											
Nov	12 (1)												

^an, number present (+ present but uncounted); x, mean diameter.

^bEstimated clutches per year based on counts of enlarged and pre-ovulatory ovarian follicles and corpora lutea.

Appendix 2. Seasonal occurrence of ovarian follicles, corpora lutea and oviducal eggs in *Elseya dentata* from the Douglas River. Rectangles enclose follicles or corpora lutea believed to be associated with a clutch of eggs that was past or pending at time of dissection

Month	Size classes of ovarian follicles (mm)											Corpora lutea (n, x) ^a	Eggs	Clutches per year ^b	
	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	20-21	22-23	24-25				26-27
Feb	17	10	7	6	13								11, 12.5	11	2
Feb	17	8	7	5	1					11					1
Mar	23 (3)	2	2	1	6	9							11, 6.0		2
Mar	19	2	3	7	10								11, 10.0	11	2
Mar	21	13	13	4									8, 6.5		1
Mar	11	3	4	1	2	2	5	6					10, 10.0	10	2
Mar	14					1	8								1
Mar	9	(2)		(2)			(1)						7, 9.5	6	1
Apr	42	6	1										11, 7.0		1
Apr	19												10, 3.5		1
Apr	34	8	(3)										11, 5.0		1
May	11	1	(2)	(1)	(6)	(7)									
May	13			(1)											
Jul	12			1 (1)	1 (1)								+, 2.0		1
Jul ^c	7 (2)		2										+, -		1
Aug	7 (1)	(1)													
Aug	4														
Sep	16 (2)	(3)	2												
Sep	11 (2)	2													
Oct	14	10	14	3											
Oct	1	3	7	7	(10)										
Dec	55	12 (2)	9 (1)	1	2	1	1	1	6	9 (1)					2
Dec	13	9	17	(1)							5	7			1

^an, number present (+ present but uncounted); x, mean diameter.

^bEstimated clutches per year based on counts of enlarged ovarian follicles (≥ 12 mm) and corpora lutea.

^cSingle egg remained in left oviduct, passage of egg was prevented by constriction in oviduct.