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Underwater Nesting by the Tropical Freshwater Turtle, *Chelodina rugosa* (Testudinata : Chelidae)

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Abstract

Nests of the northern long-necked turtle, *Chelodina rugosa*, were located by surgically implanting radio-transmitters in the oviducts of gravid females so that the transmitters were deposited in the nest with the eggs. Nests are excavated in soft substrate under shallow water in the littoral zone of seasonally flooded billabongs during the wet and early dry season. Embryonic development remains arrested until floodwaters recede in the dry season and the ground dries. Hatchling emergence presumably coincides with heavy rain or flooding at the beginning of the following wet season.

Introduction

The northern long-necked turtle, *Chelodina rugosa* (Chelidae), is widely distributed throughout the wet–dry tropics of northern Australia, inhabiting freshwater swamps, billabongs and slow-flowing rivers (Cogger 1975). The climate throughout its range is markedly seasonal, with the monsoonal 'wet' season lasting from about December to April, with little or no rain in the intervening 'dry' season.

The highest densities of long-necked turtles in the Northern Territory occur on seasonally covered floodplains (Finlayson *et al.* 1988). These floodplains have a pronounced seasonal cycle of inundation with extensive flooding over thousands of square kilometres during the wet season. Most of this area dries completely during the subsequent dry season (Finlayson *et al.* 1988). Populations retreat to more permanent waters but most of these refuges will dry, especially in years of low rainfall. Turtles in these waterholes survive by aestivating buried under the drying mud (Grigg *et al.* 1986).

Despite its abundance and the ease with which large numbers can be collected (R. Kennett, personal observation), *C. rugosa* has been little studied. Anecdotal evidence has suggested a unique nesting behaviour. While Aboriginal people do not actively search for the nests of long-necked turtles as they do the nests of other species, according to aboriginal lore *C. rugosa* bury their eggs in the ground under shallow water at the end of the wet season (J. Roberts, personal communication). Such nests would be well camouflaged and this may explain why visual searches of waterhole surrounds over two years failed to locate any nests even though gravid turtles were regularly caught in baited traps in the same waterholes.

In this study we employed a novel approach to radio-tracking in order to locate the nests of long-necked turtles. Radio-transmitters were surgically implanted in the oviducts of gravid turtles so that they would be deposited in the nest with the eggs and so reveal the nest's location. We discuss the species' nesting strategy in the context of the capriciously variable habitats exploited by the species.

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Materials and Methods

Study Site

Ironstone Lagoon is a semi-permanent waterhole near Darwin, Northern Territory, Australia (130°56′E.,12°26′S.). *Chelodina rugosa* is the only turtle species present. The lagoon follows a typical seasonal inundation cycle, with high water levels during the wet season and large areas that often dry completely during the seasonal drought (Finlayson *et al.* 1988). The waterhole contains a mixed community of emergent aquatic vegetation but there is no submergent vegetation.

Capture of Turtles

Seventeen gravid turtles were captured in baited traps in May and June 1991. The presence of eggs was determined by palpating the abdomen through the inguinal pocket. Turtles were individually marked by a unique combination of notches filed in the edge of the shell. Turtles were held in large aquaria without feeding for about 10 days to ensure that eggs would be fully calcified prior to surgery.

Additional data presented here on egg size, clutch size and oviducal period are derived from a broader study on the reproductive biology of *C. rugosa*.

Surgical Procedures

All surgical procedures were conducted in accordance with the NHMRC/CSIRO Code of Practice for the Care and Use of Animals in Research in Australia by a qualified veterinary surgeon (D.P.). Dose rates of anaesthetic were obtained from ACCART News (Arena and Richardson 1990). Turtles were given anaesthetic pre-medication by intramuscular injection of $0\cdot5$ mg kg^{-1} diazepam ('Vallium', Roche Products Pty Ltd) and anaesthesia was induced with an initial 75 mg kg^{-1} ketamine hydrochloride ('Ketamav', Mavlab Pty Ltd) injected intravenously via the jugular vein. Deep surgical anaesthesia was deemed to have been induced by the absence of corneal reflexes or cloacal sphincter reflex. If deep anaesthesia was not attained within 45 min, additional ketamine hydrochloride was administered. Four turtles failed to achieve deep anaesthesia after 3 h and operations were not performed.

The skin of the inguinal pocket was cleansed with a 1:500 solution of poly-phenolic disinfectant ('Inhibac', Ecolab Pty Ltd) and sterilised with tincture of iodine (Sigma Co. Ltd). Subcutaneous tissue was infiltrated with lignocaine hydrochloride ('Lignomav', Mavlab Pty Ltd). An incision, approximately 3 cm long, was made through the skin and abdominal muscle, and a portion of the oviduct containing an egg was gently exteriorised. A longitudinal incision was made in the oviduct, a prosthetic egg containing a transmitter was inserted towards the cloaca, and the oviduct wall, muscles and skin were sutured separately. In most cases an egg was removed to accommodate insertion of the transmitter. The wound was sprayed with an antibacterial aerosol ('Centrigen'. Vasco Animal Health Laboratories).

Postoperatively, turtles were held in individual plastic tubs in moist conditions until they became active and their skin wounds healed. One turtle died during recovery and in another the transmitter was expelled during recovery, presumably consequent to being inserted too far toward the cloaca. Eleven turtles were released at the lagoon, 4–13 days after surgery, when they had proved capable of normal swimming in a test tank.

Radio-telemetry

Transmitters were single-stage (Model SS-1, Biotrack) with an effective battery life of 12 weeks. Transmitter and aerial were wrapped in plastic parafilm and sealed in paraffin wax. The package was roughly spherical with a diameter of 20 mm, compared with a mean egg diameter of 26 mm ($n = 102$). A radio-receiver (Telonics, Arizona, U.S.A.) was used to locate the transmitters, which had a range of approximately 50 m when placed on the ground surface.

The approximate position (to ± 1 m) of each transmitter was located daily. Turbid water and dense vegetation meant that turtles were rarely sighted and transmitters were never located on dry land, hence we cannot rule out the possibility that a transmitter had already been removed from a nest and was inside a predator such as a water goanna, *Varanus mertensi*. When a transmitter did not move either between or during daily observations, a short wand antenna or cable without antenna was used to pinpoint its exact location. Attempts were made to recapture all turtles after 4 weeks from release whether they retained their transmitter or not. All recaptured turtles were X-rayed (Gibbons and Greene 1979) to determine whether any eggs had been retained.

Nest Data

Once a transmitter was determined to be in a nest, the site was tagged and protected from predators with a wire mesh cage. A type 'K' thermocouple (30 gauge) was inserted into the nest amongst the eggs when the nest was first discovered. Hourly nest temperatures were recorded over a 24-h period at monthly intervals with a Grant data logger.

Results

Location of Nests and Transmitters

Within a week of release at the lagoon two turtles deposited eggs and transmitter in nest chambers dug in flooded ground. The first nest, containing 8 eggs, was located under a clump of *Eleocharis sundaica*, 15 cm below water level and 17 m from the water's edge. The second nest, containing 7 eggs, was located under *Persicaria attentuata*, 22 cm below water level and 14·5 m from the water's edge. Rainfall between May and August was only 1·0 mm and no rain fell between the time the turtles were released and their transmitters were located in the nests, ruling out the possibility that nests were constructed in dry ground and became inundated by rising water levels during the 24 h between location checks.

Six other transmitters were recovered from under water ranging in depth from 20 cm to 2 m. One transmitter in 20 cm of water had a single egg nearby. These transmitters were not in nest holes and presumably had either been jettisoned by the turtle in response to the surgery or discarded by a predator after it raided the nest.

One transmitter presumably failed and was never recovered. This turtle was recaptured in the following nesting season and X-rayed to reveal 13 recently ovulated eggs. Two turtles that still contained transmitters 6 weeks after release were recaptured and subjected to autopsy. Oviducal adhesion had prevented the release of the transmitters and, in one turtle, six eggs.

Four of the eight turtles that were recaptured within three weeks after they released their transmitter were gravid. Two of these gravid turtles were the individuals that had nested. It is unlikely that these eggs were a subsequent clutch as the numbers of eggs (1, 3, 4 and 6 eggs) were much smaller than the mean clutch size of 12 and there was insufficient time between release and recapture for eggs to develop. As was the case for the autopsied turtles, scarring of the oviducts may have hindered release of some of the eggs but, at least in two cases, did not prevent nesting.

Details of Nests and Embryonic Development

Surface water receded from one nest by 23 June 1991, two weeks after it was constructed, and the nest was excavated one week later while the substrate was still damp. No nest chamber was apparent, as spaces between eggs were presumably filled by sediment settling after egg-laying, and the 8 eggs were encased in drying mud. The uppermost and lowest eggs were 6 cm and 15 cm from the surface, respectively. Absence of an opaque patch on the eggshell surface indicated that embryonic development had not commenced in any of the eggs (Thompson 1985). The nest and thermocouple were restored, and the nest was re-excavated and all eggs opened on 13 November, 20 weeks later. All but one egg contained live, apparently normal, late-term embryos (Yntema 1968: stages 23–27, modal stage 24, $n=7$).

Surface water receded from the other nest by 25 July 1991, four weeks after it was constructed. Three eggs were removed and opened on 20 November, 16 weeks after the surface water dried. All contained live, apparently normal, late-term embryos (Yntema 1968: stage 26, $n=3$). The four remaining eggs were left in the nest to determine when hatchlings emerged. The nest was excavated entirely on 28 January 1992, well after rising wet-season water levels would normally have flooded the ground. Despite some heavy rainfall which had softened the ground, wet-season rains were the lowest on record and the nest site was still above water. Although we are unable to confirm it, we assume that the hatchlings had escaped the nest because only broken egg shells remained.

Temperatures in both nests increased during the course of incubation, coincident with the ground drying as the water levels fell. Mean nest temperatures (averaged over 24-hourly readings) for one nest were 23·8°C (daily range 22·7–25·1°C) in August, 28·8°C (daily range 27·0–30·1°C) in September and 31·0°C (daily range 29·8–32·4°C) in November.

A further ten nests from the 1991 nesting season were located in early January 1992 in an area that was sodden but not yet covered with surface water. All had been recently excavated by predators, probably a goanna, and eggshell remains were scattered in and around the nest holes. Whether the nests were excavated before or after the hatchlings had escaped was not known. This area was covered with water for the entire 1991 nesting season (February to August, on the basis of captures of gravid females) so these eggs could have been laid only under water.

Discussion

Despite their success in exploiting a diverse range of aquatic environments, turtles remain tied to dry land for reproduction. All turtles nest on land, typically excavating a flask-shaped nest chamber which is then covered once the eggs are laid (Packard and Packard 1988). However, our results demonstrate that *C. rugosa* lays its eggs underwater, providing the only known exception to this general rule. Our results also highlight the value of traditional knowledge held by Aboriginal people, who already recognised this unique underwater nesting behaviour.

Although six turtles either failed to nest or we failed to locate their nest before a predator, there is no doubt that the two turtles that successfully nested, deposited their eggs in nest holes constructed in ground that was flooded at the time of nesting. Additional anecdotal information supports our results: *C. rugosa* was observed laying eggs in a flooded nest hole (L. Moyes, personal communication), and 10 submerged nests were located by Aboriginal people beside the Reynolds River in Litchfield National Park (D. Petherick, personal communication). On two occasions goannas were observed removing turtle eggs from submerged nests (A. Dudley and G. Wallace, personal communication).

On the basis of our results and those of other incubation experiments (Beynon 1991; Kennett *et al.* 1993) we suggest the following nesting scenario for *C. rugosa*. Eggs are deposited in nest holes dug under shallow water in the littoral zone of flooded waterholes. Embryos survive this period of immersion in a state of arrested development, and embryonic development recommences when floodwaters recede and the ground dries. Eggs of *C. rugosa* survive immersion for up to 12 weeks under experimental conditions but die if immersed after embryonic development has commenced (Kennett *et al.* 1993). How the eggs survive in waterlogged conditions that would ordinarily kill reptile eggs (Plummer 1976; Ewert 1985; Packard and Packard 1988) is unknown. Embryonic development proceeds during the dry season and may accelerate towards the end of incubation as nest temperatures increase. Embryos that complete incubation before the start of the next wet season may delay hatching by entering embryonic aestivation (Ewert 1985; Beynon 1991). Unlike most other turtle nests (Packard and Packard 1988), *C. rugosa* nests do not appear to have spaces between the eggs because eggs become encased in hardened mud as the ground dries. Substantial rain is probably required before hatchlings can break free of their egg and emerge from the nest. This may enhance survivorship of hatchlings as it reduces the chance of them emerging while the waterhole is still dry.

Underwater nesting may have evolved in response to the unpredictable availability of 'dry' nest sites in seasonally ephemeral aquatic environments. Tropical freshwater turtles typically have an extended nesting season in comparison with temperate species and may even nest all year round (Moll 1979), but the nesting season of *C. rugosa* is limited by the extreme seasonality of its habitat. During the mid-to-late dry season, water levels drop rapidly and reproductive activity is curtailed as turtles seek refuge in aestivation sites. During the wet season, on the other hand, torrential rain, rapidly rising water levels and extensive flooding limit the availability of dry nesting sites. Eggs laid in dry ground at this time will perish if rising waters flood the nest after development has begun and both the timing and extent of rainfall can vary substantially from year to year (Taylor and Tulloch 1985). Delaying nesting until the rains finish and water levels stabilise and begin to fall may mean a delay of several months and losing the chance of allocating to reproduction energy reserves obtained when food is readily available. Turtles that nest in flooded ground during the wet season, on the other hand, can exploit a longer nesting period, convert wet-season energy reserves directly into reproduction and potentially produce more clutches per year.

Our novel approach to radio-tracking proved successful in locating turtle nests. Only one animal died as a result of the operation and one turtle was recaptured in the following nesting season with a full complement of recently ovulated eggs. Refinement of the surgical procedures may reduce the chances of oviducal scarring. Oviducal adhesion prevented the release of the transmitter in two turtles and may have hindered oviposition by several others. Smaller transmitters requiring smaller incisions or insertion of the transmitter into the oviduct using a laparoscope may be preferrable. With minor modifications the procedure could be used on other oviparous reptiles with cryptic nesting behaviour.

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