First record of hatchling overwintering inside the natal nest of a chelid turtle

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Abstract. Hatchling overwintering inside the natal nest is a strategy used by several Northern Hemisphere species of freshwater turtles. We recorded hatchling overwintering in the nest by \textit{Chelodina longicollis} (Chelidae) in south-eastern Australia, during three reproductive seasons. Hatchlings spent, on average, 320 days inside the nest from the date eggs were laid until emergence. Some nests were carefully opened adjacent to the nest plug, one during winter and one in spring, to confirm that eggs had hatched and were not in diapause, although we could not precisely confirm hatching dates. Despite our small sample size, we observed a dichotomous overwintering strategy, with hatchlings from one nest emerging in autumn and spending their first winter in the aquatic environment, and hatchlings from three nests overwintering in the nest and emerging in spring. These findings expand the phylogenetic range of turtles exhibiting hatchling overwintering behaviour. Future research should evaluate whether this strategy is widespread among other long-necked turtles in temperate regions and examine physiological mechanisms involved in coping with winter temperatures.

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Introduction

Time of emergence from the nest has profound ecological and evolutionary implications for egg-laying species, including freshwater turtles (Gibbons and Nelson 1978; Costanzo \textit{et al.} 2008; Gibbons 2013). Delayed emergence has been hypothesised as one of the strategies used by turtles to better coincide emergence with resource availability (Gibbons and Nelson 1978; Mitchell 1988; Costanzo \textit{et al.} 2008; Buhlmann \textit{et al.} 2009). The delay can be of a few days to several months after hatching (Wilson \textit{et al.} 1999; Gibbons 2013; Lovich \textit{et al.} 2014; Riley \textit{et al.} 2014). Hatchling overwintering in the nest, also known as terrestrial aestivation, is one type of delayed emergence in temperate areas (Gibbons 2013), where hatchlings spend winter inside the natal nest and emerge the following spring (Costanzo \textit{et al.} 2008; Gibbons 2013). Another strategy used by some species is to emerge from the nest in the autumn, but hatchlings overwinter on land in refuges before reaching the wetland in spring (Muldoon and Burke 2012; Paterson \textit{et al.} 2012).

Overwintering in the nest has costs and benefits. Direct costs include nest mortality from freezing, flooding, predation, dehydration and energy depletion arising from extended duration of nest occupancy (Gibbons and Nelson 1978; Packard 1997; Costanzo \textit{et al.} 2008). Benefits include accelerated growth by timing emergence with an environment in which thermal and food resources are increasing rather than decreasing (Gibbons and Nelson 1978; Costanzo \textit{et al.} 1995, 2008). Hatchling overwintering in the nest has been mainly observed in turtle species in the Northern Hemisphere, especially in the family Emydidae (reviewed by Gibbons 2013), with only one record of an emydid turtle living in the Southern Hemisphere (Bager \textit{et al.} 2007).

Long-necked turtles belong to the family Chelidae, which occurs in Australia, South America, New Guinea, Timor Leste and the Indonesian island of Roti (Georges and Adams 1992; Seddon \textit{et al.} 1997). \textit{Chelodina longicollis} is a common long-necked turtle with a broad geographic distribution in south-eastern Australia, occurring in a wide range of permanent and ephemeral freshwater habitats (Kennett \textit{et al.} 2009). \textit{C. longicollis} mates in the austral autumn (April) and early spring (September), lays eggs in late spring and early summer (October–January), and emerges from nests in autumn (April–May) (Vestjens 1969; Parmenter 1985). Delayed emergence has been reported for some turtle species in Australia (Goode and Russell 1968; Kennett \textit{et al.} 1993\textit{a}, 1993\textit{b}; Doody \textit{et al.} 2001), but this adjusted timing of emergence arises through early or late embryonic aestivation in the egg. Delayed emergence via hatchling overwintering inside the nest has been suggested (Chessman 1978; Kennerson 1980; Dalem and Burgin 1996), but not documented. Here, we report, for the first time, hatchling overwintering inside the natal nest by \textit{C. longicollis}, with temperature data for the entire incubation period and nest emergence.

Methods

We monitored \textit{C. longicollis} nests in the Gungahlin region, Australian Capital Territory (ACT), south-eastern Australia, from...
November 2011 to October 2014. The ACT climate is temperate, with mean monthly maximum air temperature ranging from 11°C in July to a peak 28°C in January and February, and a mean monthly minimum air temperature ranging from 0°C to 13°C in the same months. Mean annual rainfall is 633 mm (1974–88, Australian Bureau of Meteorology). Nests were located around ponds in three locations. The Ginninderra Experiment Station operated by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) consists of areas with native grasses and eucalypts, in addition to areas with crops and pastures (Webster and Butler 1976). The Gungahlin suburb is an industrial and residential area with high road densities and managed suburban green spaces such as golf courses, suburban parks, gardens and sport ovals (see Rees et al. 2009; Roe et al. 2011). The 791-ha Mulligans Flat Nature Reserve comprises woodlands, grasslands, several ponds and the upper tributaries of Ginninderra Creek (Rees et al. 2009; Roe et al. 2011).

We monitored 10 natural nests from 2011 to 2014, and in five of them we observed nest emergence. Nests were located by walking along the perimeter of the ponds during late spring and early summer (November–December), which corresponds to the nesting season in this region (Vestjens 1969). Once a nest was found, usually by visually locating the nest plug, we measured the distance from the water and removed the nest plug to access the nest and take measurements of the length and the width of the eggs (cm), in addition to the depth of the nest cavity and the width of the nest mouth (cm). We then returned eggs to the nests, inserting an iButton® (Maxim Integrated, 160 Rio Robles, San Jose, CA 95134, USA) that recorded temperature every 2 h in the core of the nest. The nest plug was replaced and covered with wire mesh to allow us to record dates of hatchling emergence. Nests were visually monitored during the incubation period, and monitored to allow us to record dates of hatchling emergence. Nests were visually monitored during the incubation period, and monitored every other day during the expected time of emergence in autumn (March–April) (Vestjens 1969). We chose two nests from which the hatchlings did not emerge by autumn and carefully opened them adjacent to the nest plug, one during winter and the other at the beginning of spring, to confirm whether eggs were in diapause or had hatched, although we were not able to precisely detect hatching dates. Nests were monitored in the following spring every other day (September–November). Whenever there were signs of nest emergence, the mesh cover was removed and the nest accessed. We then recorded hatching success, carapace and plastron length (cm), and body mass (g).

Results

In four of the 10 monitored nests, hatchlings overwintered inside the nest and emerged in spring, and in one nest the hatchlings did not overwinter and emerged in autumn (Table 1). The other five nests showed no signs of nest emergence after 16 months. They were opened and contained unhatched eggs or hatchlings that had pipped but were dead within the shell (Table 1). We confirmed that in at least two overwintering nests (one checked in winter and one in early spring), eggs had hatched and hatchlings were in torpor, had no yolk sac, and umbilical scars were closed. Hatching possibly occurred before winter, as winter temperatures induce inactivity in turtles (Ultsch 2006), but we were not able to document the precise time of hatching. We assumed that hatching should have occurred before winter in the other two unchecked overwintering nests.

Overwintering by hatchlings inside the nest was observed in each of the three years, and emergence from the nest occurred after 320 ± 31 (mean ± s.d.) days (n = 4, range = 284–356 days), with hatching success ranging from 36 to 100% (Table 1). Incubation period of the nest that did not overwinter was 125 days, with a 92% hatching success (Table 1). Nests were 25.6 ± 24.9 m (n = 10, range = 2–70 m) away from the ponds, nest depth and nest mouth width were 8.9 ± 1.4 cm (n = 10, range = 6.8–11 cm) and 5.9 ± 1.0 cm (n = 10, range = 4.5–7.5 cm), respectively. Egg length and width were 3.17 ± 0.10 cm (n = 111, range = 2.94–3.42 cm) and 1.99 ± 0.05 cm (n = 111, range = 1.84–2.19 cm), respectively, and hatching carapace length, plastron length and mass were 2.80 ± 0.12 cm (n = 48, range = 2.4–3.0 cm), 2.16 ± 0.07 cm (n = 48, range = 1.93–2.36 cm), and 4.28 ± 0.39 g (n = 48, range = 3.0–4.9 g), respectively.

Owing to iButton failure, we were able to record nest temperatures for the entire incubation period in only two nests in the 2013–14 season, representing a clutch that did not overwinter and one that did from the same population (Table 1, Fig. 1). Temperatures inside of the nest that did not overwinter were 24.4 ± 4.3°C (n = 1, range = 14.1–36.0°C). For the overwintering nest, temperature for the first 125 days of incubation (up to the date of hatching for the non-overwintering nests) was 26.5 ± 6.1°C (n = 1, range = 13.2–44.7°C), and 11.9 ± 4.1°C (n = 1, range = 3.7–23.9°C) through the overwintering period until the time of emergence (Fig. 1). We were able to recover partial temperature data for two nests that successfully overwintered and emerged in spring, for which the recorded

### Table 1. *Chelodina longicollis* nests monitored during three reproductive seasons in Gungahlin, Australian Capital Territory, Australia

<table>
<thead>
<tr>
<th>Season</th>
<th>Area</th>
<th>Found</th>
<th>Emergence</th>
<th>Overwintered</th>
<th>Eggs (n)</th>
<th>Hatching success</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011–12</td>
<td>Csiro_4</td>
<td>29 November 2011</td>
<td>19 November 2012</td>
<td>Yes</td>
<td>13</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Csiro_5</td>
<td>29 November 2011</td>
<td>03 October 2012</td>
<td>Yes</td>
<td>11</td>
<td>82%</td>
</tr>
<tr>
<td></td>
<td>Csiro_14</td>
<td>07 December 2011</td>
<td>–</td>
<td>–</td>
<td>14</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Csiro_19</td>
<td>13 December 2011</td>
<td>–</td>
<td>–</td>
<td>12</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Reserve_1</td>
<td>08 December 2011</td>
<td>–</td>
<td>–</td>
<td>9</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Reserve_4</td>
<td>03 January 2012</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Suburb_1</td>
<td>05 January 2012</td>
<td>–</td>
<td>–</td>
<td>11</td>
<td>0%</td>
</tr>
<tr>
<td>2012–13</td>
<td>Suburb_8</td>
<td>07 December 2012</td>
<td>17 September 2013</td>
<td>Yes</td>
<td>10</td>
<td>100%</td>
</tr>
<tr>
<td>2013–14</td>
<td>Csiro_21</td>
<td>26 November 2013</td>
<td>24 October 2014</td>
<td>Yes</td>
<td>11</td>
<td>36%</td>
</tr>
<tr>
<td></td>
<td>Csiro_22</td>
<td>26 November 2013</td>
<td>31 March 2014</td>
<td>No</td>
<td>13</td>
<td>92%</td>
</tr>
</tbody>
</table>
winter temperatures were as low as 1.3°C and 2.3°C during the 2011–12 season (Table 1).

Discussion
To our knowledge, this is the first study to document hatchling overwintering inside the nest by a chelid turtle and only the second report for turtles of the Southern Hemisphere. The implications of this behaviour relate to evolutionary aspects of nest emergence in long-necked turtles, in addition to the physiology and conservation of this species. Although previous studies have suspected delayed emergence by *C. longicollis* hatchlings on finding freshly emerged hatchlings in wetlands at the beginning of spring (Chessman 1978; Kennerson 1980; Dalem and Burgin 1996), definitive evidence has been lacking. Favourable environmental conditions are needed for females to nest and hatchlings to emerge from the nest, with sometimes a considerable time gap between the two events. This gap can be bridged in Australian turtles by either early embryonic diapause such as in *Chelodina expansa* (Goode and Russell 1968; Booth 2002) and *Chelodina rugosa* (Kennett et al. 1993a, 1993b), late embryonic aestivation, as described in *Carettochelys insculpta* (Doody et al. 2001), or by delayed hatching emergence from the nest in *C. longicollis* over the winter (this study).

Current research indicates that overwintering of hatchlings inside the nest can be quite variable among and within populations (Costanzo et al. 2008; Gibbons 2013; Lovich et al. 2014; Riley et al. 2014), even including differences in timing of nest emergence among siblings sharing the same nest (see Costanzo et al. 2008). Research attempting to elucidate mechanisms and environmental cues responsible for intrapopulation variation in hatchling overwintering inside the natal nest are scarce (Riley et al. 2014). Some evidence suggests that environmental factors such as nest temperature, nest slope, percentage of bare ground surrounding the nest, and risk of predation by sarcophagid fly larvae may influence overwintering strategy in painted turtle hatchlings (Riley et al. 2014). Despite our limited sample size, we have evidence that *C. longicollis* can exhibit a dichotomous hatchling overwintering strategy within the same population, though we have no conclusive information on possible triggers and environmental conditions that affect these variable responses. For example, temperatures inside overwintering nests tend to be cooler than in non-overwintering nests (Riley et al. 2014), but we found an opposite result, with mean temperatures higher for the overwintering nest up to the time of emergence in the non-overwintering nest. We also observed hatchling overwintering in the nest in each year of the study (2011–14) but, interestingly, such behaviour was not reported in a previous investigation in the same region, where only autumn emergence was documented (Vestjens 1969). Such findings suggest that hatchling overwintering in *C. longicollis* may vary among years, as reported for other species (Lovich et al. 2014). In addition, further research
is needed to evaluate whether the effect of drought–flood cycles in the Australian environment could influence responses of hatchling overwintering in chelid turtles, as there is evidence that rainfall and cool temperatures play a role in delayed emergence by hatchling turtles (Lovich et al. 2014).

The incubation period of 125 days for C. longicollis that did not overwinter was in the range reported by others (Vestjens 1969; Parmenter 1985). The extended period inside the nest by C. longicollis hatchlings that overwintered (incubation plus overwintering = 320 days, on average) is similar to the period experienced by species in the Northern Hemisphere (Gibbons and Nelson 1978; Costanzo et al. 1995; Wilson et al. 1999; Gibbons 2013), but temperatures experienced inside the nest during winter in our study were milder than those in the USA and Canada (Packard 1997; Costanzo et al. 2000a; Riley et al. 2014). North American turtle hatchlings use physiological mechanisms, such as supercooling, to tolerate freezing temperatures (Costanzo et al. 2000b; Packard and Packard 2005), though further investigation would be necessary to investigate whether C. longicollis performs such physiological mechanisms. While our nests did not experience freezing temperatures, C. longicollis populations elsewhere in more southerly locations, higher elevations and colder climates would likely have to cope with freezing (e.g. winter temperatures as low as –13.9°C at the Cooma Airport Weather Station, Australian Bureau of Meteorology: Dunsmore 1966).

Previous studies have detailed the importance of terrestrial habitats for numerous aspects of the behaviour and population dynamics of C. longicollis (Roe and Georges 2000a, 2000b; Rees et al. 2009). Hatching overwintering is yet another critical aspect of ecology that occurs in terrestrial environments. Consequently, hatchlings may be vulnerable while in the nest to terrestrial disturbances outside of typical activity periods, such as changes in soil management practices, in addition to increased risk of predation by invasive predators, such as foxes (Vulpes vulpes) (Thompson 1983; Spencer et al. 2006) and fire ants (Solenopsis invicta) (Gibbons 2013).

Overwintering in the nest was once thought to be an unusual phenomenon observed in only a few North American turtle species, but after extensive research it is now believed to be a much more common behaviour in many species worldwide (Gibbons 2013). Our findings of a chelid species engaging in this behaviour increases our knowledge of the phylogenetic range of turtles exhibiting hatchling overwintering behaviour, and we suspect that this should also occur in other chelid species inhabiting temperate regions of South America and Australia. Further studies are needed to investigate whether hatchling overwintering in the nest is more widespread in C. longicollis (Chessman 1978; Kennerson 1980; Dalem and Burgin 1996) and other species of long-necked turtles, in addition to the environmental context in which it occurs.

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