

Nesting habitat of the broad-shelled turtle (*Chelodina expansa*)

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Abstract. Turtles have persisted for over 220 million years, despite facing threats at every life-history stage. In Australia, nest predation by introduced foxes has driven severe declines in some populations. Our project quantified the nesting habitat of the endangered broad-shelled turtle (*Chelodina expansa*) to facilitate protection of critical nesting grounds. We determined the nesting preferences of *C. expansa* at five distinct wetlands on the Murray River from 2011 to 2014. We identified environmental variables associated with nest sites in different habitats and compared those at nests and non-nest sites to determine nesting preferences. Kernel density estimates were used to identify important nesting grounds. Our study has important implications for conservation of *C. expansa*. Habitat preferences for nest sites of *C. expansa* are predictable both within and across sites, with females preferring to nest ~50 m from shore (~4 m elevation), in open habitat with little vegetation. Based on these habitat preferences, kernel density estimates showed that *C. expansa* may select the same nesting beaches in subsequent years. Fox depredation of nests (and nesting adults) drives turtle declines in Australia, so identifying nesting areas for protection is a first step in turtle conservation.

Additional keywords: conservation ecology, habitat preference, natural selection, reproduction.

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Introduction

In turtles, embryo and hatchling success are significantly influenced by nest-site selection (Wilson 1998; Kolbe and Janzen 2002a; Spencer 2002a; Micheli-Campbell *et al.* 2013). Nest micro- and macrohabitats influence life-history traits, such as survival and growth (Wood and Bjorndal 2000; Kolbe and Janzen 2002a; Spencer 2002a; Micheli-Campbell *et al.* 2013). Selecting an appropriate nest site is thus fundamental for embryonic development and increasing offspring survival (Hughes and Brooks 2006; Tamplin and Cyr 2011). The effects of incubation temperature, in particular, pervade turtle life histories. Incubation temperature affects embryonic developmental rates, body size, performance, thermoregulation behaviour, and sex (Wood and Bjorndal 2000; Kolbe and Janzen 2002a; Dormer *et al.* 2016). Incubation temperature also affects long-term survival and age at maturity (Spencer 2002a; Spencer and Janzen 2010). Environmental conditions at potential nest sites can vary considerably and one variable, vegetation cover, is an important cue that females use to select nesting locations, because it directly affects the temperature of the nest (Janzen 1994; Wilson 1998; Janzen and Morjan 2001; Kolbe and Janzen 2002a).

Predation is another factor that may significantly influence nest-site selection. In general, nesting further away from shore decreases predation of freshwater turtle nests (Kolbe and Janzen 2002b; Marchand *et al.* 2002; Marchand and Litvaitis 2004) because nests are less clustered and predators cannot use linear search patterns to detect them (Robinson and Bider 1988; Marchand and Litvaitis 2004). However, nesting behaviour is also influenced by mothers' perception of their own predation risk (Wood and Bjorndal 2000; Spencer 2002a), thus the ultimate position of a nest is likely to be a balance between maximising offspring success and minimising adult female mortality (Spencer and Thompson 2003).

In Australia, non-native species such as the European fox (*Vulpes vulpes*) have a significant impact on native freshwater turtle populations. The Murray River turtle (*Emydura macquarii*) and the common long-necked turtle (*Chelodina longicollis*), which inhabit the Murray River and adjoining waterways, are intensively depredated (nests and adults) by foxes (Thompson 1983; Spencer and Thompson 2005). Nest depredation rates are over 90% annually for both of these species (Thompson 1983; Spencer 2002a; Spencer *et al.* 2017). The broad-shelled turtle

(*Chelodina expansa*) is sympatric with both *E. macquarii* and *C. longicollis*, and is listed as endangered in Victoria (e.g. DSE 2013). *Chelodina expansa* nests after rain and prefers to nest uphill, 30–300 m from the water's edge (Booth 2010). However, *C. expansa* employs solitary nesting strategies and nest in autumn, unlike both *E. macquarii* and *C. longicollis*, which nest more communally in spring (Bowen *et al.* 2005). This solitary autumn nesting strategy may, to some degree, reduce nest predation rates of *C. expansa* (Spencer and Thompson 2005; Spencer *et al.* 2016). However, *C. expansa* embryos undergo diapause over the winter after oviposition and eggs are underground for up to 12 months before they hatch (Booth 2002). Given that incubation spans several seasons and is far longer than that of most other species of turtle, it is critical for females to select nest sites that both facilitate embryonic development and protect eggs from predators. Developing protocols to predict preferred nest sites of this cryptic endangered species is thus an important tool for its conservation.

Here, we determine the maternal nesting preferences of *C. expansa* in a range of natural and modified habitats on the Murray River, Victoria. We tested three aims over subsequent years: (1) in 2013 we determined the macro- and microhabitat characteristics of depredated *C. expansa* nests at Cockatoo Lagoon and compared these characteristics to non-nest locations at Cockatoo Lagoon; (2) in 2014, we determined how the locations of depredated *C. expansa* nests varied across multiple sites located along the Murray River, Victoria, by comparing macro- and microhabitat data collected at each nest, and (3) we

determined whether populations of *C. expansa* nest at similar nesting beaches, within a site, across multiple years. Notably, our study focussed on the characteristics of nests depredated by invasive red foxes, because non-depredated nests are highly cryptic. Prior studies indicate that invasive foxes destroy 95% or more of all turtle nests present in the Murray catchment (Thompson 1983; Spencer and Thompson 2005; Spencer *et al.* 2016, 2017), so we assume that these depredated nests represent most of the nesting effort by *C. expansa* at our study sites.

Methods

Study species and sites

Chelodina expansa is the largest Australian chelid turtle (Goode 1967). It inhabits permanent water bodies along the Murray–Darling river system and coastal rivers of south-eastern Queensland, from the Logan–Albert drainage in the south to the Fitzroy drainage in the north. Offshore populations occur on Fraser, Moreton, and Stradbroke Islands, Queensland (Chessman 1988). *C. expansa* is a specialised predator and is solely carnivorous, predominantly consuming crustaceans, small fish, and aquatic insects (Chessman 1983). Clutch size is closely correlated with body size, and *C. expansa* delays maturity to 14–15 years to increase its reproductive output (Spencer 2002b).

Our study sites were five separate wetlands located in Gunbower Forest on the Murray River floodplain near Gunbower and Cohuna, Victoria (Fig. 1). The Gunbower Forest wetlands are

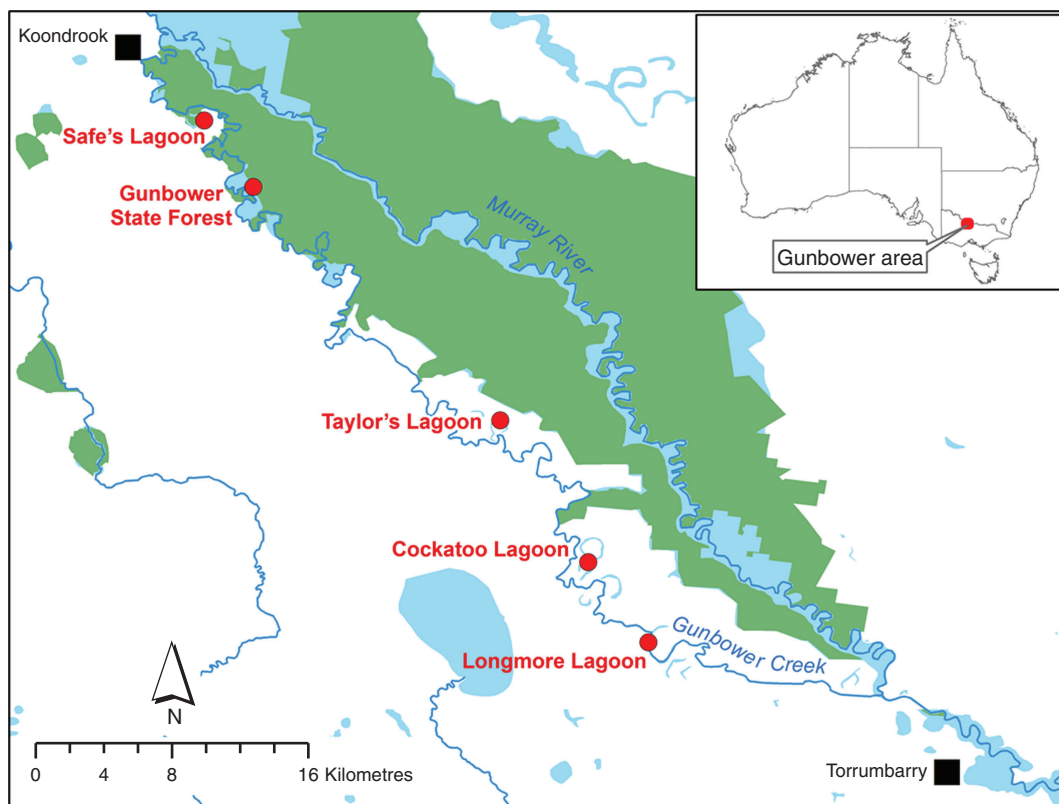


Fig. 1. Locations of study sites near Gunbower, in north-central Victoria, Australia.

protected under the Convention on Wetlands of International Importance, i.e. Ramsar Convention (Department of Primary Industries 2013). The surrounding area is a mix of woodland and agricultural pastures. Data on *C. expansa* nesting have been collected at the Gunbower Lagoons of this study since 2011 (Turtles Australia Inc., pers comm.) and will be analysed as part of this current study.

Within-site comparison of C. expansa nest characteristics

In 2013 we determined the macro- and microhabitat characteristics of depredated *C. expansa* nests at Cockatoo Lagoon and compared these characteristics to non-nest locations at Cockatoo Lagoon. Cockatoo Lagoon is an oxbow of Gunbower Creek and is characterised by irrigated pasture, stubble sandhills and cattle grazing. Between 11 April and 10 May 2013 riparian zones of Cockatoo Lagoon were searched for depredated nests. Site surveys were undertaken during the known *C. expansa* nesting season, which occurs from March to June in Queensland populations (Booth 2010). We focussed our surveys on March–May due to previous reports of *C. expansa* nesting during this period at the Gunbower Lagoons (Turtle Australia Inc., pers comm.). Study sites were walked by a team of four people, with inspections undertaken across the various sites for up to 8 h per day, between 0800 and 1800 hours. Depredated turtle nests were identified as shallow, bare-dirt holes in the ground, ~12–15 cm deep, with scattered turtle eggshells nearby. *C. expansa* nests were specifically distinguished from nests of *E. macquarii* and *C. longicollis* through the presence of fresh eggshells, since *C. expansa* nests 4–6 months after the other species. Notably, the bare dirt hole and bright white eggshells of a depredated turtle nest contrast sharply with the adjacent ground, which is typically flat and brown-rust in colour, or covered with vegetation and/or leaf litter. Because all our surveys took place during or just after the nesting season, there is little opportunity for dirt, leaf litter, or vegetation to fill in the holes. Foxes are capable of destroying nests rapidly, including within 24 h of laying, and we have observed a single fox destroying 15 nests in just 5 h (Spencer *et al.* 2016). For these reasons, we argue that our ability to detect depredated nests is consistent across all habitat types and sites in our study. Latitude and longitude of nests were recorded with a Garmin GPSmap 62 Series, with an accuracy of 3 ± 1 m. Once recorded, nests were either filled or a marker stake was placed at the nest to avoid it being recorded twice.

During April–May 2013, macro- and microhabitat characteristics of nest sites were recorded and compared with non-nest sites at Cockatoo Lagoon. Non-nest sites were located 1–10 m, 11–25 m, 26–40 m, 41–60 m, 61–80 m, 81–100 m, 101–120 m before the nest, towards the water's edge. Another category, 5 m beyond the nest, in the direction away from the water's edge, was also sampled. Each category was not included if an individual nest was located closer than 120 m from shore. By using this approach, we assume that turtles would walk from the water to their nesting site in a straight line, and we acknowledge that our results may be biased as a consequence. However, it would be impossible to remove this bias without knowing the exact path each turtle took. Thus, we

assume the impact of our bias here is similar across all nests included in our analysis due to our consistent approach.

At each nest and non-nest site, we characterised the general macro- and microhabitat type (Table 1). Additional macro- and microhabitat characteristics of nest sites were then quantified and compared with measurements of the same variables for sites that were not selected by the nesting turtles. Macrohabitat variables measured were elevation and distance from the nest to the shore at each nest and non-nest site. Distances (m) to the shore were measured using a measuring wheel from the nest to the shore in a straight line. Elevation was measured using a laser level. Microhabitat variables that were measured were percentage coverages of vegetation, leaf litter and bare ground, percentage canopy cover, distance to the nearest vegetation that was less than 2 m tall, distance to the nearest vegetation that was greater than 2 m tall, and distance from the selected nest to the nearest nest and non-nest site. Percentage coverages of vegetation, leaf litter and bare ground were visually estimated after placing 1-m² quadrats over each nest and non-nest point. We measured canopy cover above the nests and non-nest sites using a densitometer (Janzen 1994; Wilson 1998; Janzen and Morjan 2001). The densitometer has a convex mirror that reflects the overstorey vegetation. We held the densitometer over the nest and counted the number of squares covered by vegetation and multiplied this figure by 1.04 to obtain the percentage of the densitometer not covered by vegetation. This figure was then subtracted from 100% to obtain the canopy cover percentage. Distances (m) to the nearest shrub or tree, and nest, from each nest and non-nest site were measured in a straight line, using a measuring wheel. All variables measured were chosen because of their potential to impact nesting success, such as the thermal and hydric effects of canopy and/or ground cover, or the potential for nearness to the water level to indicate potential flooding risk.

Table 1. Micro- and macrohabitat codes for characterisation of nest sites and non-nest nests

Habitat	Code
Microhabitat	
Mudflat	0
Clumping grass	1
Leaf litter	2
Saltbush	3
Stubble	4
Chenopod	5
Bare ground	6
Road	7
Running grass	8
Sedge	9
Herbs	10
Macrohabitat	
Open woodland	0
Irrigated pasture	1
Riparian strip	2
Sparse chenopod sandhill	3
Stubble sandhill	4
Open forest	5
Grassland	6

Across-site comparisons of *C. expansa* nest characteristics

In March–April 2014, we determined how the locations of depredated *C. expansa* nests varied across multiple sites located along the Murray River, Victoria, by comparing macro- and microhabitat data collected at each depredated nest. We aimed to determine whether microhabitat nesting preferences of *C. expansa* differ across a larger area. Between 25 March and 22 April, riparian zones of Cockatoo Lagoon, Gunbower State Forest, Longmore Lagoon, Safe's Lagoon, and Taylor's Lagoon were searched for depredated nests. The main land practices at Cockatoo Lagoon, Longmore Lagoon, Safe's Lagoon, and Taylor's Lagoon are irrigated pasture and cattle grazing. Gunbower State Forest is characterised by red gum (*Eucalyptus camaldulensis*) forest. Sites were walked by a team of three people, with inspection undertaken across the various sites for up to 8 h per day, between 0800 and 1800 hours. At each nest, TurtleSAT, a Citizen Science tool that utilises the GPS function of mobile phones, was used to record nest location, date and time observed and micro- and macrohabitat nest data (TurtleSAT 2014). TurtleSAT is valuable for identifying hotspots of adult mortality (e.g. road deaths), as well as identify nesting grounds, and can be applied to any turtle species in Australia. TurtleSAT asks the user a series of questions relating to nest characteristics (see Table 2). The user answers the questions from a list of pre-empted answers, each of which was assigned a categorical code for analysis (Table 2). Nest data were also imported into Google Earth™ and qualitatively characterised based on the macrohabitat (Table 3). Distance to water, nearest tree and riparian zone of each nest were also determined by drawing a straight line from the nest to each variable using the ruler tool in Google Earth™. We acknowledge that mobile phone GPS locations may be accurate to only 5–10 m, but we assume that this inaccuracy applied randomly to all of the locations uploaded to TurtleSAT such that any significant differences that we detected in our analyses are real.

Table 2. TurtleSAT questions and pre-empted answers
Categorical codes for analysis are in parentheses at the end of each answer

Questions	Pre-empted answers
What is the soil type at the nest?	(a) Sandy (1) (b) Brown clay (2) (c) Red clay (3) (d) Brown soil (4) (e) Unknown (5) (f) Other (6)
What is the slope of the soil at the nest?	(a) Flat (1) (b) Medium (2) (c) Steep (3)
What is the ground habitat at the nest?	(a) Open (1) (b) Grassy (2) (c) Shrubs (3) (d) Other (4)
How much overstorey vegetation cover is at the nest?	(a) No tree cover/open (0) (b) Some tree cover (1) (c) Lots of tree cover (2) (d) Other (3)

Data analyses

Microhabitat variables of nesting and non-nesting areas were compared using Analysis of Similarity (ANOSIM) and SIMPER analysis in PRIMER software (Clarke and Warwick 2001). The same analysis was used to compare nesting habitats among wetlands in 2014.

Latitude and longitude data from all nests found during April–May of 2013 and March–April of 2014, as well as data collected in an identical manner in 2011–12 (Turtles Australia Inc., pers comm.) were imported into ArcMAP (ESRI, ver. 10). To determine whether populations of *C. expansa* nest at similar nesting beaches within a site, across multiple years, we collated data from 2011–14 and used Spatial analysis (kernel density estimates, KDE). KDE calculate the density of nests within an area and overlay data from consecutive years to determine nesting hotspots within the study sites. Statistically, the kernel density technique is a better hot spot identifier than cluster analysis techniques (Shahrabi and Pelot 2009). Kernel density methods create a continuous surface to represent density variability over the entire study area, not just in certain clusters as in other hot spot techniques (Shahrabi and Pelot 2009). Kernel density methods allow density surfaces of incidents and activities to be easily compared (Shahrabi and Pelot 2009). We have chosen to show only KDE analysis from Cockatoo and Safe's Lagoons in our results due to high densities of *C. expansa* nests over the 2011–14 period.

Results

Nesting preferences

Chelodina expansa nesting occurred any time from mid-March to late April and was often triggered by rain. During our study, all nesting events we observed occurred between 1200 and 1800 hours. Nests were generally clustered along elevated sections of the bank of the wetlands or on adjacent sandhills.

In 2013 habitat variables of nest sites were significantly different from those of areas that turtles might traverse before nesting, and from those beyond the nesting site (Global $R=0.337$, $P=0.001$). As female *C. expansa* moved farther from the water to nest, the elevation of the nest increased (Fig. 2). Nest sites were significantly farther from trees and shrubs compared with areas ≥ 10 m closer to water (Fig. 3). Thus, habitat variables related to tree and shrub cover, such as canopy cover, were significantly reduced over nests compared with at non-nesting sites (Fig. 4). Overstorey cover at the nest (10%) was generally twice that of 5 m beyond the nest (5%) and the average distance to trees was ~ 10 m further. Bare ground also consistently increased as distance from water increased, such that nests usually had little grass or leaf litter cover (Fig. 5).

Table 3. Macrohabitat codes for characterisation of nest sites during the 2014 nesting season

Habitat	Code
Pasture	1
Woodland	2
Open woodland	3
Riparian	4

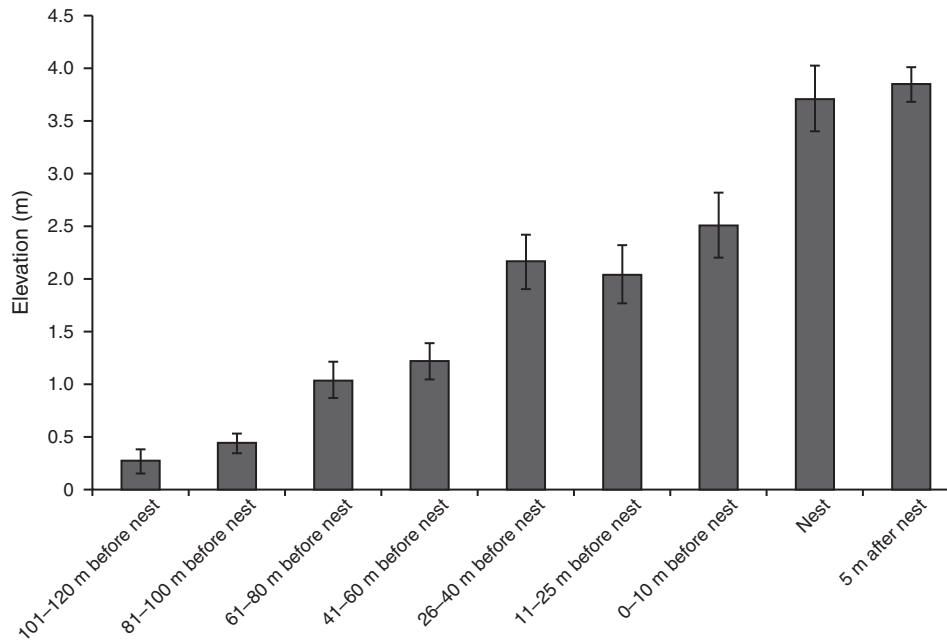


Fig. 2. Average elevations of nests and non-nest sites in distance categories from 5 m past nest to shoreline.

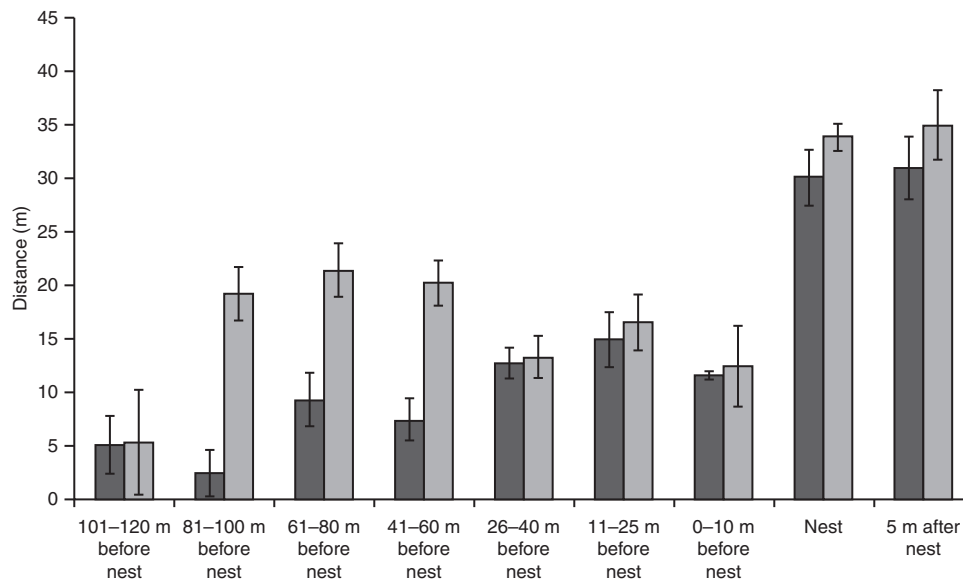


Fig. 3. Comparison of distance to trees and shrubs from nests and non-nest sites. Dark grey, trees; light grey, shrubs.

In 2014, 197 depredated nests were recorded across all sites. Habitat variables at nesting locations differed among sites (Global $R=0.098$, $P=0.001$) with nest sites at Gunbower State Forest differing from the pasture-based sites, Cockatoo, Longmore, Safe's and Taylor's Lagoons (Table 4). Nests were located in a narrow range, 50–100 m from the water and in relatively open areas. Nests were generally clumped, with most nests located 10–80 m from another nest. Nests were located well away from trees, although in Gunbower State Forest, where tree densities were higher than at other sites, nests were placed

closer to trees (Table 4). The main difference between Gunbower State Forest and the other pasture-based wetlands was related to distance to trees and riparian zones (Table 4). Gunbower State Forest had fewer open areas and higher densities of trees, so nests were closer to shore and trees than at Cockatoo, Longmore, Safe's and Taylor's Lagoons (Table 4).

Nesting preferences were consistent across wetlands, despite wetland differences in surrounding habitat type. Hotspot analysis using KDE showed that the same nesting beaches were consistently used from 2011 to 2014 (Fig. 6). The habitats of

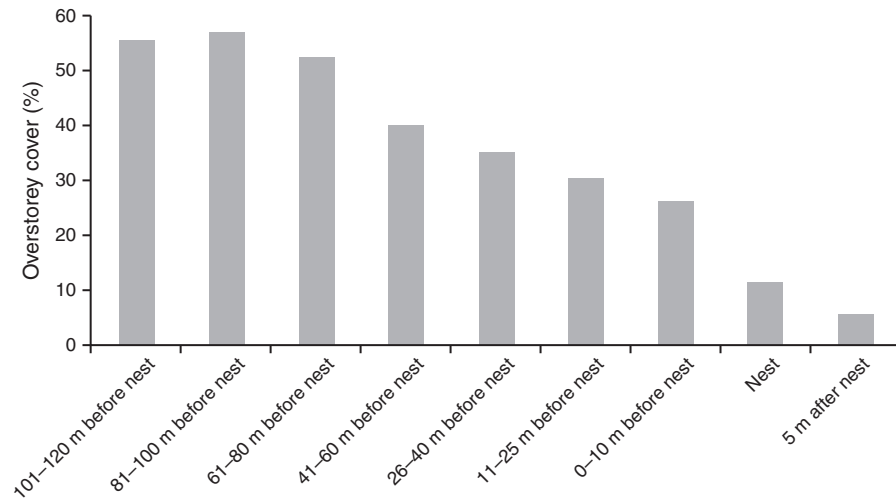


Fig. 4. Relative percentage of overstorey cover of all non-nest sites and nests.

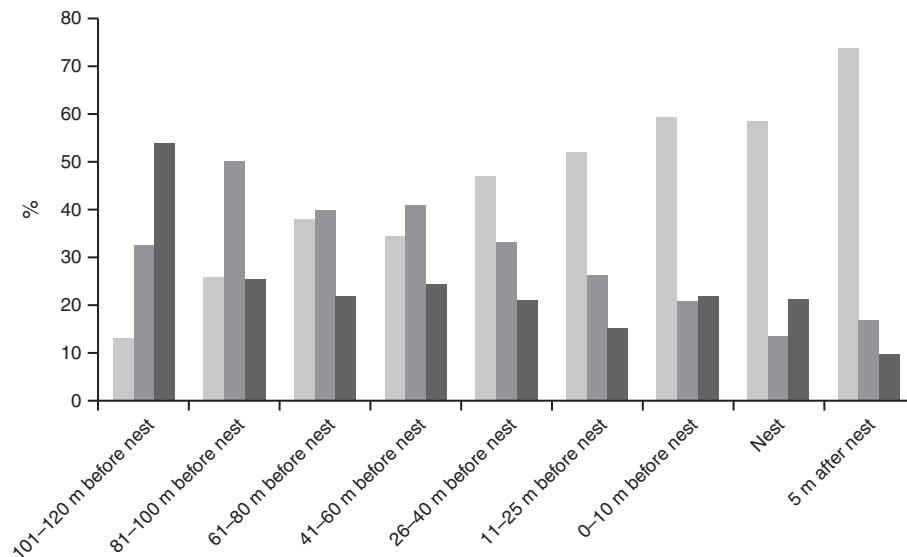


Fig. 5. Relative percentages of bare ground at non-nest sites and nests. Light grey, percentage bare ground; mid grey, percentage vegetation; dark grey, percentage leaf litter.

these nesting beaches were similar despite wetlands being located in broad habitat types of woodland forest to highly modified pastures ($P > 0.05$) (Fig. 3).

Discussion

Freshwater turtle populations are in crisis. In total, 63% of the assessed species and ~42% of all known species are considered threatened (Baillie *et al.* 2004). Australian turtles are not immune; with 69–91% of Murray River turtle populations having declined over the last 40 years (Chessman 2011). The results from our study have several important implications for the conservation and management of the endangered *C. expansa*. We found that populations of *C. expansa* have

preferred nesting areas, which allows for targeted management of nesting grounds. Habitat preferences for nesting sites of *C. expansa* are predictable, with females preferring to nest ~50 m from shore, in open habitat of low vegetation stubble (Fig. 7). Predictable nesting requirements allow for identification of potential nesting grounds in a region. The timing of nesting is also predictable, coinciding with rain in March/April, although some nesting may occur outside of this period (Booth 2010; Cann and Sadler 2017). Most environmental and conservation management agencies have access to a suite of environmental and geological databases that could be utilised for GIS spatial modelling of potential nest locations in their region, using our data. For example, multivariate spatial interpolation modelling (e.g. CoKriging) combines spatial data (e.g. elevation) with

Table 4. SIMPER analysis comparing habitat variables among wetlands

	Average values		Dissimilarity		Cumulative contribution (%)
	Average	s.d.	Average	s.d.	
	Cockatoo	State Forest			
Distance to water (m)	83.72	52.26	20.92	1.39	45.23
Distance to riparian (m)	42.98	15.73	14.24	1.48	76.01
Distance to closest tree (m)	26.85	4.94	9.94	1.25	97.49
Habitat	2.01	1.69	0.67	0.95	98.93
Overstorey	0.42	0.70	0.49	0.84	100.00
	Cockatoo	Longmore			
Distance to water (m)	83.72	59.94	17.11	1.23	43.33
Distance to riparian (m)	42.98	33.51	12.53	1.40	75.06
Distance to closest tree (m)	26.85	25.25	9.44	1.31	98.97
Overstorey	0.42	0.10	0.24	0.47	99.58
Habitat	2.01	1.90	0.16	0.31	100.00
	Cockatoo	Safe's			
Distance to water (m)	83.72	72.84	19.89	1.30	45.78
Distance to riparian (m)	42.98	28.54	12.87	1.46	75.38
Distance to closest tree (m)	26.85	24.05	10.03	1.32	98.46
Habitat	2.01	2.20	0.35	0.46	99.26
Overstorey	0.42	0.44	0.32	0.61	100.00
	Longmore	Safe's			
Distance to water (m)	59.94	72.84	17.94	1.20	44.123
Distance to riparian (m)	33.51	28.54	12.02	1.42	73.71
Distance to closest tree (m)	25.15	24.05	10.03	1.30	98.40
Habitat	1.90	2.20	0.40	0.42	99.38
Overstorey	0.10	0.44	0.25	0.57	100.00
	Longmore	State Forest			
Distance to water (m)	59.94	52.26	17.93	1.33	41.28
Distance to riparian (m)	33.51	15.73	13.43	1.47	72.20
Distance to closest tree (m)	25.15	4.94	10.99	1.45	97.48
Habitat	1.90	1.69	0.67	0.90	99.03
Overstorey	0.10	0.70	0.42	0.90	100.00
	Safe's	State Forest			
Distance to water (m)	72.84	52.26	20.98	1.27	48.84
Distance to riparian (m)	28.54	15.73	11.53	1.38	75.67
Distance to closest tree (m)	24.05	4.94	9.08	1.15	96.81
Habitat	2.20	1.69	0.89	0.85	98.88
Overstorey	0.44	0.70	0.48	0.87	100.00
	Cockatoo	Taylor's			
Distance to water (m)	83.72	96.64	21.57	1.25	50.36
Distance to riparian (m)	42.98	34.88	12.28	1.43	79.02
Distance to closest tree (m)	26.85	25.00	8.57	1.32	99.03
Overstorey	0.42	0.23	0.26	0.45	99.63
Habitat	2.01	2.08	0.16	0.21	100.00
	Longmore	Taylor's			
Distance to water (m)	59.94	96.64	21.20	1.23	51.07
Distance to riparian (m)	33.51	34.88	11.73	1.46	79.32
Distance to closest tree (m)	25.15	25.00	8.22	1.24	99.13
Habitat	1.90	0.20	0.20	0.21	99.61
Overstorey	0.10	0.23	0.16	0.50	100.00
	Safe's	Taylor's			
Distance to water (m)	72.84	96.64	23.96	1.27	53.25
Distance to riparian (m)	28.54	34.88	11.40	1.47	78.59
Distance to closest tree (m)	24.05	25.00	9.03	1.37	98.67
Habitat	2.20	2.08	0.34	0.52	99.42
Overstorey	0.44	0.23	0.26	0.60	100.00
	State Forest	Taylor's			
Distance to water (m)	52.26	96.64	26.59	1.38	54.33
Distance to riparian (m)	15.73	34.88	11.97	1.46	79.08
Distance to closest tree (m)	4.94	25.00	9.06	1.57	97.67
Habitat	1.69	2.08	0.73	0.89	99.17
Overstorey	0.70	0.23	0.41	0.86	100.00

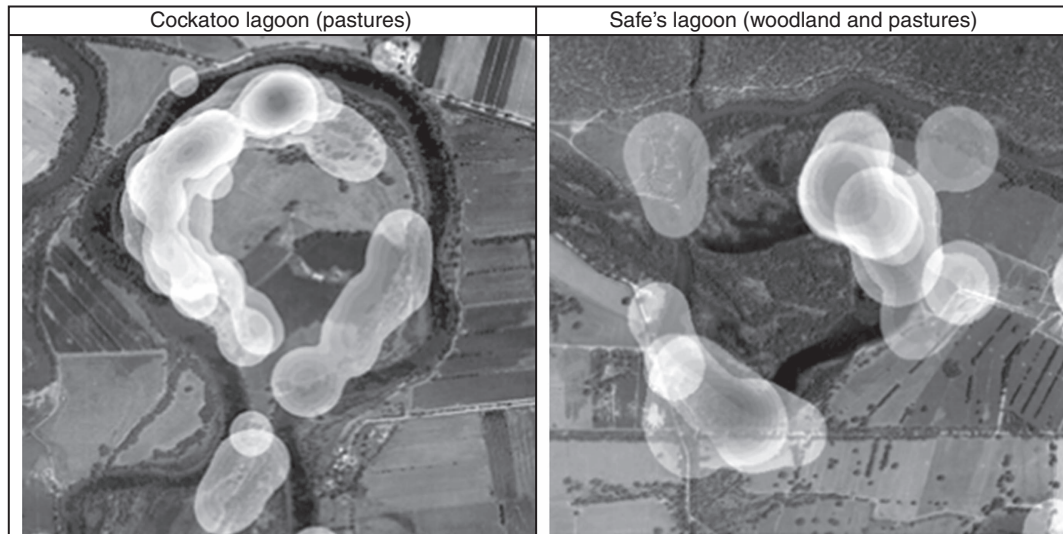


Fig. 6. KDE hotspots of nest distribution and density at Cockatoo and Safe's lagoons. Hotspots were created using data collected from 2011–14. High-intensity nesting spots over the four years are indicated by darker areas in the hotspots.



Fig. 7. Schematic diagram of a typical transect from shoreline to nest site (above arrow) at an average elevation above the shore of 3.7 m.

variables of interest to make a single map based on correlates of all included variables (Krivoruchko 2011).

Preference for nesting in open areas is common in freshwater turtles, primarily because the thermal properties of open nests enhance hatchling developmental rates (Janzen 1994; Wilson 1998; Janzen and Morjan 2001; Kolbe and Janzen 2002a). The construction of nests in open microhabitats maximises hatchling survival partly because of the reduction in the incubation period (Spencer and Thompson 2003; Micheli-Campbell *et al.* 2013). Maternal selection of open nest sites allows eggs to reach optimal temperatures more rapidly (Wilson 1998) and facilitates the synchronous breaking of secondary diapause developmental stage (Booth 2002). However the long diapause experienced by *C. expansa* potentially increases

susceptibility of the egg to bacterial and fungal infections. Ground with minimal vegetation also aids in nest cavity construction and receives higher levels of sunlight than does herbaceous cover (Flitz and Mullin 2006).

Elevation and distance to water are important characteristics that affect nesting. Nest sites at lower elevations are more likely to be inundated by flooding (Spencer and Thompson 2003), while those at higher elevations can increase the risk of hatchling disorientation (Warner and Mitchell 2013) and predation on adults (Spencer and Thompson 2003; Zare *et al.* 2012). Maternal preference of higher elevated sites (Fig. 7) may be an adaptation to flooding associated with winter and spring rainfall patterns. Because distance from the water's edge and elevation are correlated, it is assumed that *C. expansa* selects a nest site based

on elevation in order to avoid inundation. In our 2013 study two nests <10 m from shore were inundated with water from winter flooding.

Nesting behaviour and location may also offer some relief from predation. None of the nests in this study were found intact without the female present, which may reflect actual predation rates or be a function of sampling bias due to cryptic nature of intact nests. *Chelodina expansa* populations do not appear to be declining at rates similar to those of *E. macquarii* and *C. longicollis* on the Murray River (Chessman 2011), but broad-shelled turtles occur at much lower densities than the other two turtle species. *Chelodina expansa* nests during autumn, six months out of phase with both *E. macquarii* and *C. longicollis* (Cann 1998). Its nesting biology is also different. Individual *C. expansa* nest in response to rain (Booth 2010), but not all females respond to the same rain events (Bowen *et al.* 2005). Thus, the nesting season occurs over an ~4–6-week period in March–April but can extend from March to June, with some *C. expansa* nesting as late as November (Booth 2010). Nest densities of *C. expansa* are generally lower than those of the other two species because their population numbers are lower. Female *C. expansa* also nest far from shore, which scatters their nests widely.

Although natal homing is rarely observed and documented in freshwater turtles (Micheli-Campbell *et al.* 2013), the consistent use of nesting hotspots by *C. expansa* may indicate natal homing. Nest-site selection is highly heritable (Valenzuela and Janzen 2001; McGaugh *et al.* 2010) and closely related females nest close to one another (Freedberg *et al.* 2005). Unfortunately, this study largely identified nests after they were destroyed by foxes. Further research that identifies individuals is required to determine individual plasticity of nesting behaviour over several years, let alone the complexity of multigenerational natal homing.

Despite consistency in microhabitat selection by *C. expansa*, the mother's perceived risk of predation can alter nest-site selection (Spencer 2002a). High nest densities close to shore provide more linear search patterns and facilitate detection by foxes (Robinson and Bider 1988; Marchand *et al.* 2002; Marchand and Litvaitis 2004). Soil disturbance and maternal cloacal secretions increase predation by foxes that use both visual and olfactory cues to detect nests (Spencer 2002a). In agricultural pasture areas, nests were clumped in distinct nesting areas, with most nests 0–30 m away from one another. In most cases, nest predation rates are highest during the first 24 h and up to five days after oviposition (Robinson and Bider 1988; Spencer 2002a; Wirsing *et al.* 2012), thus highlighting the importance of management strategies mitigating fox predation before and during oviposition.

Although populations of *C. expansa* appear stable, populations of sympatric species have declined by up to 91% in some areas (Chessman 2011). Foxes pose the greatest threat to freshwater turtle populations in southern Australia, but current management strategies to reduce their numbers have been largely ineffective (Spencer and Thompson 2005; Spencer *et al.* 2016). Our study significantly aids targeted management of likely nesting grounds on the Murray River. Once nesting areas are identified in a region, more dynamic and integrated strategies of fox mitigation can be implemented, because turtle

populations and nesting grounds are often discrete and can be micromanaged. Currently, the primary strategy for reducing fox activity (if any control is implemented) is to run lethal baiting transects along fence lines or established paths, rather than specifically targeting nesting areas. However, foxes are extremely efficient at detecting nests and although transect baiting may result in some patchy reductions in fox activity, even a small number of foxes in an area can destroy most of the turtle nests (Spencer *et al.* 2016, 2017).

In conclusion, our study is a major step forward for conservation of *C. expansa* because it demonstrates a model for nest-site selection by *C. expansa* that can be used to identify critical nesting grounds for the species. The life history of freshwater turtles involves high but fluctuating rates of egg and juvenile mortality, balanced by extreme iteroparity (i.e. long-lived, highly fecund), in which threats to adult survival are low (Gibbons and Semlitsch 1982; Shine and Iverson 1995). The life history of *C. expansa* may differ from the life history of other freshwater turtles (i.e. *E. macquarii*) in that *C. expansa* matures later and has lower adult survival. *C. expansa*, however, appears to have lower rates of nest predation and higher rates of juvenile survival (Spencer and Thompson 2005), but these observations on *C. expansa* life history are limited to populations in the Albury region only. If we assume that *C. expansa* experiences the same risk of extinction as its sympatric species (*E. macquarii* and *C. longicollis*) then the mortality of eggs and young has increased, primarily because of predation by foxes (Thompson 1983), and adult mortality is increasing (Spencer and Thompson 2005). A major key for conserving *C. expansa* is to assess population numbers and nest predation rates along the Murray River and implement management strategies to reduce nest predation rates in locations that can be identified using our data (Spencer *et al.* 2017). Reducing nest predation rates may involve 'mosaic' fox management strategies where specific nesting areas are the focus for intensive and integrated fox control every 2–6 years (Spencer *et al.* 2017). Tools such as TurtleSAT, which are developed to identify areas of nest predation and high adult turtle mortality, are also applicable to the conservation of other species.

Conflicts of interest

The authors declare no conflicts of interest.

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