# Threats to the early life stages of the Mary River Turtle (*Elusor macrurus*) from Queensland, Australia

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**Abstract**

The Mary River turtle (*Elusor macrurus*) is a unique, endangered species that is endemic to the Mary River in southeast Queensland, Australia. Tiaro & District Landcare Group operates a nest protection program and supports research on this turtle. In this report, two potential threats to the early life stages of the Mary River turtle, predation and global warming, are investigated. Also, existing management strategies are evaluated and new ones proposed.

*Project 1: Predation*
Many predators are known to predate turtle eggs, hatchlings and adults. In this research, wildlife cameras were installed on a nesting bank along the Mary River to determine which animals are specific threats to the Mary River turtle. Many animals that could be passive or active threats to this turtle were found on the nesting bank, but only Red Foxes and Lace Monitors were photographed predating a nest. Only two photographs were taken of hatchlings, which limits any analysis on the predation of hatchlings. However, the Eastern Water Dragon and Nankeen Night Heron, both known to predate hatchlings, were found on the nesting bank. No evidence was found of adults being predated, although some photographs indicate that foxes do not show interest in adult turtles, as they were found on the same photograph without any further interaction. The nest protection program run by Tiaro & District Landcare Group protects individual nests with a plastic mesh and baits for foxes. Those seem to be the optimal strategies to minimize the predation of the eggs. More research with more optimal camera setting is needed to establish the threats to the hatchlings on the nesting bank.

*Project 2: Global warming*
Global environmental changes challenge many species to respond to those changes. In southeast Queensland, Australia an increase of 2.5 °C in ambient temperature and decrease of 20% in the amount of rainfall is expected within 50 years. Research indicates that temperature influences hatching success and the performance of the Mary River turtle hatchlings. To deal with global warming, some species are known to migrate to colder regions or advance their laying date. However, this turtle is restricted to the river and seems therefore not able to migrate. Rainfall determines the start of the nesting season by giving the nest stability and provides embryos with the right moisture level for development. The Mary River turtle seems therefore also unable to advance its nesting date. This study hypothesized that if the Mary River turtle can adjust its nesting behaviour to increasing ambient temperatures, nest temperature during the nest season should be constant, despite the increase in ambient temperature. This study further investigated whether this turtle can and does influence its nest temperature by influencing nest depth or the amount of sun on the nest. To find answers to those questions, temperature loggers were placed in 23 natural nests to collect nest temperatures during the nesting season. It was not found that the Mary River turtle changed nest depth or the amount of sun on the nest throughout the nesting season. However, all mean nest temperatures were in the presumed optimal range of 38 ± 3 °C, so they are likely to already nest in optimal conditions. In addition, some anecdotal evidence suggests that the Mary River turtle influences its nest temperature by controlling the amount of sun exposure on the nest by varying vegetation cover and cardinal direction of the nesting bank. More research is needed to confirm this. If long-term studies show that turtles are not adapting well, Tiaro & District Landcare Group could consider adding nest shading to their already existing nest protection program as this research showed that a decrease in the amount of sun exposure on the nest lowers nest temperatures. Long-term research is needed to establish whether the Mary River turtle can adapt fast enough to increasing ambient temperatures and if this shading strategy is necessary.

## Introduction

The Mary River is an important water supply for life within the river's catchment in southeast Queensland, Australia (MRCCC, 2012, Fig. 1). Eleven dams are built in the river to provide water for irrigation and urban water supplies (Johnson, 1997). Besides the importance for humans, the river is home to many endemic, endangered species, such as the Mary River cod (*Maccullochella mariensisis*), the Australian lungfish (*Neoceratodus forsteri*) and the Mary River turtle (*Elusor macrurus*, Box 1) (IUCN, 2015).

The small community group 'Tiaro and District Landcare Group' made the protection of the Mary River turtle its main project and has operated a conservation program since 2001 which includes research on the Mary River turtle and in situ protection of the nests. This is necessary, because the population of the Mary River turtle has crashed up to 95% mainly due to intensive commercial egg selling between 1962 and 1974 (Flakus, 2002). While the turtle is trying to recover from this impact, other threats, such as the reduction of suitable nesting sites, predation and global warming, are threatening its existence.


*Fig. 1. Geographical location of the Mary River in Southeast Queensland, Australia. Red dot shows the locations of the nesting banks used in this study (Design Unit ©, Australian Museum; Google Maps, 2015)*

*Box 1*

The Mary River turtle is a unique turtle species, which was scientifically described in 1994 (Cann & Legler, 1994). Unlike other freshwater turtle species, the Mary River turtle is a sexual dimorphic species, with the males not only being bigger than the females, but also having an unusually large tail which has the ability to hook (Fig. A). The turtle can reach an age of 30-80 years, with an average carapace length of 380 mm (males) and 326 mm (females) and feeds upon green algae and aquatic invertebrates (Cann & Legler, 1994). Females start breeding when they are 15-25 years old (Flakus, 2002). They nest between October and December at night at the sandy banks of the Mary River after periods of rainfall, which makes the sand stable enough to prevent collapsing of the nest chamber (Flakus, 2002; Micheli-Campbell et al., 2013a). Rainfall also provides the eggs with optimal moisture conditions for development (Booth & Yan Yu, 2008). Their nests are on average 15 cm deep and contain on average 15 rigid-shelled eggs (Flakus, 2002). It takes on average 55 days for the eggs to hatch, although this is dependent on temperature (Micheli-Campbell, 2011). The gender of the hatchlings is genetic and therefore independent of nest temperature (Georges & McInnes, 1998). It is a highly mobile turtle, traveling up to 4 km a day and relies on riffles for their food supply (Flakus, 2002; Campbell, 2012; Micheli-Campbell *et al.,* 2013b). It basks on instream rocks or timber structures in the river (personal observation). The turtle is a bi-modal respirator: it has the ability to take oxygen from the air with its lungs and from the water with gill-like structures in the cloaca. Therefore the hatchlings can stay submerged for up to 72 hours (Clark *et al.,* 2008).

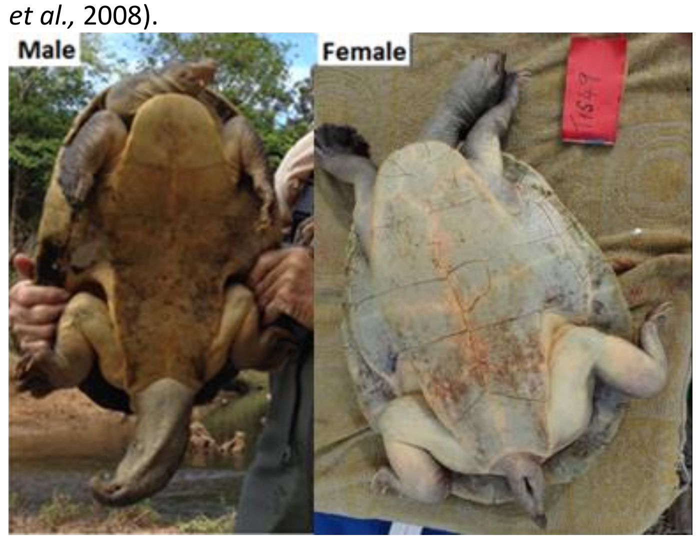


*Fig. A. Male and female Mary River turtle (Elusor macrurus)*

The first aim of this research was to gain insight into and evidence of the actual predators of the eggs, hatchlings and female adults on the nesting bank and evaluate effectiveness of existing protection strategies. This was done by analysing photographs from four wild life cameras that were placed on a productive nesting bank. Tiaro & District Landcare Group operates a nest protection program, by placing plastic meshes over natural nests. Some years, the Group also baits for foxes. Despite these measures taken, nests of the Mary River turtle are still found being predated, presumably by Red foxes (*Vulpes vulpes*) and goannas (which includes the Lace Monitor (*Varanus varius*) and the Yellow-Spotted Monitor (*Varanus panoptes*), fig. 2&3). Other possible predators are dingo's (*Canus lupus;* e.g. Doody *et al.,* 2006), wild pigs (*Sus scrofa;* e.g. Whytlaw *et al.*, 2013), and several bird species, such as eagles (e.g. Burger & Gochfield, 2014). The fact that some nests are still being predated is mainly because not all nests can be found as not all nesting banks are patrolled and sometimes turtle tracks are obliterated by turtles arriving later on the bank. However, some nests are being predated while already protected (personal observation). Therefore, it is needed to gain insight into the activity on the nesting bank and evaluate existing protection methods.

The second aim of this resarch is to study the effects of global warming as this might also form a threat to the Mary River turtle. In southeast Queensland, Australia, an increase

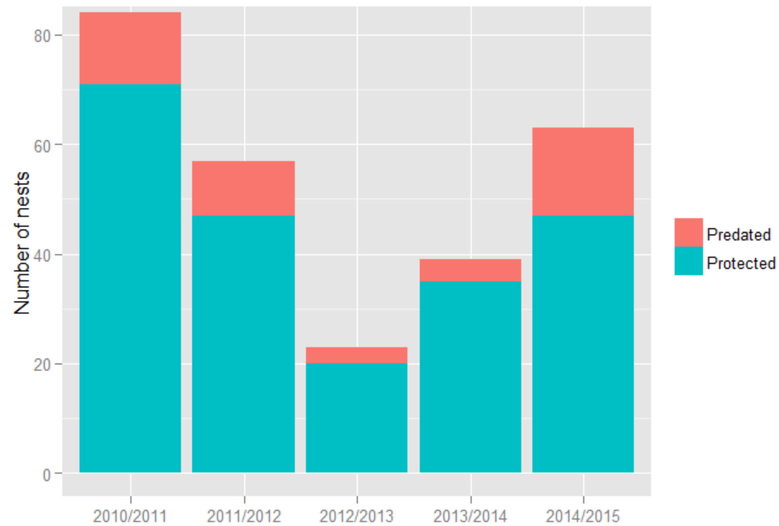


*Fig. 2. Number of protected and predated nests of the last five seasons.*

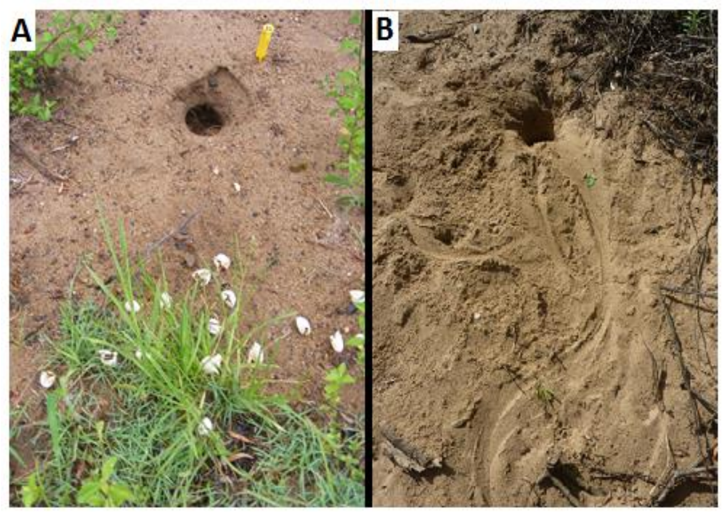


*Fig. 3. Predation of two nests by presumably a) a Red fox (Vulpes vulpes) and b) a goanna (*which includes the Lace Monitor (*Varanus varius*) and the Yellow-Spotted Monitor (*Varanus panoptes*).

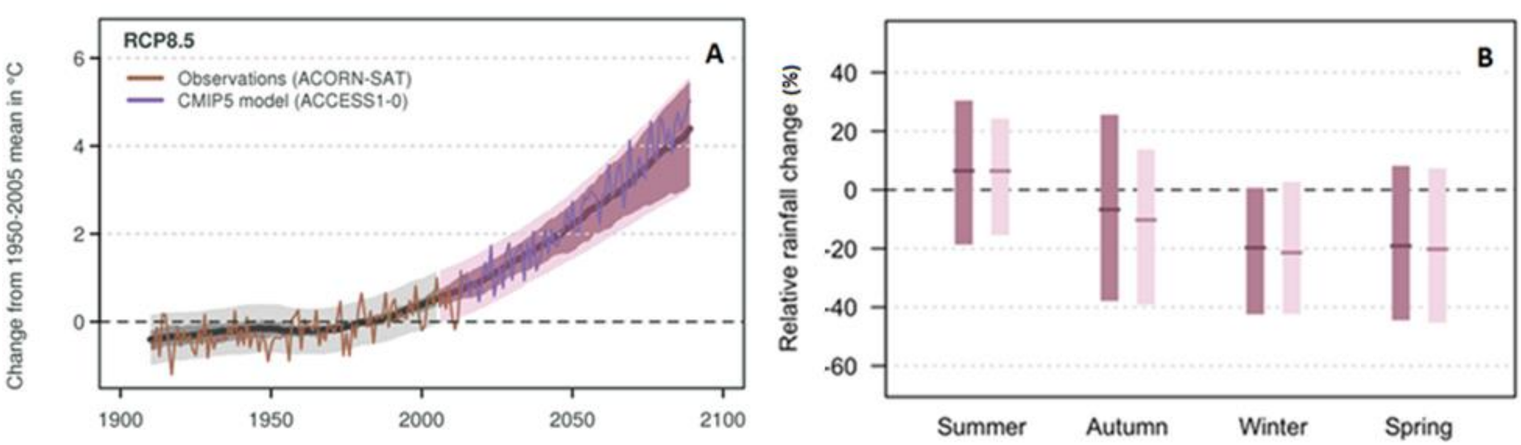


*Fig. 4. a) An expected increase in ambient temperature of 2.5°C in 2065 in Australia, b) a 20% decrease in the amount of rainfall in winter and spring expected by 2080-2099 in % of 1986- 2005 in Eastern Australia. For details on colours and models, please see original report (Australian Government, 2015).*

of 2.5 °C in ambient temperature and a decrease of 20% in rainfall is expected within 50 years from now (Australian Government, 2015, fig. 4). Laboratory experiments performed by Micheli-Campbell and colleagues (2011) showed that temperature influences the early life stages of the Mary River turtle: incubation temperatures of Mary River turtle eggs when kept constant had a large influence on hatching success and the performance of the hatchlings. While a constant temperature of 26 °C prolonged the incubation time with ten days, the effects of a constant temperature of 32 °C were more dramatic: the chances of hatching reduced by 95% and the 5% that hatched were 27% smaller than normal and swam and right themselves much slower (Micheli-Campbell *et al.*, 2011). A year later, the research was repeated with fluctuating incubation temperatures (28± 3 and 28 ± 6 °C) (Micheli-Campbell *et al.*, 2012a). They found that fluctuating temperatures had less impact on hatching success and hatchling performance, although they still found a lower stroke force for hatchlings from eggs exposed to incubation temperatures of 28 ± 6 °C. Although temperatures in nature are fluctuating and this seems to have less impact on the early life stages of the Mary River turtle (Micheli-Campbell *et al.*, 2011, 2012a), the experiment in 2011 shows that eggs and embryo development are sensitive to temperatures.

The question rises whether global warming, which will lead to more extreme peak temperatures and higher mean temperatures in the nest, will threaten the existence of the species or whether there is a way that female Mary River turtles can adjust their nesting behaviour to influence incubation temperatures in the nest. Other species are known to adapt to increasing temperatures by migrating to colder regions (e.g. butterflies: Parmesan *et al.*, 1999; marine fishes: Perry e*t al.,* 2005) and birds are known to advance their laying date (Dunn& Winkeler, 1999; Both *et al.*, 2004). However, migration or advancing its laying date seem unlikely adaptations for the Mary River turtle. Migration seems no option, because the turtle is an endemic species that only comes on land to nest and seems therefore restricted to a single river system. Also, the Mary River turtle is dependent on season-dependent rainfall for the stability of its nest, which will even decrease while temperatures are increasing (fig. 4). Therefore it is not likely that it will be able to shift its nesting date. However, there are two other potential adaptations. The Mary River turtle could regulate nest temperatures by digging its nest deeper (e.g. Shine & Harlow, 1996; Telemeco *et al*, 2009) or changing its nesting location, for example towards shadier places (Kolbe & Janzen, 2002; Refsnider, 2012; Wood *et al.*, 2014).

In this research, the natural increase in ambient temperature between spring and summer was used to study the behavioural responses to increasing temperatures of female Mary River turtles in the wild. The range of 28± 3°C used in the lab was assumed to be the optimal range of incubation temperatures (Micheli-Campbell *et al.*, 2012a). Three hypotheses with two assumptions were explored: (1) If the Mary River turtle can adjust its nesting behaviour to increasing temperatures, nest temperatures throughout the nesting season will be constant, despite the increase in ambient temperature. It is further hypothesized that if turtles can keep nest temperatures constant while ambient temperatures are increasing, this study will find (2) an increase in nest depth or (3) a nest location with less sun exposure throughout the nesting season. It is hereby assumed that nest depth and/or the amount of sunshine predict nest temperature. To test those hypotheses and assumptions, temperatures of wild clutches was monitored and corresponding nest depth and sun exposure was determined.

## Methods

*Data collection*

To get more insight in the actual predators of the Mary River turtle on the nesting bank, four wildlife cameras (Reconyx, Inc. PC800 Hyperfire Professional IR) were placed at nesting bank A, starting two weeks before the nesting season (September 29, 2014) until after all clutches had hatched (February 18, 2015) (fig. 5&6). The motion trigger of the cameras was always enabled and was activated when it detected a change in the heat profile of its detection zone. However, as turtles are ectothermic and they take over the environmental temperature, they are hard to distinguish from their background by heat profile detection used by the motion trigger (Welbourne, 2013). To overcome this problem, the cameras were set to take pictures every two minutes between 7 PM and 6.15 AM (time lapse). Camera 1, 2 and 3 where placed on 2.3m high posts, facing towards the river, while camera 4 faced the length of the bank and was 50cm above ground (fig. 6). The posts were placed so that the cameras would photograph the areas where turtles had nested in previous seasons. There were two other cameras (5&6) that were set up opportunistically at different times, scattered at different nesting banks with different time settings at nesting bank A and at a smaller nesting bank, 80 meter downstream. All pictures were analysed manually and all animals, their number, date and time of appearance and their activity was recorded. Sometimes, footprints appeared in the sand without a corresponding picture. This occurs because the detection zone of the cameras does not cover its total view, although the Reconyx wildlife cameras used here have the biggest detection zone obtainable (Paul Meek, 2014, personal communication). These occurrences of 'missed animals' were also recorded. Insects, spiders, toads, frogs and ducks were not included in the analysis, as they are not known to be a potential danger to turtle eggs, hatchlings or adults. Besides the presence of animals, the wild life cameras were also able to capture nesting turtles.

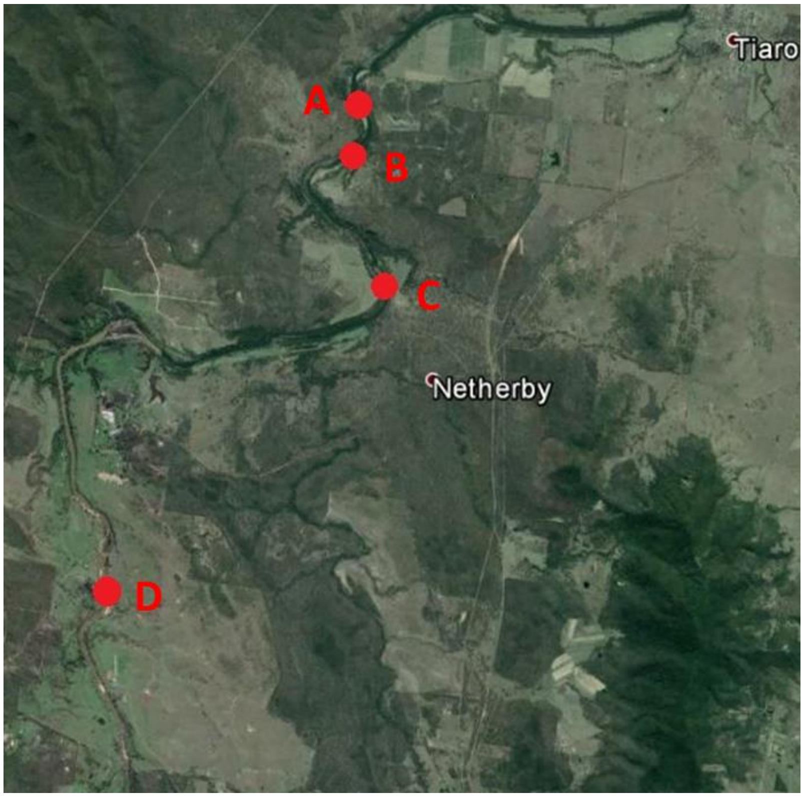


*Fig. 5. The specific stretch of the Mary River with the four nesting banks which were used in this research. Tiaro is located in the upper right corner (25.764 °S, 152.528 °E, Google Earth, 2014).*


*Fig. 6. Four wild life camera posts (red) and their area of view (blue)*

To determine whether global warming might be a possible threat to the Mary River turtle, temperatures of real nests were collected. In the nesting season, between October and January, after at least 10 mm ($10L/m^2$) of rainfall measured at Tiaro, Australia, four suitable nesting banks (fig. 5) were checked for two consecutive days (a total of 26 days) to locate freshly laid nests by searching for nesting imprints. When located, we carefully dug by hand until we reached the first eggs and measured the straight distance between the top of the eggs and the sand surface. We continued digging adjacent to the nest to locate the bottom of the nest and measured the distance between the bottom and the sand surface. A total of 23 nests were tooled with two temperature loggers (i-Button®) corresponding with the depth of the bottom and the top of the nest, adjacent to the original nest so as not to disturb the clutch. The loggers were set to start at least three hours after placement and set to measure the temperature every 20 minutes. It was assumed that the small amount (~15) of eggs with developing embryos was not influencing the nest temperature and that sand temperatures adjacent to the nest were similar to those found in the nest (personal communication David Booth, 2015). Displaced sand was replaced and a 900 x 900mm plastic mesh with 50 x 50mm gaps to let emerging hatchlings through was placed over the nest pinned with eight plastic 300 mm pegs to protect the nest from predators. Three artificial nests with loggers at 5, 10, 15, 20 and 25 cm were constructed at three random locations to measure temperatures at a wider range of depths than the natural nests. The measure for sun exposure was determined per nest as the sum of the amount of sunshine (full=2, medium=1, none=0) at December, 19, 2014, measured every 30 minutes between 6:30 AM and 7:00 PM. It was impossible to determine incubation time, as we were not able to visit the nests daily and there are usually no signs of hatching on the surface. Therefore, a mean of 55 days was used for all nests as standard incubation time (Micheli-Campbell *et al.*, 2012a). 65 days after oviposition (a mean of 55 days plus 10 extra days to give the eggs enough time to hatch), hatching success was determined for intact nests ($H=N/(N+U)$), where N is the number of empty eggshells (> 50% visible), U the number of unhatched eggs and N+U the total number of eggs that were laid.

*Statistical analyses*

Camera pictures were analysed manually. It was impossible to identify whether a turtle on a picture was the same turtle as the turtle on the picture two minutes before, except from when they were nesting, as no identifying features were observable. Therefore, nesting analyses include the real number of turtles, while in the other analysis, the total numbers of turtles found on pictures (numbers of pictures * numbers of turtles on the image) was used. Also other animals were not individually identified, but as they were occurring less frequently, they were analysed as 'unique events'. A unique event was determined when there were at least 30 minutes between the first picture and the following picture of the same animal. Sightings within 30 minutes of the same type of animal were excluded. As all camera pictures were analysed together, this determination of unique sightings also removed the bias of the same animal being captured by several cameras. To keep all night activity together during the analysis, the time after midnight until sunrise was called the same date as the date before midnight.

For the impact of global warming, linear regression was used to estimate five relations: (1) mean nest temperature (top-bottom/2) per day (°C) ~ ambient temperature per day (°C), (2a) mean nest temperature of logger (°C) ~ nest depth of logger (cm), (2b) mean nest depth (top-bottom/2 in cm) ~ day in nesting season (3a) mean nest temperature (top-bottom/2, °C) ~ sun exposure (3b) sun exposure ~ day in nesting season. Hourly ambient temperatures were retrieved from Queensland Department of Natural Resources and Mines, which were measured at nesting bank D, located 5.73 km as the crow flies, upstream from the nesting bank with the cameras (fig. 5).

R programming (R Development Core Team, 2014) was used to perform statistical analyses.

**Results**

Predation

I analysed a total number of 227,529 photographs which included 2813 photographs (12.4%) that captured animals. From the photographs that had animals on them, 590 were turtle photographs (20.1%) and 382 were unique events of other animals (13.6%).

*Camera settings*

Figure 7 shows how often turtles, foxes and goannas (which include Lace Monitors and Yellow-Spotted monitors), activated the motion trigger, how often they were captured by the time lapse pictures or when only footprints appeared in the sand, but nothing was visible on the pictures. As expected (Paul Meek, 2014, personal communication), the motion trigger was almost never activated (1.5%) when a turtle passed by. The scarce triggers were only by post 4, the camera that was low above the ground (personal observation). However, the choice of time lapse at night seems to be the right method: turtles were captured 93.7% of the occurrences and were only missed in 4.7%. Goannas have the highest percentage of being missed (12.2%). This is probably because they are diurnal, when most of the time, the camera did not take time lapse pictures. However, they are triggering the motion trigger pretty well despite being ectothermic (63.4%). At some moments, some cameras were also set at two minute time lapse pictures during the day and this shows that goannas are also easily captured by time lapse pictures (24.4%). Red foxes activated the motion trigger easily (82.6%), most likely because they are endothermic and are probably too fast to be captured regularly by time lapse (13%). They are almost never missed (4.3%).

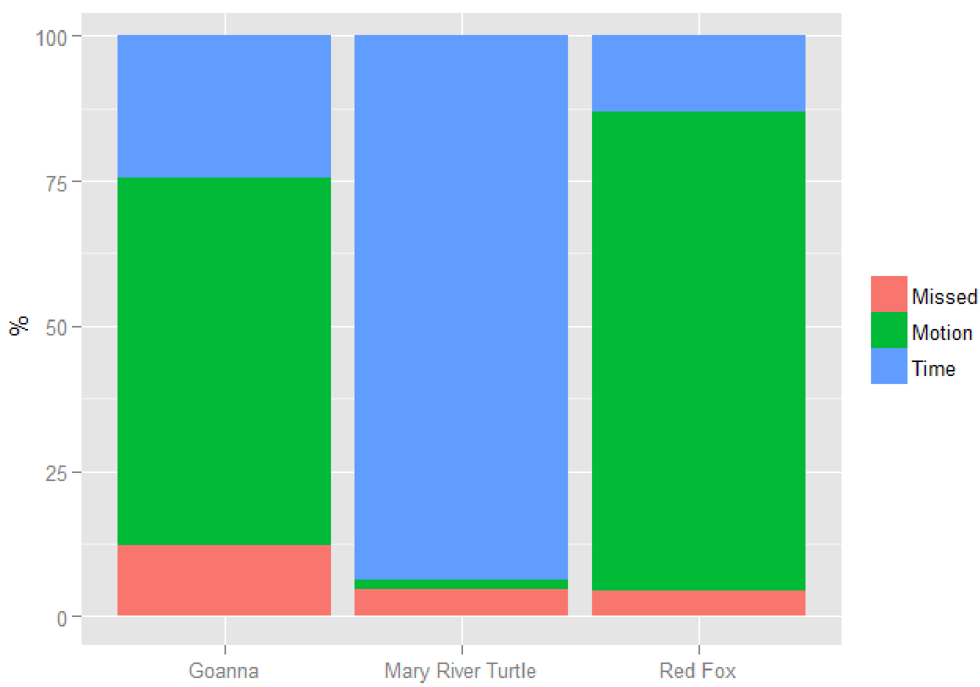


*Fig. 7. Percentage of goanna, turtle and fox pictures captured by motion trigger, time lapse or was missed.*

*Turtles*

All turtles photographed were identified as adult female Mary River turtles (fig. 8a). Although the camera's field of view was directed at nests, they only took two (time lapse) photographs of hatchlings on December 4, 2014, around 21.45 (fig. 8b). There is a decrease in female adult turtle appearances as the nesting season continues. Turtles came en masse on the nesting bank after the first seasonal rainfall mid-October and a second large peak is visible a month later (fig. 9). As known from previous years and found again in this study, the appearance of turtles on the nesting bank is dependent on (even small amounts of) rainfall (fig. 9). However, the actual nesting (in front of the camera) only occurred after at least 10 mm of rainfall (fig. 9, red dots). The last turtle picture was taken on January 9, 2015 despite further occurrences of rainfall after this date (fig. 9). Assuming that the nesting season stopped at January 9, a Chi-square analysis based on the percentages of the presence of turtles the night after rain until the end of the nesting season, reveals indeed a relation between the presence of turtles after a day with at least one mm of rain ($X^2$ (1, N=102) = 6.2, $p$<0.05).


*Fig. 8 a). Five turtles on one picture after the first seasonal rainfall. b) Hatchlings on their way to the water*

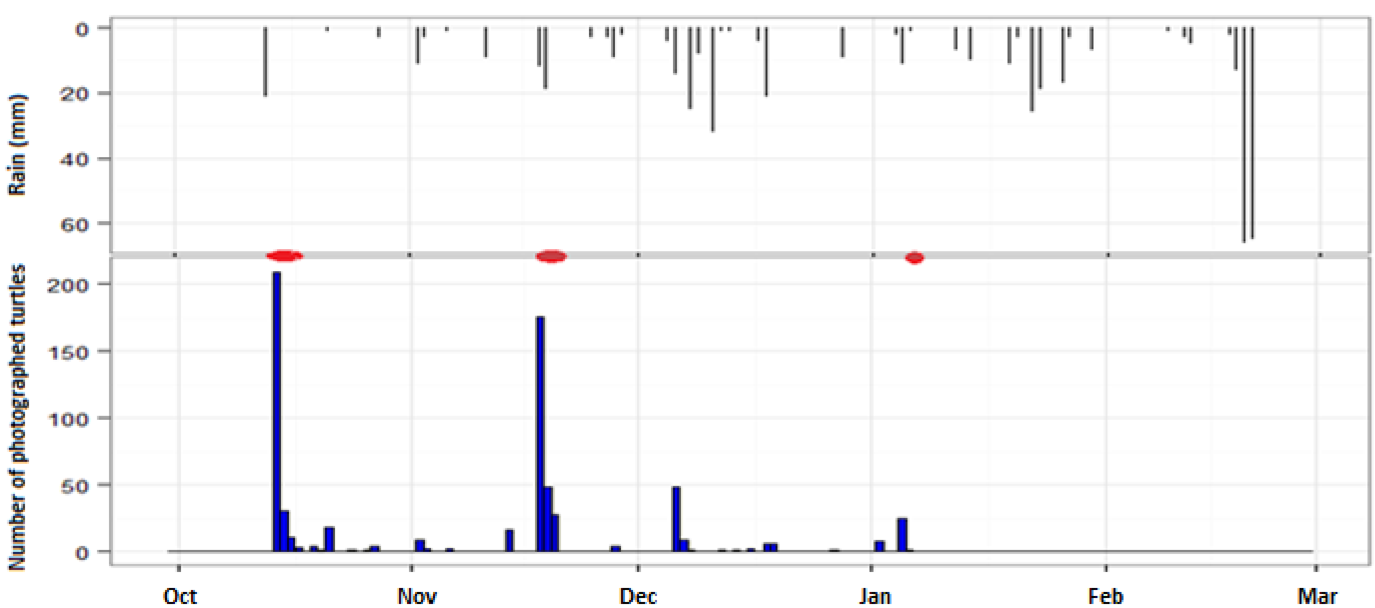


*Fig. 9. Number of photographed turtles and the daily rainfall. Red dots show the occurrences of nesting in front of the camera.*

The wildlife cameras photographed 17 nesting turtles. Nesting time in October (49.8 min) is almost significantly longer than in November (32.1 min) (Two Sample t-test: t(8)=2.29, *p*=0.05; fig. 10a). There are two outliers: one with a nesting time of 12 minutes, which probably was only a test hole without eggs, and a second one of 88 min where it was visible that it was disturbed by other turtles (not shown here). When removing those outliers, the difference in nesting time between October and November becomes significant (Oct: 49.7 min, Nov: 32.1 min, t(11.2)=5.43, *p*<0.001, fig. 10b). When six other observations from previous years were included, the same pattern was found (Oct: 50.9 min, Nov: 32.9 min, t(14.6)=7.0113, *p*<0.001, fig. 10c).

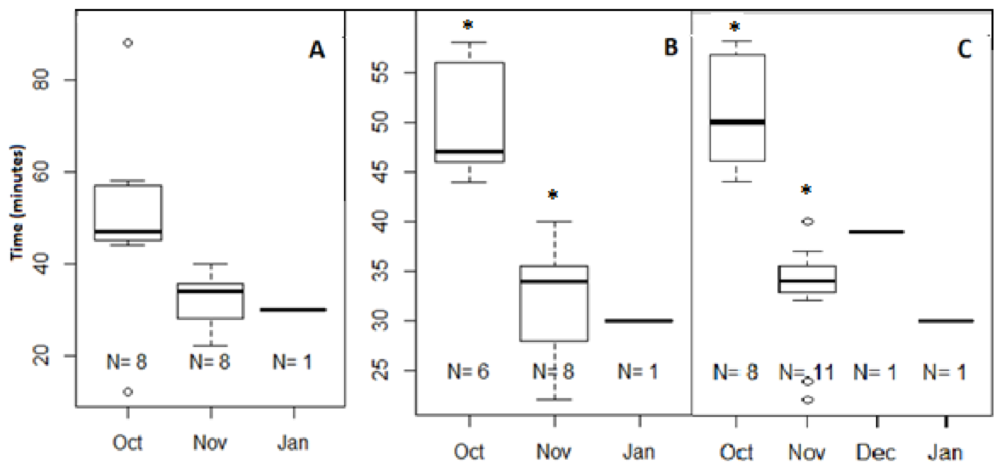


*Fig. 10. Nesting time per month a) all data points included, b) outliers removed, c) including six nests from previous years*

An obvious explanation of the difference in nesting time, might be a difference in the amount of eggs. Unfortunately, only the number of eggs of four nests that were laid in front of the camera could be determined, which leads to a non-significant prediction (Linear model: $R^2$=0.537, *p*=0.268, fig. 11a). However, when including all nests of this nesting season (N=54), no differences are found between October, November and December (One-way anova: mean=15, *F*(2, 54) = 0.12, *p* = 0.887, fig. 2.11b).

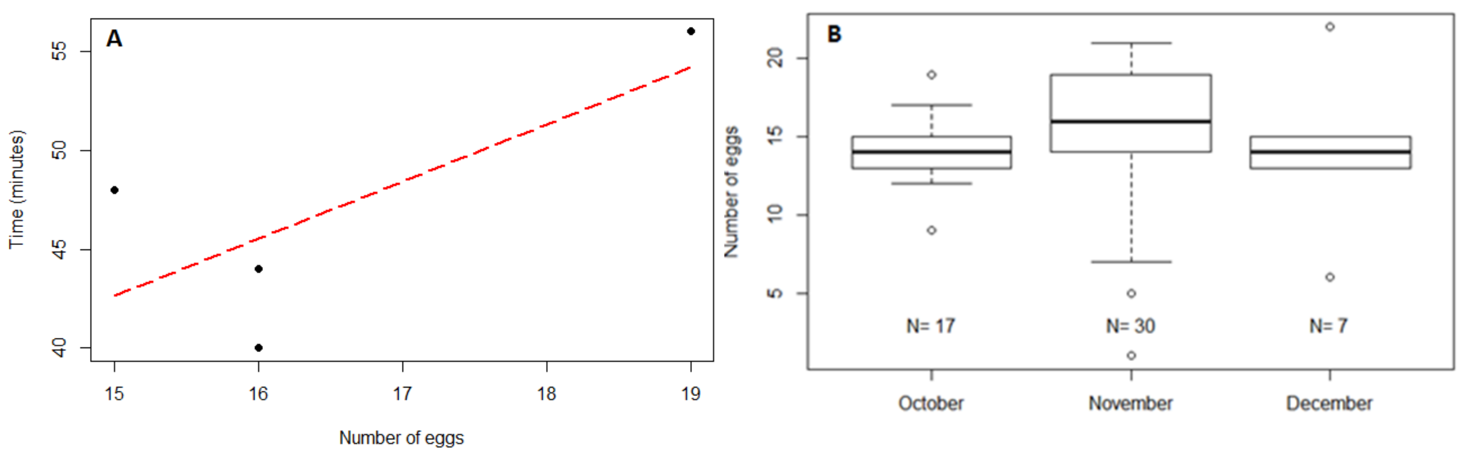


*Fig. 11a) Non-significant relation between the number of eggs in a nest and the nesting time ($R^2$=0.537, p=0.268), b) Boxplot showing no difference in the number of eggs between months*

*Predators*

Figure 12 shows the animals, except for turtles, that were found on the photographs and their potential threat to the Mary River turtle. People do not appear as a threat, as most images were of the Project team members. Lace monitors and Red foxes seem to be the main nest predators as they were found in respectively 80 and 23 unique events and each one was photographed predating one nest (fig. 13 b, d). Only foxes and Lace Monitors are known to predate eggs, but other animals, such as the Eastern Water dragon (*Intellagama lesueurii lesueurii,* 8 unique events, WAZA, 2015) and the Nankeen Night-Heron (*Nycticorax caledonicus,* 1x, Limpus *et al*., 2003) are known to actively predate on hatchlings. Cows (3x) are seen as passive threats, as they accidentally break eggs while walking on the nest. Several large snake species, such as the Carpet Phyton (*Morelia spilota*, 5x) and the Red-Bellied Black Snake (*Pseudechis porphyriacus*, 3x), Brushed-Tailed possum (*Trichosurus Vulpecula*, 4x), platypus (*Ornithorhynchus anatinus*, 1x) and water rat (*Hydromys chrysogaster,* 2x) and the owl species Tawny Frogmouth (*Podargus strigoides,* 1x), will not actively hunt, but might eat an hatchling when their paths cross. They are therefore not seen as an active threat to the hatchlings (P. Couper, Queensland Museum, 2015, personal communication). However, I did not find evidence that any of these animals predated on hatchlings, although this lack of evidence might be more due to the fact that hatchlings were only captured twice on the pictures. None of these animals were found attacking adults turtles, even when foxes were close to adult turtles (fig. 13 a, c).

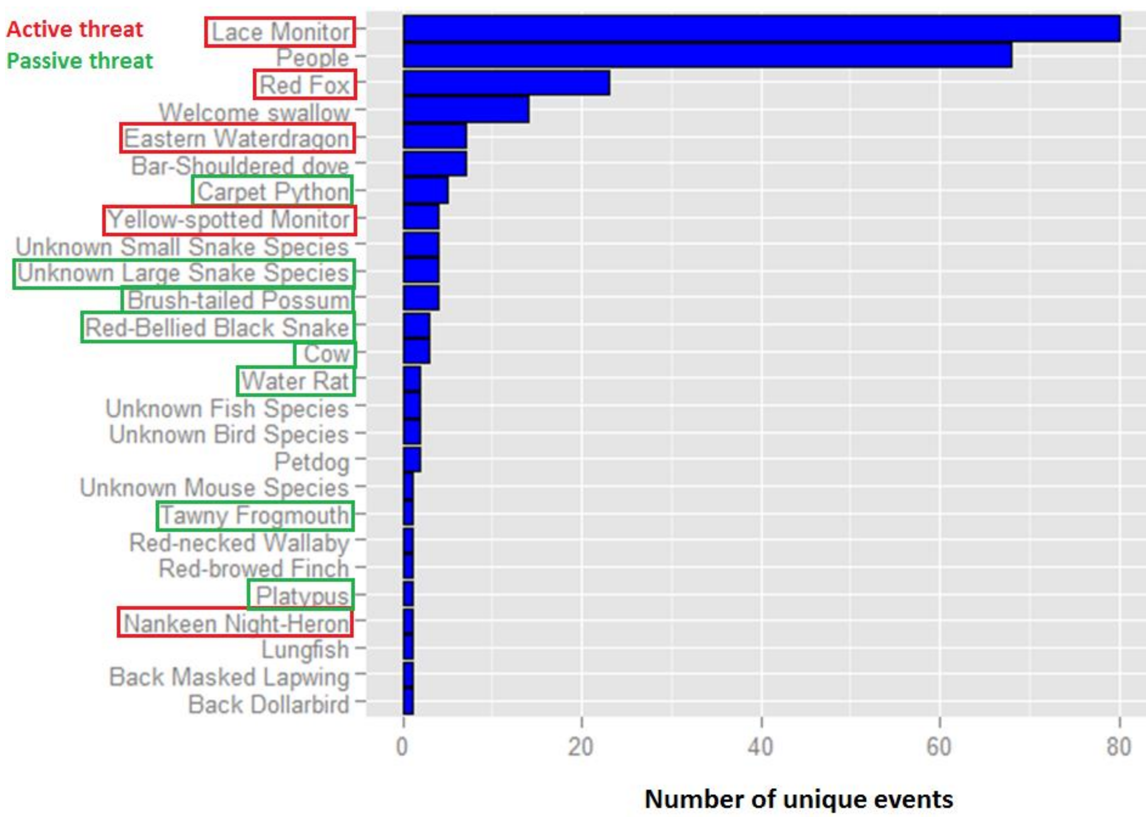


*Fig. 12. Animals, except turtles, found on the nesting bank with their potential threat (red=active, green=passive, no color=no known threat) to the Mary River turtle.*

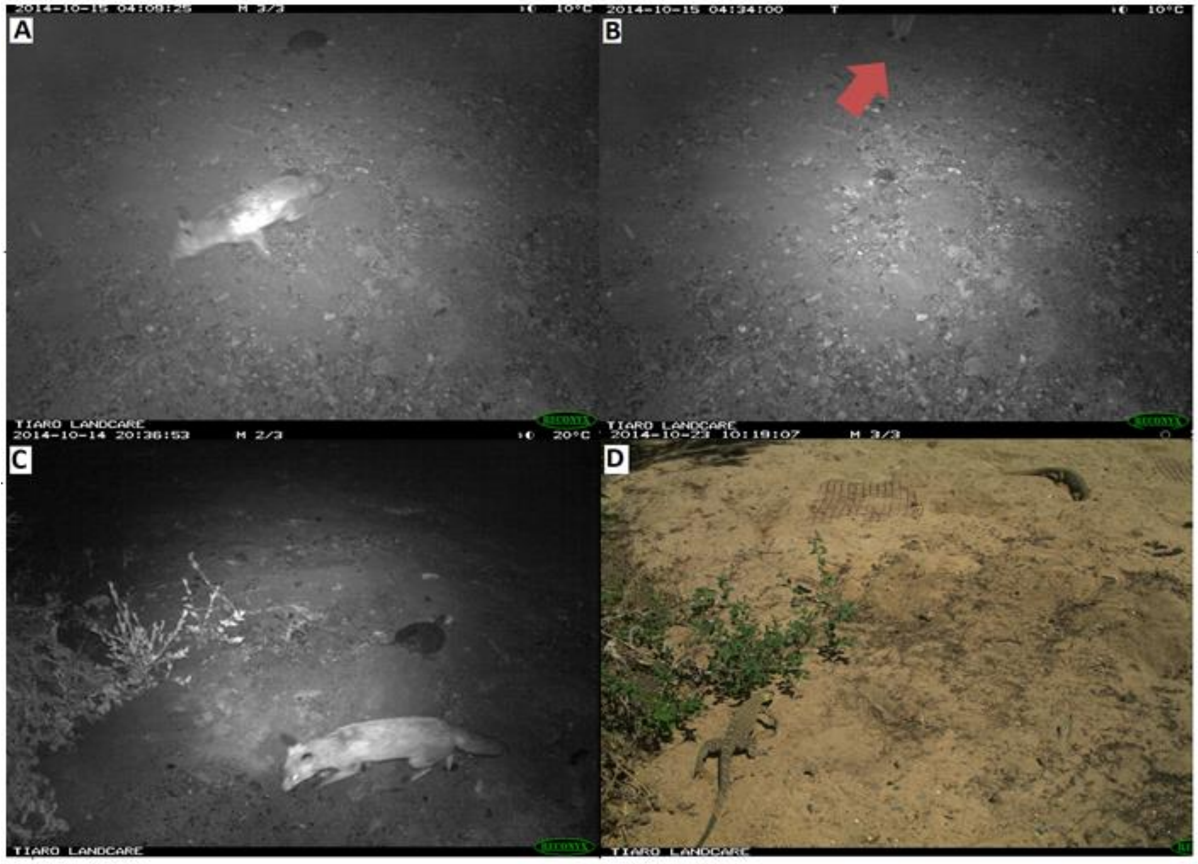

*Fig. 13 a). A Mary River turtle is nesting while a fox walks by. b) Two minutes after the turtle from picture A left, a fox is predating the nest. c) A turtle and fox close to each other without visible interaction. d) Two Lace monitors, one is predating a nest that was laid one week before.*

When focussing on the activity patterns of foxes and Lace monitors appear at random hours during the night, while Lace Monitors are diurnal and show a peak in occurrences before noon (fig. 14).

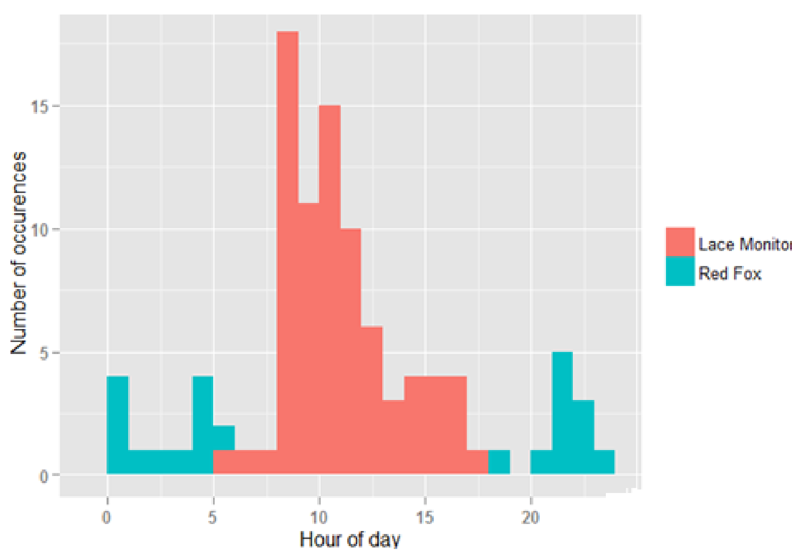

*Fig. 14. Activity patterns of Lace Monitors and Red Foxes, the main predators of the Mary River turtle nests*

Figure 15 shows whether the occurrences of the Mary River turtle and its two main predators overlap around the nesting season or if the predators are present at random times. 'Overlapping' is used to mean that foxes and turtles are found on photographs in the same night (with the date after midnight until sunrise the same date as before midnight). For Lace Monitors overlapping with turtles means the day after that night. Lace Monitors seem to occur without the presence of turtles and are already present before the nesting season. Foxes seem to arrive when the first turtles come onto the nesting bank. A Chi-square analysis based on the percentages of occurrences of turtles and Lace monitors and turtles and foxes, reveals indeed a relation between the presence of turtles and foxes ($X^2$ (1, N=117) = 4.8, $p<0.05$), but not between turtles and Lace Monitors ($X^2$ (1, N=117) = 1.8, $p>0.05$). The Mary River flooded between January 24 and February 4 and therefore the cameras were removed. They were replaced after the flood, but did not capture anymore animals on the camera.

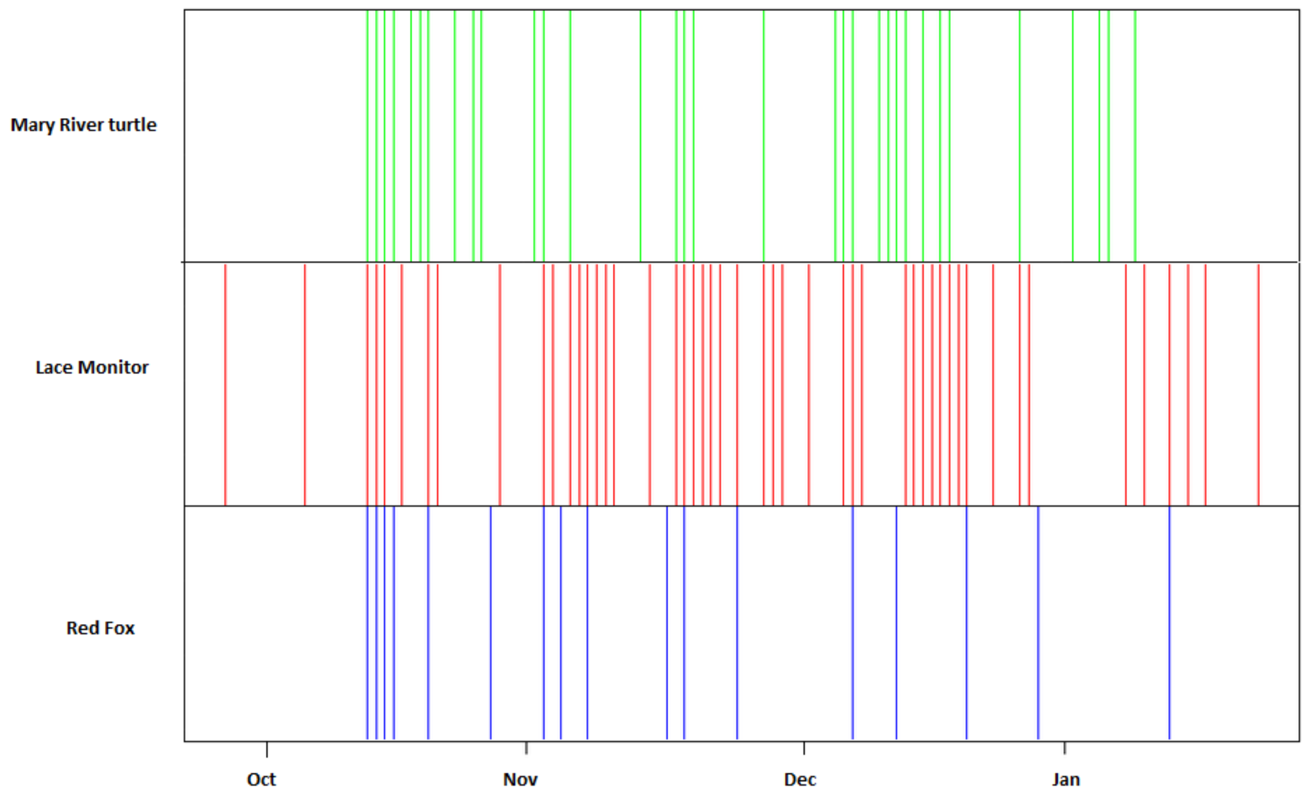

*Fig. 15. Occurrences of Mary River turtles, Lace monitors and Red foxes around the nesting season.*

## Global warming

Although temperature loggers were placed in 23 nests, due to broken temperature loggers and problems with the logger's memory, not all nest temperatures were recorded during the incubation period. Nests that had more than 49% data of the 55 incubation days for both top and bottom were included in data analysis (14 nests, table 1). 8, 13 and 29 days of data was collected from the three artificial nests. Measured temperatures from real nests ranged from 18.5 to 40.5 °C with a mean of 28.35°C.

*Table 1: nest used for data analysis with laying date, nesting bank and the % of available data*

| Nest | Laying date | Nesting bank | Available data (% from 55 days) |
|---|---|---|---|
| **1** | 15 oct | A | 51.9 |
| **4** | 15 oct | A | 51.9 |
| **6** | 15 oct | C | 53.6 |
| **7** | 15 oct | C | 66.1 |
| **9** | 15 oct | C | 53.6 |
| **11** | 4 nov | B | 53.6 |
| **12** | 20 nov | C | 53.6 |
| **14** | 20 nov | C | Bottom: 49.3<br>Top: 50.4 |
| **16** | 20 nov | B | 53.6 |
| **18** | 29 nov | B | 55.4 |
| **19** | 8 dec | C | 89.3 |
| **21** | 18 dec | C | 94.6 |
| **22** | 18 dec | C | 91.1 |
| **23** | 20 dec | C | Bottom: 78.6<br>Top 76.8 |

Mean ambient temperature per day increased significantly throughout the nesting season, but day does not explain all the variation (only days with data from three nests or more, Linear model: $\beta$=0.026, $R^2$= 0.123, $p$<0.001, fig. 16, blue). A similar pattern is found for mean nest temperature per day (Linear model: $\beta$=0.022, $R^2$=0.155, $p$<0.001, fig. 1.3, red). Notice that this is only a linear regression in the nesting season. After the nesting season (March) temperatures are dropping. Figure 16 also shows that nest temperatures follow ambient temperature fluctuations, and when performing a linear model, mean daily ambient temperature predicts mean daily nest temperature ($\beta$=0.584, $R^2$=0.412, $p$<0.001, fig. 17). Figure 17 shows that most of the mean daily nest temperatures are within the optimal range of 28± 3°C (83%), 8% are below and 9% are above this optimal range.

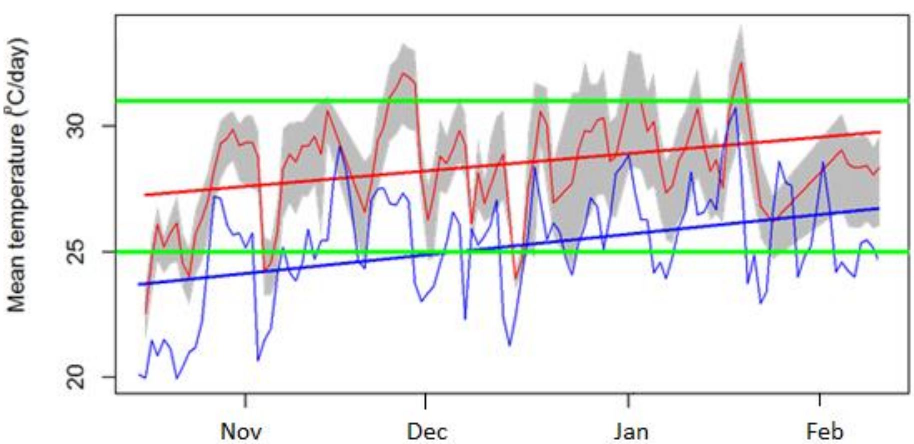


*Fig. 16. Ambient temperature (blue) and nest temperature (red) with minimum and maximum values (grey) throughout the nesting season. Both do change significantly over time (ambient: $\beta$=0.026, $R^2$= 0.155, p < 0.001, nest: $\beta$=0.022, $R^2$=0.123, p<0.001). The green lines show the optimal nest temperature range.*

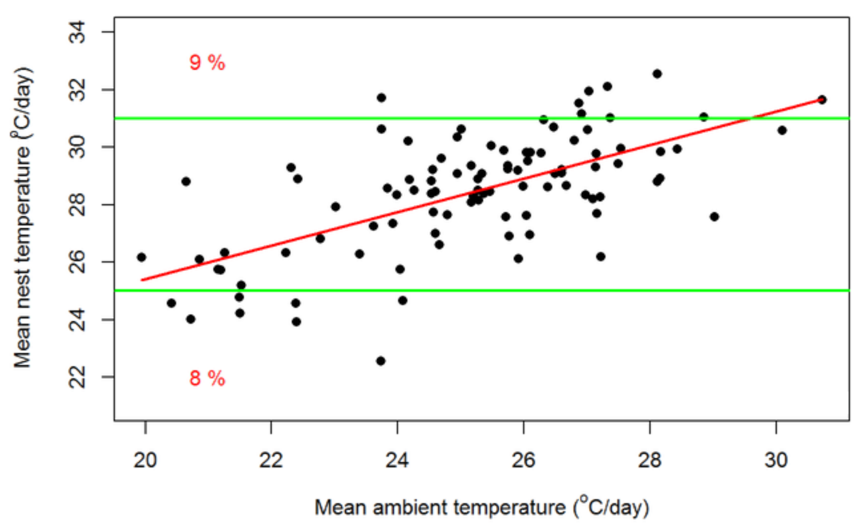


*Fig. 17. Mean ambient temperature per day predicts mean nest temperature per day ($\beta$=0.584, $R^2$=0.412, p<0.001). Red numbers show the % of data points above and below the optimal nest temperatures (green).*

*Nest depth – Fluctuations*

Bottom nest depths range from 17-22 cm, top nest depths range from 11-17 cm and mean nest depth ranges from 14.5-19.25 cm (table 2, fig. 18). For all nests, temperatures at the top of the nest fluctuate more than the temperatures at the bottom of the nest (fig. 19).

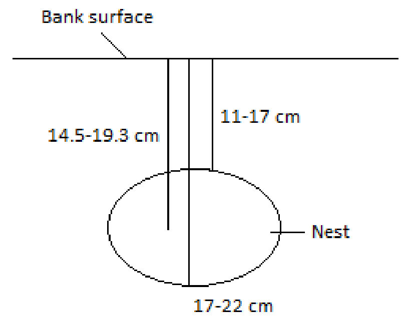


*Fig 18. Nest depths*

When nest depths are divided in shallow (<17cm) and deep nests (>= 17 cm), fluctuations differ significantly (2-sample t-test: below mean t(217.7)=-6.8266, p<0.001; above mean t(210.2)=-8.3614, p<0.001, fig. 20). This gives shallow nests a mean fluctuation of 4.70 °C and deep nests a mean fluctuation of 3.67°C. Despite the difference in fluctuations, the difference between mean top and bottom temperature is sometimes significant, but small (mean absolute temperature difference = 0.29°C, table 2).

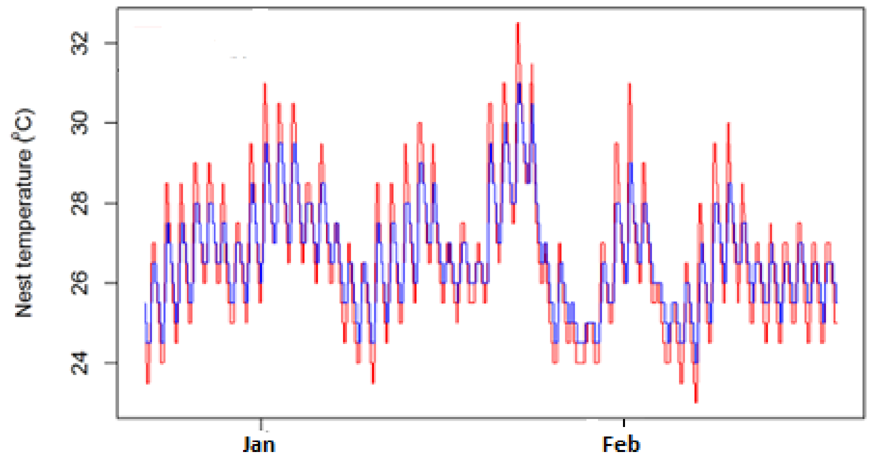


*Fig. 19. An example of a nest (22) where top temperatures (red) fluctuate more than bottom temperatures (blue).*

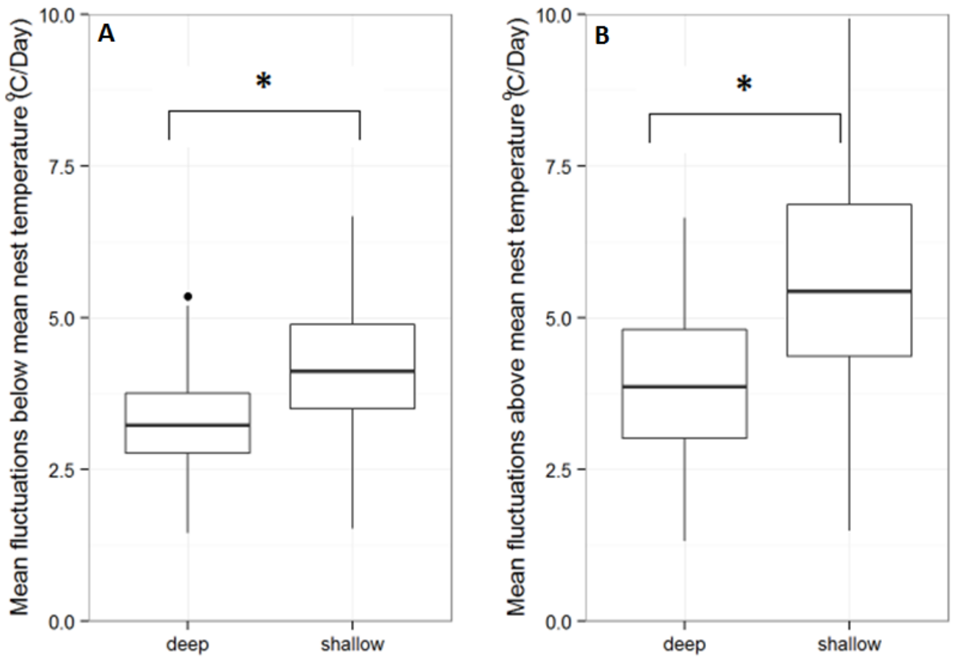


*Fig. 20. Mean temperature fluctuations per day a) above the mean nest temperature per day and b) below the mean nest temperature per day for shallow nests (< 17 cm) and deep nest (=> 17 cm)*

*Table 2. Nest temperatures and depths and the paired t-test statistics for the difference between mean top and bottom nest temperatures*

| Nest | | Mean nest temperature (°C) | Mean nest temperature total (°C) | Temperature difference (Top-bot) | Depth (cm) | Depth difference (Top-bot) | *t* | *df* | *p* |
|---|---|---|---|---|---|---|---|---|---|
| **1** | Top | 25.69 | 25.70 | -0.01 | 11 | 7.5 | 0.236 | 664 | 0.813 |
| | Bottom | 25.70 | | | 18.5 | | | | |
| **4** | Top | 27.56 | 27.40 | 0.33 | 13.5 | 6.5 | -4.756 | 664 | <0.001 * |
| | Bottom | 27.23 | | | 20 | | | | |
| **6** | Top | 28.35 | 28.15 | 0.40 | 13 | 5 | -16.116 | 682 | <0.001 * |
| | Bottom | 27.95 | | | 18 | | | | |
| **7** | Top | 29.59 | 29.21 | 0.76 | 13 | 6 | -11.401 | 821 | <0.001 * |
| | Bottom | 28.83 | | | 19 | | | | |
| **9** | Top | 27.64 | 27.64 | 0.01 | 12.5 | 6.5 | -0.084 | 682 | 0.933 |
| | Bottom | 27.64 | | | 19 | | | | |
| **11** | Top | 30.69 | 30.40 | 0.58 | 14.5 | 7.5 | -12.351 | 682 | <0.001 * |
| | Bottom | 30.11 | | | 22 | | | | |
| **12** | Top | 27.55 | 27.51 | 0.07 | 15 | 7 | -2.338 | 682 | 0.020 |
| | Bottom | 27.48 | | | 22 | | | | |
| **14** | Top | 28.46 | 28.01 | 0.89 | 13 | 6 | -26.361 | 486 | <0.001 * |
| | Bottom | 27.57 | | | 19 | | | | |
| **16** | Top | 29.81 | 29.76 | 0.08 | 12 | 5 | -1.718 | 682 | 0.086 |
| | Bottom | 29.72 | | | 17 | | | | |
| **18** | Top | 29.25 | 29.26 | -0.01 | 13 | 6 | 0.318 | 686 | 0.751 |
| | Bottom | 29.26 | | | 19 | | | | |
| **19** | Top | 25.45 | 25.61 | -0.32 | 17 | 4.5 | 12.237 | 192 | <0.001 * |
| | Bottom | 25.76 | | | 21.5 | | | | |
| **21** | Top | 29.29 | 29.94 | -0.27 | 14 | 6 | 4.284 | 538 | <0.001 * |
| | Bottom | 29.57 | | | 20 | | | | |
| **22** | Top | 26.62 | 26.63 | -0.02 | 14.5 | 3.5 | 1.169 | 1179 | 0.243 |
| | Bottom | 26.64 | | | 18 | | | | |
| **23** | Top | 30.49 | 30.62 | -0.29 | 16 | 2 | 13.897 | 902 | <0.001 * |
| | Bottom | 30.77 | | | 18 | | | | |
| **Mean** | | | | 0.29 | | 5.6 | | | |

*Nest depth - Mean nest temperature*

All total mean nest temperatures are within the optimal range of 28± 3°C (fig. 21, table 2). Mean nest temperatures at nesting bank A seem to be lower than temperatures at nesting bank D, but the number of nests is too low to perform a statistical test. Nesting bank C shows variation in the mean nest temperatures.

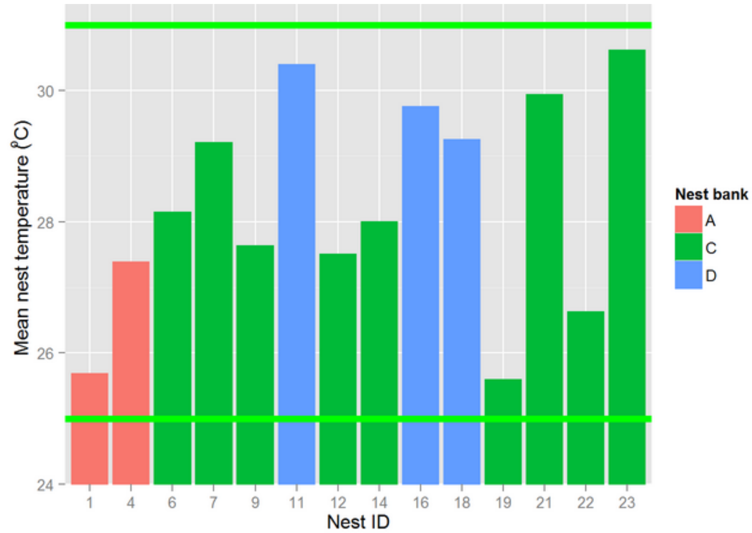


*Fig. 21. Mean nest temperatures per nest and nesting bank, green line indicates the optimal range of 28± 3°C.*

Nest depth does not predict mean bottom or mean top nest temperature ($R^2$= 0.007, $p$> 0.05, fig. 22a). This is the same for depths outside the natural range ($R^2$= 0.005, $p$>0.05, fig. 22b). One artificial nest was constructed on a spot on nesting bank A where no nesting turtles were observed although the sand substrate seemed suitable. This nest showed higher mean nest temperatures than average (fig. 22b, blue). Figure 22 also shows that nest size differs but this is not related to the number of eggs in the nest ($R^2$=0.001, $p$=0.932, not shown). The day of the nesting season does not predict mean nest depth (fig. 23, $R^2$= 0.140, $p$=0.188). Also mean ambient temperature at day or one day before day of nesting does not predict mean nest depth (Appendix I).

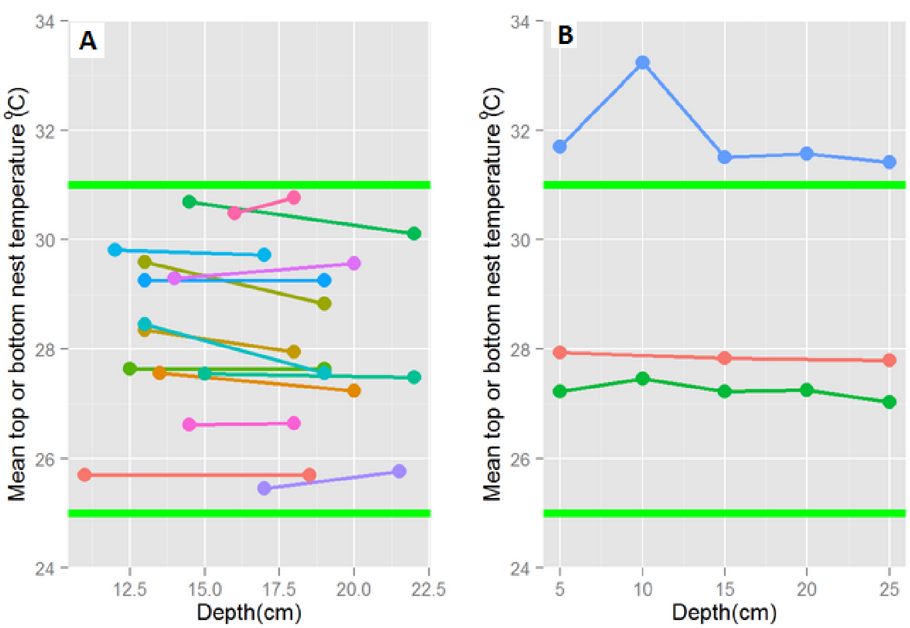


*Fig. 22. Nest depth does not predict mean nest temperature in a) real nests ($R^2$= 0.007, p> 0.05) and b) three artificial nests ($R^2$= 0.005, p>0.05). Same colors belong to the same nest.*

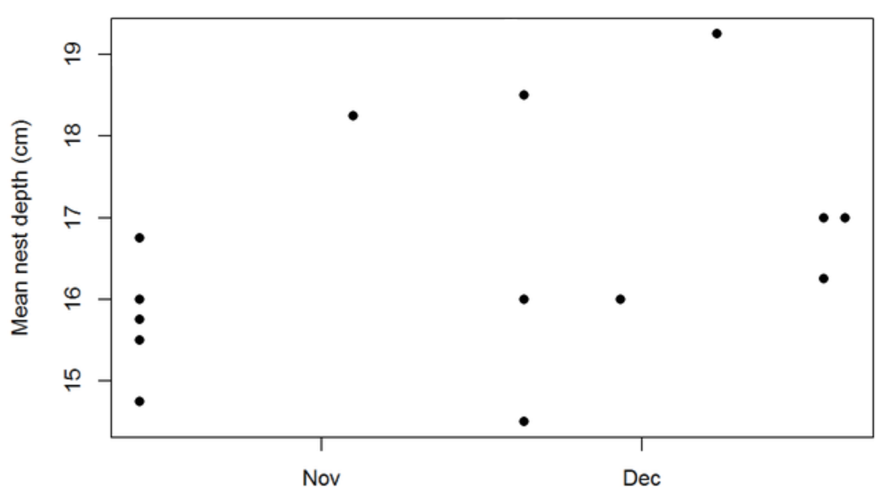


*Fig. 23. The day in the nesting season does not predict mean nest depth ($R^2$= 0.140, p=0.188).*

*Sun exposure*

Mean nest temperature per nest increases significantly with increasing sun exposure on the nest, with sun exposure explaining 40.2% of the variation ($\beta$=0.184, $R^2$=0.402, $p$=0.027, fig. 24). The day in the nesting season on which the nest is laid does not predict the amount of sun exposure on the nest ($R^2$= 0.112, $p$=0.241, fig. 25). Also mean ambient temperature at day or one day before day of nesting does not predict mean nest depth (Appendix I).

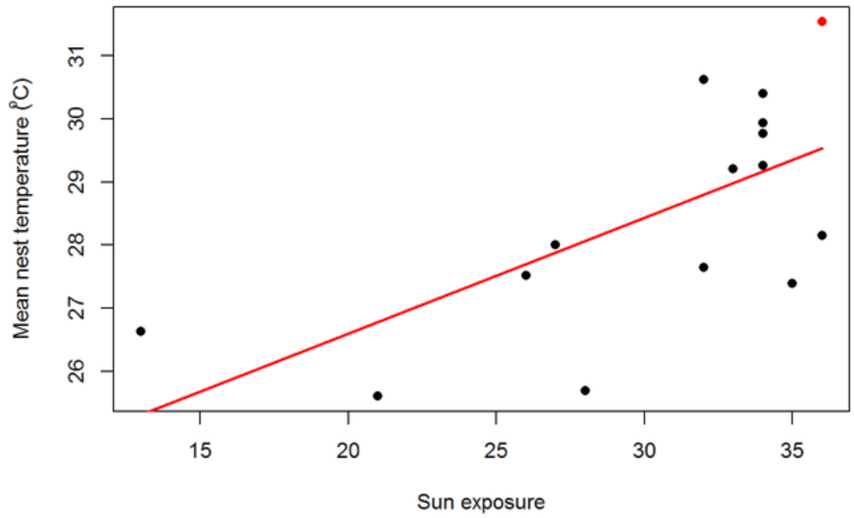


*Fig. 24. Sun exposure predicts mean nest temperature for real nests (black dots) and one artificial nest (red dot) (β=0.184, $R^2$=0.402, p=0.027).*

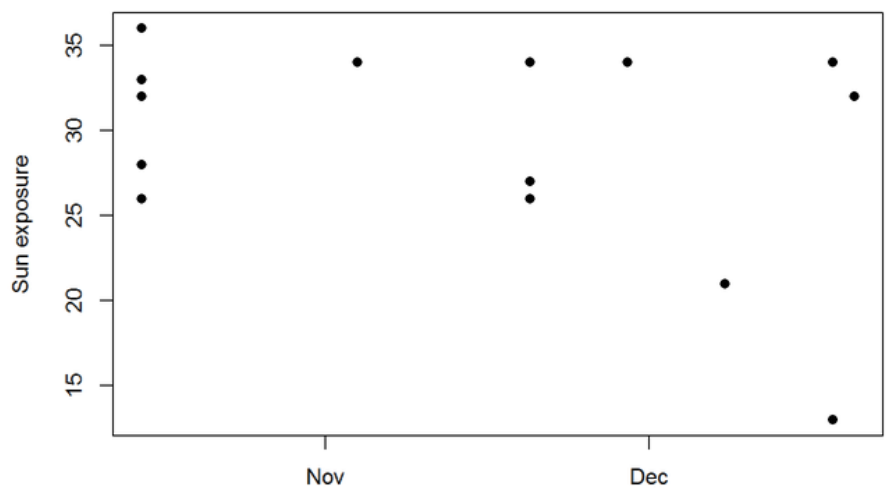


*Fig. 25. Day of nesting season does not predict the amount of sun exposure on the nest ($R^2$=0.112, p=0.241).*

*Hatching success*

Seven nests had a hatching success of 100%, six nests had a hatching success higher than 65% and only one nest had a hatching success of 15% which was due to the flooding of the nest and it was therefore removed from further analysis on hatching success (fig. 26). Mean hatching success was 91.1%.

Mean nest temperature predicts hatching success (β=-4.63, $R^2$=0.352, *p*=0.042, fig. 27a), but the percentage of nest temperatures per nest above 34 °C, which is the maximum temperature Micheli-Campbell *et al.*, (2012a) measured in the lab (fig. 27b), did not. Also mean nest depth (fig. 27c) or sun exposure (fig. 27d) did not predict hatching success. A linear model with all four variables and their interactions included, reduced using backwards selection led to the same results (Appendix II).

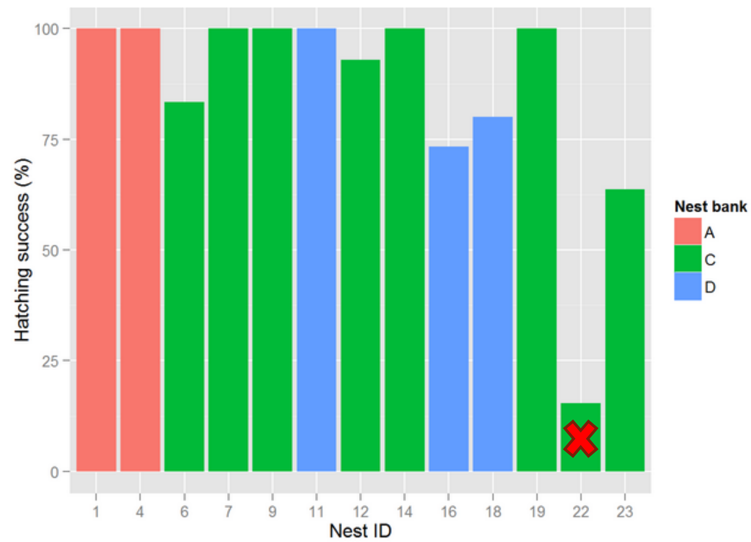


*Fig. 26. Percentage of hatching success per nest. Nest 22 was drowned by a flood and removed from further hatching success analysis.*

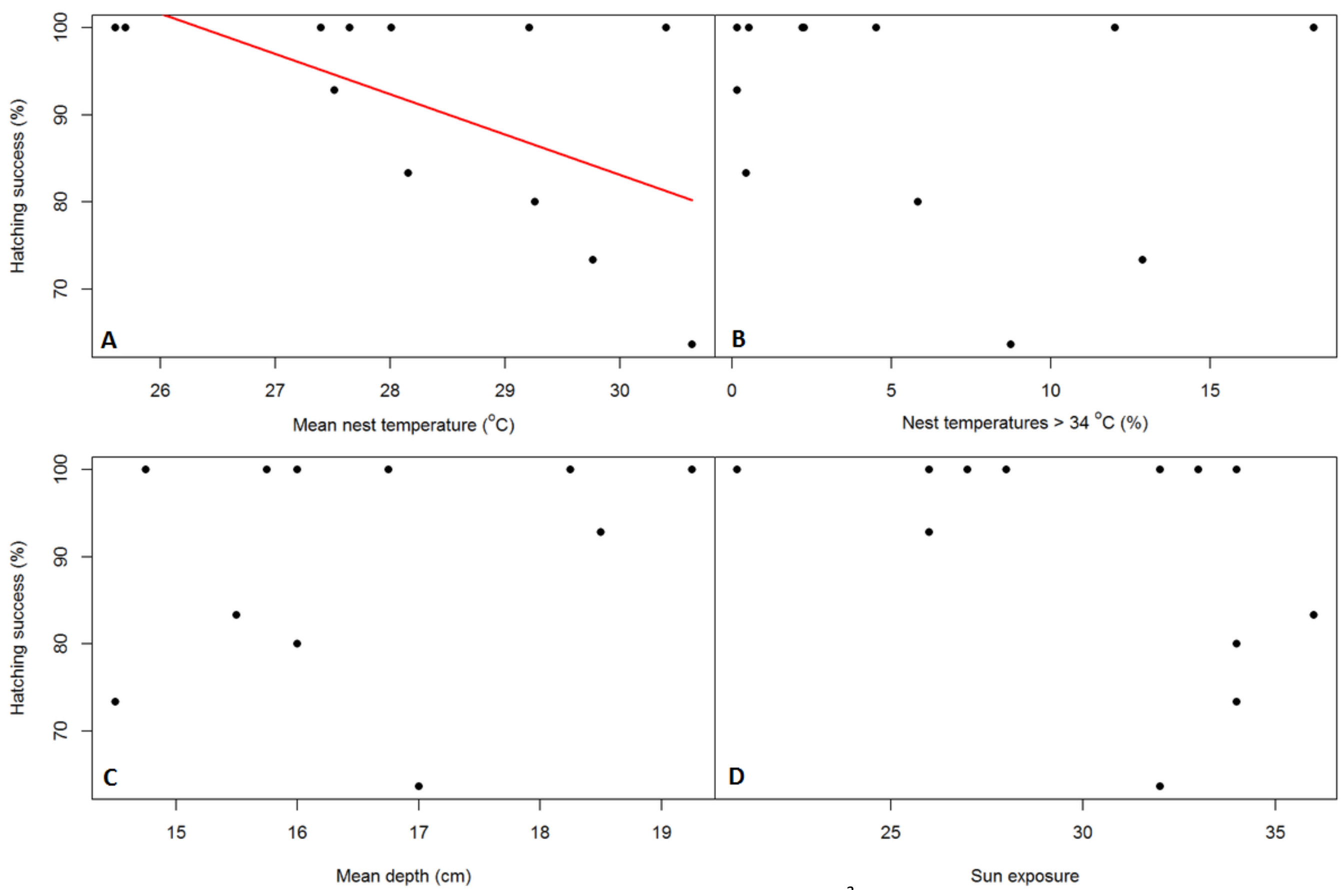


*Fig. 27. Hatching success is predicted by a) mean nest temperature (β=-4.63, $R^2$=0.352, p=0.042) but not by b) percentage nest temperatures > 34°C ($R^2$=0.263, p=0.088), c) mean depth ($R^2$=0.056, p=0.458) or d) sun exposure ($R^2$=0.226, p=0.119).*

**Discussion**

This research aimed to determine the impact of two possible threats, predation and global warming, to the Mary River turtle.

*Predation*

As known from previous years, but now for the first time captured on camera, turtles only come onto the nesting bank after rainfall. This is consistent with other riverine species in the tropics (Moll & Moll, 2004). Frits *et al.*, (1982) found that nesting in sea turtles was more successful after a period of rainfall, because the sand was more stable. This is also suggested as an explanation as to why the Mary River turtle chooses to come on land after rainfall. Two nesting peaks were found, despite occurrences of rainfall between those peaks. This might suggest a second laying by the same female, which is not unusual in turtles (Whitfield Gibbons *et al.*, 1982). However, individual turtles could not be identified in this study, so a genetic analysis or individual tracking study needs to confirm this suggestion.

Red foxes and Lace Monitors were photographed predating nests and therefore seen as the main nest predators. Red foxes are more active when turtles are on the nesting bank, while Lace Monitors are also on the nesting bank when turtle have not been there the previous night. This is in disagreement with Booth & Lei (personal communication, 2015), who found that goannas at beaches started their visits when sea turtles were actually nesting. Both predators were not photographed after the last nests hatched. However, the nesting bank flooded for a week in this period. So whether the absence of predators is related to the absence of turtles or whether their absence was due to the flood remains unclear. There were no photographs of hatchlings being predated on. However, this is most likely due to the fact that hatchlings were only photographed twice, although cameras were pointing at nests. Although hatchlings emerge worldwide at night (e.g. Salmon & Reising, 2014), Plummer (2007) found that freshwater turtle hatchlings emerge from their nest at sunset, when it is not totally dark. When patrolling the nesting bank in the current study, two nests were observed hatching at December 16, 2015, around 16.30. This might suggest that the hatchlings of the Mary River turtle hatched at times when the camera was not active. This is most likely from the late afternoon until 7 PM. Although there was no evidence of the predation of hatchlings, Eastern water dragons and Nankeen night herons, which are known to predate hatchlings (Patrick Cooper, 2015, personal communication), were found on the nesting bank. Two pictures showed foxes and turtles in the same picture without any further interaction and we never found a dead adult on the nesting bank. This might suggest that foxes are not interested in adult Mary River turtles, although there is evidence of foxes attacking nesting Murray turtle females (Thompson & Spencer, 2015). It is therefore still something to be aware of in the future.

In addition to photographs of predators, also nesting turtles were photographed. It was found that nesting time in the second peak was significantly shorter than during the first peak. The literature does not report abundantly about nesting times, but Withfield Gibbons *et al.* (1982) found a decrease in the amount of eggs throughout the nesting season. The number of eggs might be a logical explanation to predict nesting time, but the current study did not find that the number of eggs predicted nesting time. Another possibility for a longer nesting time during the first peak is disturbance due to intraspecific competition. During the first seasonal rainfall many turtles came onto the nesting bank at once to look for a suitable nesting place, while mid-November the number of turtle was lower. Further research should confirm this suggestion.

*Global warming*

It was hypothesized that if the Mary River turtle could adapt its nesting behaviour to increasing ambient temperatures, a constant nest temperature would be found throughout the nesting season, despite increasing ambient temperatures. It was found that ambient temperatures increased throughout the nesting season, but that nest temperatures followed this pattern instead of being constant as hypothesized. It was also found that mean daily ambient temperature did predict mean daily nest temperature. This means that turtle eggs are likely to experience higher incubation temperatures with increasing ambient temperatures.

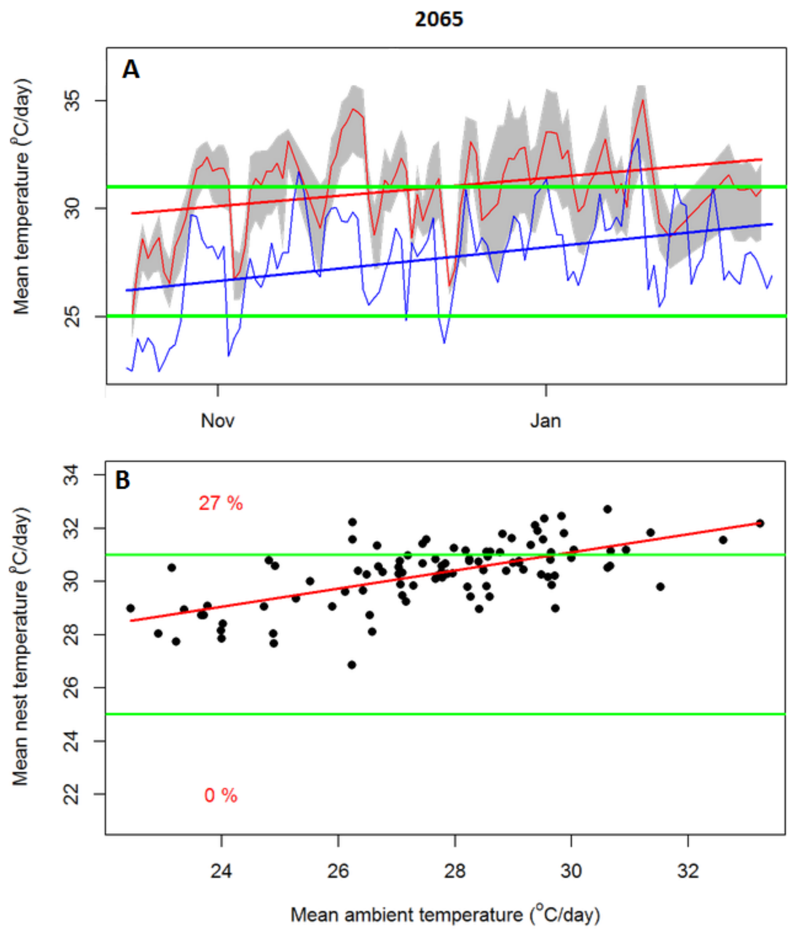


*Fig. 28. Expected scenario with an increase of 2.5 °C in ambient temperature a) Ambient and nest temperatures expected throughout the nesting season and b) Prediction of nest temperature by ambient temperature following the estimated prediction:*

$$\textit{nest temperature} = 13.7 + 0.412 * \textit{ambient temperature}$$

Figure 28 shows the potential scenario in 2065 with the expected increase of 2.5 °C in ambient temperature in Australia. Halfway the nesting season (mid-December), mean nest temperatures will rise above the optimal range (fig. 28a). In the current season 9% of the mean daily nest temperatures were above the optimal range and 8% below (fig. 17). In 2065, 27% will be above the optimal range, while no temperatures are below this range (fig. 28b). Both graphs show that the increase in temperature will have an impact on the nest temperatures. This is especially true in combination with the possible lower hatching success that was found with increasing nest temperatures, which was not found by Micheli-Campbell *et al.* (2012a) in their lab study. However, I should be cautious to draw conclusions about this, as only 50% of the nest temperatures were available for data analysis. This can cause a bias when for example only nest temperatures were measured in the beginning of the season in the first period of the incubation period, where ambient temperatures and therefore nest temperature were cooler.

To keep nest temperatures within the optimal range despite the increase in ambient temperature, two possible adaptations for adult females to regulate nest temperatures were studied, namely influencing nest depth and the amount of sun exposure on the nest.

To be able to regulate nest temperature with nest depth it was assumed that deeper nests had lower mean nest temperatures. This assumption partly did not hold as there was no relation found between nest depth and mean nest temperature. However, nest depth determined the size of temperature fluctuations: temperatures in shallower nest fluctuated more than they did in deeper nests. This is found by many other studies (e.g. Roosenburg, 1996; Glen *et al.*, 2006). These findings mean that turtles can influence the size of temperature fluctuations by changing nest depth, but they cannot influence mean nest temperatures. The question rises which of the two is most important to regulate. This is a complicated question to answer, because mean nest temperature determines the impact of the fluctuations. Small fluctuations at higher mean nest temperatures will have a different influence than large fluctuations at lower mean nest temperatures. Campbell (2012a) found that fluctuating temperatures had less negative impact on the eggs and hatchlings of the Mary River turtle than constant high temperatures. Refsnider (2012) found that hatchlings of the Painted turtle performed faster with larger fluctuating incubation temperatures. It is however yet unknown whether more active hatchlings have a higher fitness. One might speculate that when a hatchling rights itself faster, it is stronger and can escape easier from a predator. However, Janzen (1994) showed that slower hatchlings had a higher first-year survival, because they were less visible for predators. So whether turtles should influence fluctuations to increase fitness remains unclear.

To be able to adapt to global warming, it was hypothesized that the Mary River turtle should dig its nest deeper throughout the nesting season as ambient temperatures were increasing. Turtles did not dig their nest deeper throughout the nesting season, neither did the mean ambient temperature on the day of oviposition or the day before predict nest depth. This can have four possible explanations: (1) it is not important to regulate fluctuations and although important, it is impossible to control mean nest temperatures by changing nest depth. (2) The Mary River turtle is not capable of influencing nest depth because nest depth is restricted by its rear limb length (Refsnider, 2012). (3) It is unlikely that turtles can predict ambient temperatures during the incubation period and can therefore not respond with adjusting nest depth (Warner & Andrews, 2002). (4) They already have adapted well, and there was no threat this year as all mean nest temperature were within the optimal range, and hatching success in all our nest was reasonable high. Adding up these facts I conclude that turtles will most likely not use nest depth to keep nest temperatures within the optimal range.

To be able to influence nest temperature with the amount of sunshine on the nest, it was assumed that sun exposure predicted mean nest temperature. This assumption was correct: a higher mean nest temperature was found with an increasing amount of sun exposure on the nest. This is consistent with many other studies such as Morjan, 2003; Refsnider *et al.,* 2013 and Wood *et al.*, 2014. To be able to adapt to global warming, it was hypothesized that Mary River turtles should choose a nesting site with less sun exposure throughout the nesting season. This was not found to be true, neither did they use the mean ambient temperature at the day of or day before nesting to control the location of the nest. It was also not found that the amount of sun exposure on the nest predicted hatching success. This might mean that (1) turtles are not able to determine the amount of sunshine needed on the nest to control nest temperatures or (2) they already nest in the nest location with optimal temperatures, as all mean nest temperatures this year were within the optimal range of 28± °C (fig. 1.10) and only 5% of the fluctuations were above 34°C, from which it is unclear what influence it has on the eggs and hatchlings (Micheli-Campbell *et al.*, 2012a).

Although the data in this research seem to be insufficient to answer the question whether Mary River turtles can adapt their nesting behaviour to increasing temperatures, there might be a presumption from a result of one of the artificial nests placed at a

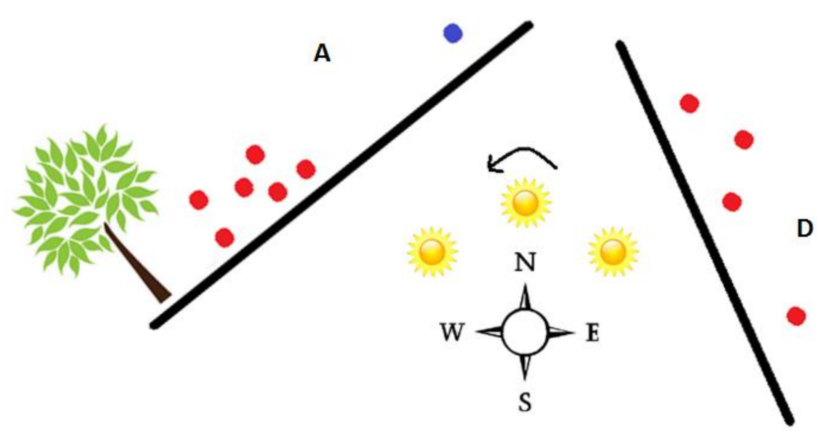


*Fig. 29. Nesting bank A & D with different nesting patterns (real nests (red) and one artificial nest (blue)) and different sun exposure.*

location where turtles were not observed nesting this year. Figure 29 shows two nesting banks from the study (see also fig. 5). Nesting bank A faces north-westwards and will therefore catch most sun in the afternoon, when ambient temperatures are highest. This nesting bank is surrounded by trees and turtles nest almost on top of each other under the trees on the western side of the nesting bank (red dots). Nesting bank D faces north-eastwards and therefore catches most of the morning sun, when ambient temperatures are lower than in the afternoon. Here, there are no trees and turtles are nesting all over the nesting bank. So, there is a clear difference between the two nesting banks in vegetation cover and nesting behaviour. However, they have in common that the sun is not shining on the nests after 3 PM. On nesting bank A, this is because the nests are shaded by the tree, on nesting bank D, because of the sun disappearing behind the nesting bank, because of the cardinal direction of the nesting bank. Although mean nest temperature seem to be a little higher at nesting bank D, they still fall within the optimal range (fig. 21). The artificial nest (blue dot) that was placed at a location where no turtles nested although the sand substrate was the same, had nest temperatures above the optimal range (fig. 22b & 24). It seems therefore that Mary River turtles do choose a nesting site. Many other studies found that turtles seem to be able to choose the habitat with the right temperatures. The snapping turtle is found to choose a habitat with shorter vegetation and open sand (Kolbe & Janzen, 2002). The terrapin turtle selects nesting sites with higher temperature and lays bigger eggs in warmer locations (Roosenburg, 1996). Resnider (2012) found that painted turtles chose the amount of sun cover on the nest. Micheli-Cambell *et al.* (2013a) found that mean nest temperatures in artificial nests were lower than real nests. Wilson (1998) showed that the striped mud turtle selected nesting sites that were cooler than non-nesting sites. Those studies all show that turtles are able to choose the optimal conditions for their eggs and might suggest that the Mary River turtle is also able to do this. It is yet unknown how the Mary River turtle choose the right nesting site. It might be hard for female turtles to use visual cues  as they are nesting at night. It is possible that they use the temperature of the sand to determine the right incubation temperature, as lizards are found to do (Warner & Andrews, 2002). More research is needed to establish how Mary River turtle choose the nest location with optimal temperatures.

**Management implications & further research**

The Mary River turtle is a unique turtle species that is endemic to the Mary River in Queensland. It is trying to recover from many years of egg removal by the now illegal pet trade, but is in the meantime facing other threats, such as the reduction of suitable nesting sites and also the threats studied in this research: predation and global warming.

As presumed, Lace monitors and foxes seem to be the main predators of the nests of the Mary River turtle. Foxes are nocturnal, seem to respond to turtles coming on the nesting bank and were photographed predating nests within minutes after turtles laid their eggs. On the other hand, Lace monitors are diurnal, predated nests up to a week after laying of the eggs and seem to be around continuously, especially during the mornings. The best option to save the eggs seems to protect the nest right after the laying of the eggs. This would mean that the project team would need to be on site during the night when turtles are expected to nest. However, previous trials have shown that turtles do not come onto the nesting bank when people are around (Marilyn Connell, 2015, personal communication). The second best option is to protect the nests as early in the morning as possible, as this will reduce the chances of being predated by a Lace monitor. This is already done by the Tiaro & District Landcare Group. However, diurnal nest protection will not help to protect nests against foxes. Tiaro & District Landcare Group irregularly baits for foxes. As foxes are introduced in Australia and therefore seen as an invasive species, I would recommend to bait every year before the nesting season as this seems to be the only way to protect nests from being predated by foxes. This study is inconclusive as to which threats hatchlings are exposed to on their way to the water, because not enough hatchlings were photographed, although many nests were laid within the camera's field of view. To gain more insight in the possible threats, I would suggest to extend the time lapse setting of the camera to the early afternoon. The time lapse interval of two minutes at night seem to be sufficient to capture most ectothermic animals on the nesting bank, although I cannot be sure about what the camera missed, except from turtles, goannas and foxes. Camera 4 that was placed 50 cm above ground, photographed a few turtles with the motion trigger, but still captured most turtle by time lapse. Further research regarding turtles using wildlife cameras should therefore not rely on the motion trigger.

This study further shows that global warming might be a threat to the early life stages of the Mary River turtle, because of the relation between mean daily ambient and mean daily nest temperature. In 2065, half of the mean nest temperatures will be above the optimal temperature if nothing changes. Although the nest location, which determines the amount of sun exposure on the nest, could be used by the Mary River turtle to influence nest temperature, this research remains inconclusive about whether the Mary River turtle is able to adjust its nesting behaviour to increasing temperatures. However, some anecdotal evidence in this research and other studies indicate that turtles do adjust their nesting behaviour to increasing temperatures by changing their nest location towards places with less sun exposure, so this might also be the case for the Mary River turtle. Long-term studies are needed to collect yearly nest temperatures and hatching success to confirm these indications. A small note on collecting nest temperatures: hourly measurements are enough (David Booth, personal communication) and reduce the change of full loggers as I experienced during this study. It also would be very important to map temperature regimes and moisture levels at nesting banks, to determine whether there are enough locations with the optimal temperatures and moisture levels available.

If future studies show that the Mary River turtle is not adapting well and/or there is not enough habitat with optimal temperatures available, another temporary solution can be conducted by Tiaro & District Landcare Group. As it already operates a nest protecting program, it can consider including a shade cover over the nests to reduce the amount of sun exposure on the nest and thereby lowering nest temperatures. However, I would suggest one other important future research topic, which is to determine whether more active hatchlings have a higher fitness and thereby determine whether an increase or a decrease of nest temperatures is desirable, using maximum temperatures from the field (40.5°C) and higher. Other subjects that need further investigation are (1) Are there other ways that the Mary River turtle can regulate nest temperature, such as for example moisture levels (Morjan, 2003)? (2) Why is there a difference in nesting time between the first mass laying and the second? (3) Does the same female nest multiple times in one season? Only then are we able to form a complete picture and can we determine which management strategies are best to be used to preserve this unique turtle species.

**Acknowledgements**

All activities were undertaken in accordance with the standard practices approved under de DAFF Animal Experimentation Ethics Committee: Queensland Turtle Conservation Project SA 2012/11/394, 395, 398, 399, 400 (Authority No. BNE24185, Department of Environment and Heritage Protection, Queensland Government)

The R script used for data analysis can be found at http://www.rpubs.com/Rosanne3/MaryRiverTurtle.

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**Appendix I**

Mean ambient temperature at day of nesting ($R^2$=0.221, $p$=0.09, fig. Ia) nor mean ambient temperature at day before nesting ($R^2$=0.000, $p$=0.967, fig. Ib) does predict the amount of sun exposure on the nest. The same non-significant relations were found for nest depth (day of nesting: $R^2$=0.055, $p$=0.422, fig. Ic; day before nesting ($R^2$=0.034, $p$=0.53).

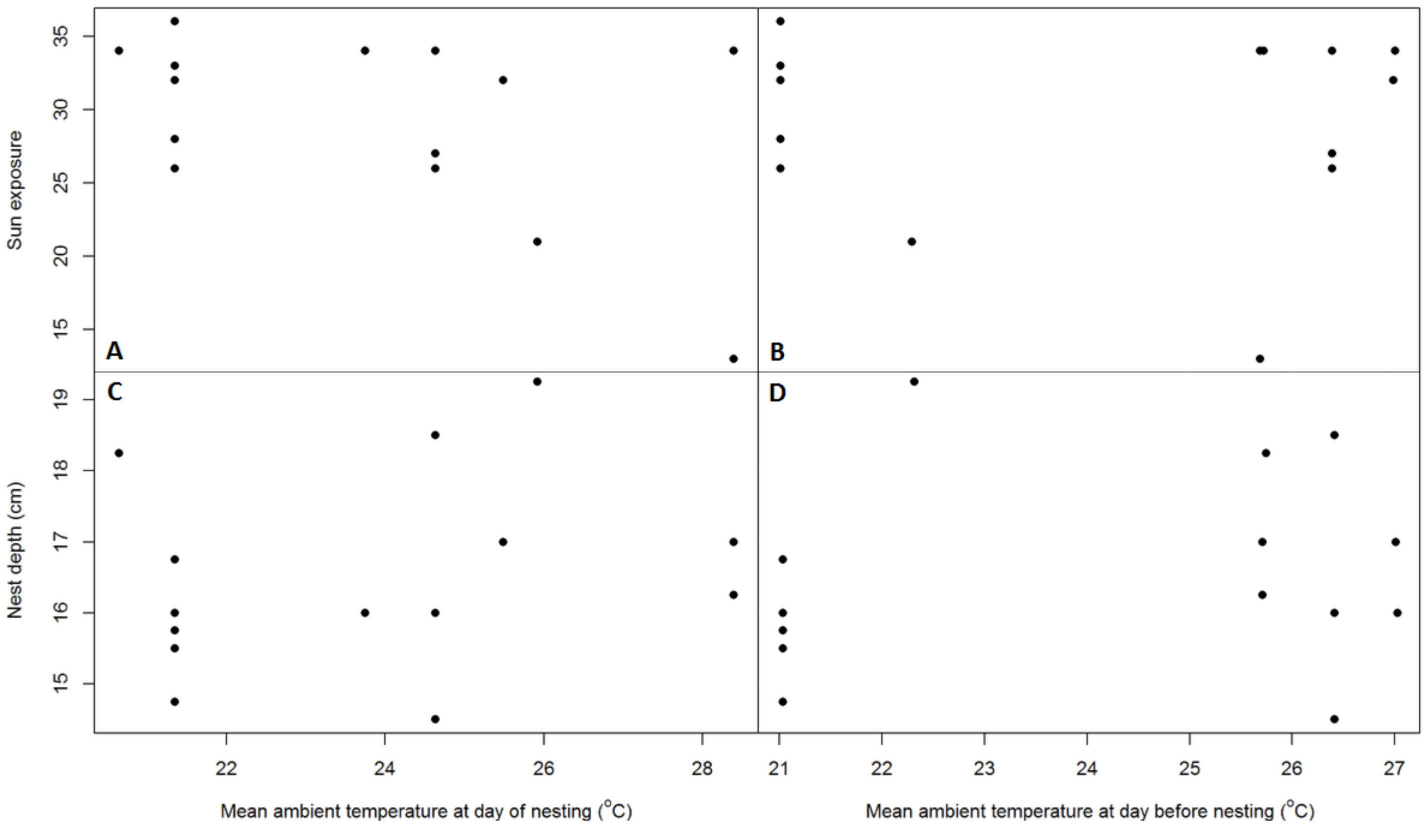


*Fig. I. Sun exposure is not predicted by (a) mean ambient temperature at day of nesting, (b) mean ambient temperature at day before nesting, and nest depth is not predicted by (c) mean ambient temperature at day of nesting or (d) mean ambient temperature at day before nesting.*

**Appendix II**

The following linear model was performed:

hatching success ~ (1) Sun exposure +
(2) Mean nest temperature +
(3) Percentage above 34% +
(4) Sun exposure: Mean nest temperature +
(5) Sun exposure: percentage above 34% +
(6) Percentage above 34%: mean nest temperature +
(7) Sun exposure: Mean nest temperature: Percentage above 34%

Non-significant terms were removed one by one using backwards selection starting with the interactions. This led to the following slightly significant model: hatching success ~ Mean nest temperature, $R^2$=0.287, $p$=0.042.

**References**


Australian Government, Department of the Environment Bureau of Meteorology (2015) "Technical report: Climate change in Australia. Projections for Australia's NRM regions"

Booth, D.T. & C. Yan Yu (2008). "Influence of the Hydric Environment on Water Exchange and Hatchlings of Rigid-Shelled Turtle Eggs" *Physiological and Biochemical Zoology*, 82(4):382-387

Both, C. *et* al. (2004). "Large–scale geographical variation confirms that climate change causes birds to lay earlier" *Proceedings of the Royal Society B,* 271(1549):1657-1662

Burger, J. & M. Gochfeld (2014). "Avian predation on olive ridley (*Lepidochelys olivacea*) sea turtle eggs and hatchlings: Avian opportunities, turtle avoidance, and human protection" *Copeia* 1:109-122

Campbell, M. (2012). "Analysis of the tracking data collected by acoustic telemetry for two species of freshwater turtle (*Elusor macrurus* and *Elseya albagula*) along the Tiaro stretch of the Mary River (QLD)" *Internal report for Tiaro & District Landcare Group*

Cann, J. & J.M. Legler (1994). "The Mary River Tortoise: A New Genus and Species of Short-Necked Chelid from Queensland, Australia (Testudines: Pleurodira)" *Chelonian Conservation and Biology,* 1(2):82-96

Clark, N.J., M.A. Gordos & C.E. Franklin (2008). "Diving behavior, aquatic respiration and blood respiratory properties: a comparison of hatchling and juvenile Australian Turtles" *Journal of Zoology,* 275(4):399-406

Dunn, P. O. & D.W. Winkler (1999). "Climate change has affected the breeding date of tree swallows throughout North America" *Proceedings of the Royal Society B,* 266(1437):2487-2490

Doody, J.S, B. Green, R. Sims, D. Rhind, P. West & D. Steer (2006). "Indirect impacts of invasive cane toads (*Bufo marinus*) on nest predation in pig-nosed turtles (*Carettochelys insulpta*)" *Wildlife Research*, 33(5):349-354

Flakus S.P. (2002). "Ecology of the Mary River Turtle, Elusor macrurus" Master of Sciences Thesis (Zoology), Department of Zoology and Entomology, The University of Queensland, Brisbane, QLD, Australia

Frits, T.H., M.L. Stinson & R. Márquez (1982). "Status of Sea Turtle Nesting in Southern Baja California, Mexico" *Bulletin of the Southern California Academy of Sciences*, 81(2):51-60

Georges, A. & S. McInnes (1998). "Temperature fails to influence hatchling sex in another genus and species of chelid turtle, *Elusor macrurus* (Chelonia: Chelidea)" *Journal of Herpetology,* 32(4):596-598

Glen, F., A.C. Broderick, B.J. Godley & G.C. Hays (2006). "Thermal control of hatchling emergence patterns in marine turtles" *Journal of Experimental Marine Biology and Ecology*, 334:31-42

IUCN (2015). “IUCN Red List of Threatened Species. Version 14.2.” <http://www.iucnredlist.org/details/7664/0>, Downloaded on November 10, 2014

Johnson, D. (1997). “State of the rivers: Mary river and major tributaries: an ecological and physical assessment of the condition of streams in the Mary River Catchment” *Department of Natural Resources*, Resource Sciences Centre, Brisbane

Kolbe, J.J. & F.J. Janzen (2002). “Impact of nest-site selection on nest success and nest temperature in natural and disturbed habitats” *Ecology*, 83(1):269-281

Limpus, C.J., J.D. Miller, E.J. Parmenter & D.J. Limpus (2003). “The green turtle, *Chelonia mydas*, population of Raine Island and the northern GBR : 1843-2001” *Memoirs of the Queensland museum*, 49 (1): 349-440.

Micheli-Campbell, M.A., H.A. Campbell, R.L. Cramp, D.T. Booth & C.E. Franklin (2011). “Staying cool, keeping strong: incubation temperature affects performance in a freshwater turtle” *Journal of Zoology*, 285: 266-273

Micheli-Campbell, M.A., M.A. Gordos, H.A. Campbell, D.T. Booth and C.E. Franklin (2012a). “The influence of daily temperature fluctuations during incubation upon the phenotype of a freshwater turtle” *Journal of Zoology*, 288:143-150

Micheli-Campbell, M.A. (2012b). “Habitat requirements for nesting and early life-stages of the endangered Mary River turtle (*Elusor macrurus*): Insights for conservation” PhD Thesis, The University of Queensland, Australia

Micheli-Campbell, M.A., T. Baumgartl, D.T. Booth, H.A. Campbell, M. Connell & C.E. Franklin (2013a). “Selectivity and Repeated Use of Nesting Sites in a Freshwater Turtle” *Herpetologica*, 69(4):383-396

Micheli-Campbell, M.A., H.A. Campbell, M. Connell, R. G. Dwyer & C.E. Franklin (2013b). “Integrating telemetry with a predictive model to assess habitat preferences and juvenile survival in an endangered freshwater turtle” *Freshwater Biology*, 58:2253-2263

Moll, D. & E.O. Moll (2004). “The Ecology, Exploitation, and Conservation of River Turtles" Oxford University Press: New York

Morjan, C.L. (2003). “Variation in nesting patterns affecting nest temperatures in two populations of painted turtles (*Chrysemys picta*) with temperature-dependent sex determination” *Behavioral Ecology and Sociobiology*, 53:254-261

MRCCC, 2013. ‘Something about Mary’

Parmesan, C. *et* al. (1999). “Poleward shifts in geographical ranges of butterfly species associated with regional warming” *Nature*, 399: 579-583

Perry, A.L., P.J. Low, J.R. Ellis & J.D. Reynolds (2005). “Climate Change and Distribution Shifts in Marine Fishes” *Science*, 308(5730): 1912-1915

Plummer, M.V. (2007). “Nest emergence of smooth softshell turtle (*Apalone mutica*) hatchlings” *Herpetological Conservation and Biology*, 2(1):61-64

R Development Core Team (2014). “R: A language and environment for statistical computing, reference index version 3.0.2” R Foundation for Statistical Computing, Vienna, Austria <http://www.R-project.org>

Refsnider, J.M. (2012). “Effects of climate change on reptiles with temperature-dependent sex determination and potential adaptation via maternal nest-site choice” *Graduate Theses and Dissertations*, paper 12570

Refsnider, J.M., B.L. Bodensteiner, J.L. Reneker & F.J. Janzen (2014). “Nest depth may not compensate for sex ratio skews caused by climate change in turtles” *Animal Conservation*, 16:481-490

Roosenburg, W.M. (1996). “Maternal Condition and Nest Site Choice: An alternative for the Maintenance of Environmental Sex Determination?” *America Zoologist*, 36:157-168

Salmon, M & M. Reising (2014). “Emergence Rhythms of Hatchlings Marine Turtles: Is a Time Sense Involved?” *Chelonian Conservation and Biology*, 13(2):282-285

Shine, R. & P.S. Harlow (1996). “Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard” *Ecology*, 77:1808–1818

Telemeco, R.S., K.C. Abbott & F.J. Janzen (2009). “Modeling the Effects of Climate Change-Induced Shift in Reproductive Phenology on Temperature-Dependent Traits” *The American Naturalist*, 181(5):637-648

Thompson, M.B. & R-J. Spencer (2015). “Murray River Turtle Information Package” *Turtlesat*: http://turtlesat.org.au/docs/turtleinformationpack_v3.pdf

Warner, D.A. & R.M. Andrews (2002). “Nest-site selection in relation to temperature and moisture by the lizard *sceloporus undulates*” *Herpetologica*, 58(4):399-407

Welbourne, D. (2013). “A Method for Surveying Diurnal Terrestrial Reptiles with Passive Infrared Automatically Triggered Cameras”, *Herpetological Review*, 44(2):247-250

Whitfield Gibbons, J., J.L. Greene & K.K. Patters (1982). “Variation in Reproductive Characteristics of Aquatic Turtles” *Copeia*, 4:776-784

Whytlaw, P.A., W. Edwards & B.C. Congdon (2013). “Marine turtle nest depredation by feral pigs (*Sus scrofa*) on the Western Cape York Peninsula Australia: Implications for management” *Wildlife Research*, 40(5):377-384

Wilson, D.S. (1998). “Nest-site selection: microhabitat variation and its effects on the survival of turtle embryos” *Ecology*, 79(6):1884-1892

Wood, A., D.T. Booth & C.J. Limpus (2014). "Sun exposure, nest temperatures and loggerhead turtles hatchlings: Implications for beach shading, management strategies at sea turtle rookeries" *Journal of Experimental Marine Biology and Ecology*, 451: 105-114

World Association of Zoos and Aquariums, WAZA (2015). http://www.waza.org/en/zoo/select-a-region/oceania/reptiles/lizards-and-tuatara/physignathus-lesueuri, consulted May 5, 2015