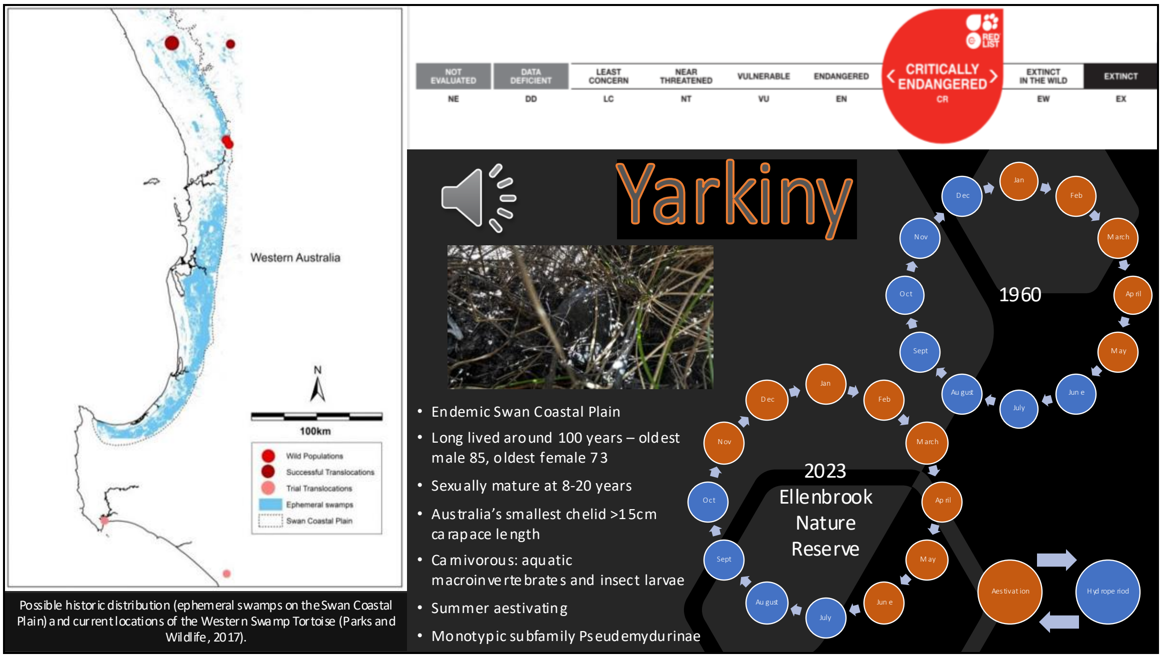
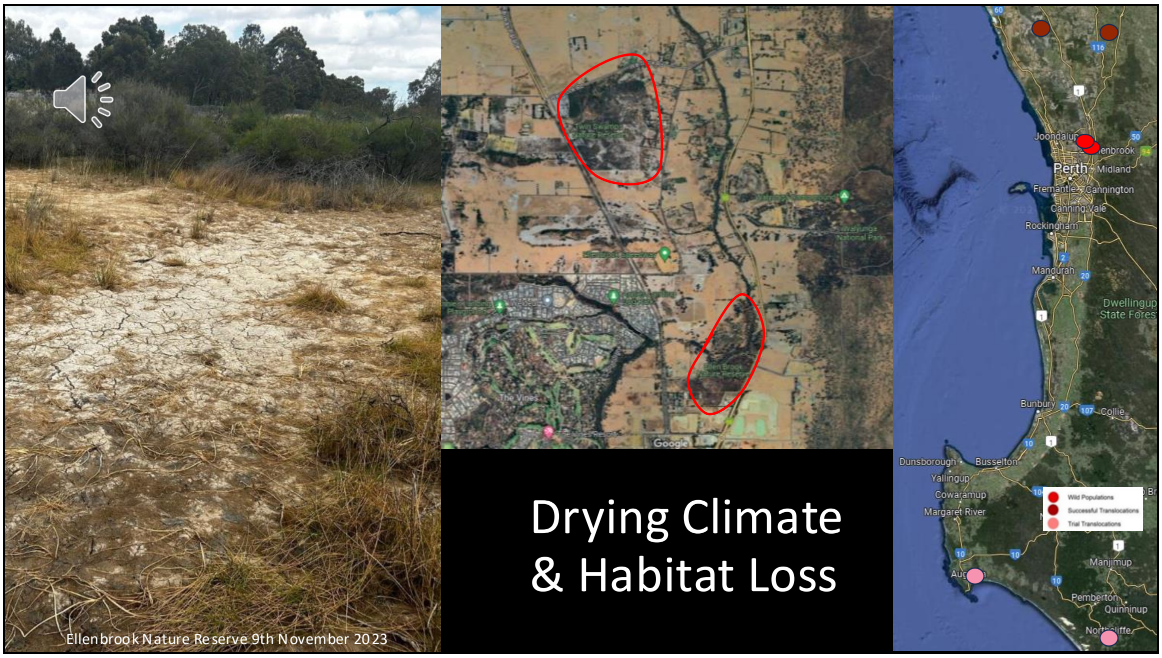
Investigating egg incubation strategies for the Western Swamp Turtle to optimise fitness for individuals released to the wild.



- One of Australia's most critically endangered reptiles, endemic to ephemeral clay-based wetlands along the Swan Coastal Plain, north of Perth in Western Australia.
- Smallest Aust. Chelid
- Long lived
- Winter active, aestivate underground or under leaf litter for 6-8 months
- Thought to be extinct for around 100 years until chance rediscovery in the 1950's
- By the late 1980s, when the Recovery Team was formed, less than 50 individuals were remaining
- Perth Zoo breeding program commenced as part of the recovery strategy and has played a key role in the recovery of the species for over 30 years.

What ideal habitat looks like in hydroperiod: water to depth of ~50cm water present for 7 months of the year.



This species is facing many challenges:

- Habitat is becoming progressively drier, with climate change an immediate threatening process
- The changing hydrology and land use in Swampy habitat has increased the difficulty in finding suitable release sites.

Only 2 remaining natural wild populations in original habitat:
Ellenbrook NR: 50ha. ~30 adults, ~100 juveniles
Twin Swamps NR: 155 ha. Formerly extinct, until reintroduced in 1994. Now ~15 adults, ~35 juveniles

Other local sites have been established with translocated zoo bred animals, with an estimated total population now at around 500 individuals.

There have also been a small number of animals translocated to assisted migration trials sites, over 300km south of their natural range. These sites have a longer hydroperiod and lower temperatures and may provide a viable longer-term solution in the face of climate change.

- Remaining local habitat sites are unlikely to be viable in the long term without intensive management intervention including water supplementation & access to lined ponds or to farm dams as drought refuges.
- A rapid evolutionary response is unlikely as they've remained relatively unchanged for over 20 million years.
- There are many human imposed barriers and dangers associated with natural dispersal.
- Post release monitoring is difficult, the turtles are cryptic, far moving & don’t enter baited traps.
- Trackers can be used if funding & resources are available, these fall off when scutes are shed.
- New eDNA methodology can detect presence or absence of WST in water, can’t detect individuals or indicate the number of animals present.

- In saying all that, there is hope for this species
- Since 1989, the program has bred and released 1145 of these turtles to the wild.
- Over the past 3 decades, breeding management strategies and artificial incubation procedures have been refined to achieve a high level of hatching success, with release targets exceeded each year.
- The original 1980s goal of the zoo breeding program was to increase the world number of the species to a more secure level, which has been achieved.
- The conservation challenge is no longer to maximise the numbers bred in captivity, but to produce a fitter translocation stock and to find new translocation sites to support a viable population in a rapidly changing world.
- As part of this review, it was proposed by DBCA Senior Research Scientist and turtle expert, Dr Gerald Kuchling to trial natural incubation in the Perth Zoo enclosures as a starting point to see if any of these factors could be improved.
- So, we listened to the expert and changed course.

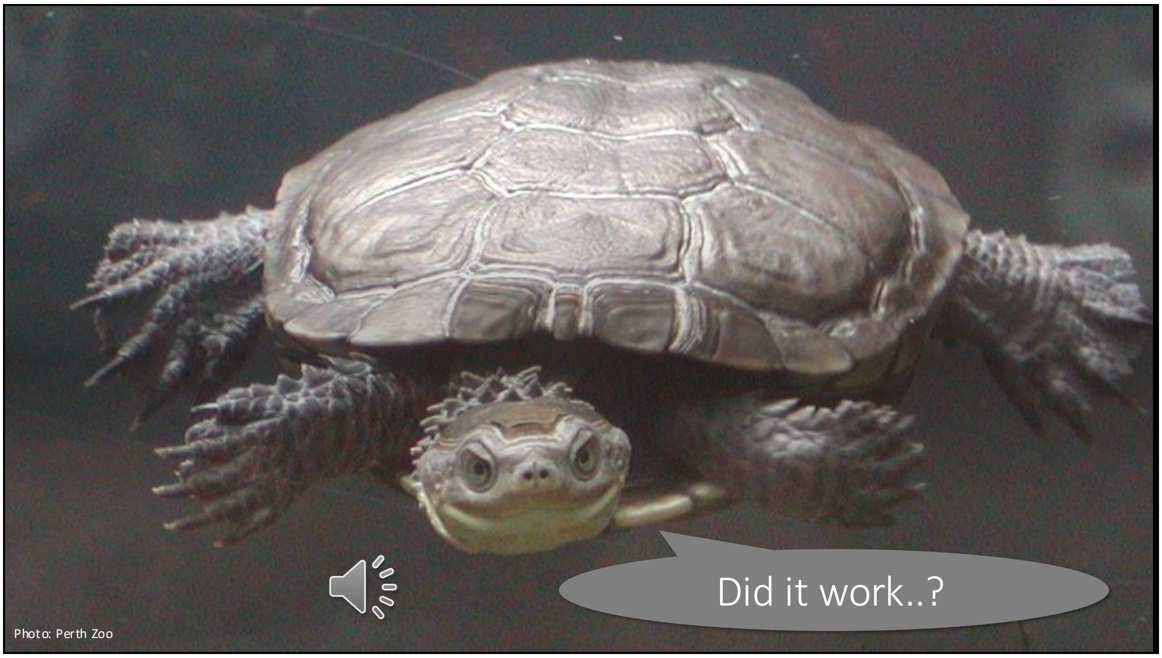
- From the start of the program until 2022, all eggs have been artificially incubated. This is a typical approach for reptile breeding programs.
- Prior to this project, a small dataset from wild nests indicated there was an egg mortality threshold of 34 degrees. There were concerns that nests left in-situ in the breeding complex would be exposed to unsuitable temperatures.
- No evidence of Temperature Dependent Sex Determination in this species, however the 3 temperatures are used as a precaution.
- 3 incubation treatments: 24 degrees static, 22-26 degrees fluctuating, 25-29 degrees fluctuating.
- At end of 170/150 days, chambers removed from incubator and placed into 20°C room, and a simple but innovative vibrational device to simulate rain is used, which can trigger hatching and reduce incidence of assist hatch cases to zero.
- The team had perfected artificial incubation for the WST, finally achieving consistent high numbers of hatchlings.

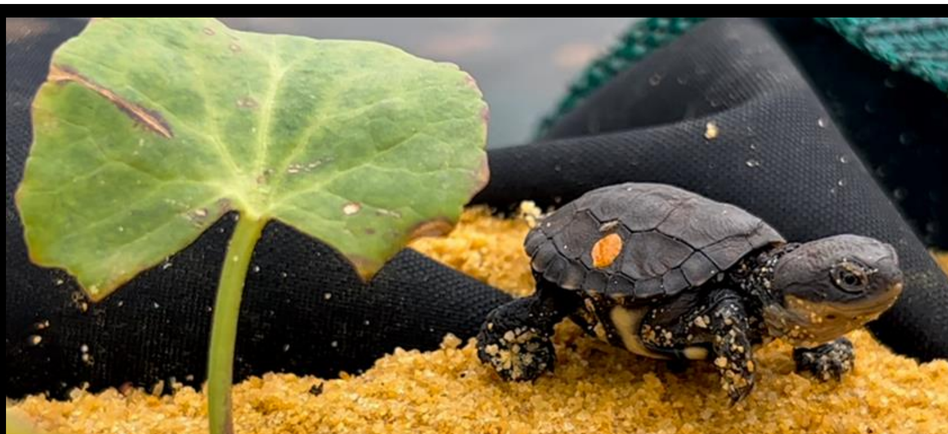
- We wanted to find out about the viability of translocating eggs as a recovery strategy for WST, which currently remains unstudied & it's not yet known if the southern assisted migration sites have suitable environmental parameters for incubation – which is critical for the long-term survival of these populations. This also raised a number of other questions about incubation strategies for the WST.
- There has been limited research on the impact of artificial incubation for chelonians, however some research into other reptilian species suggested that incubation temperature including fluctuating environmental conditions can influence hatchling sex ratio, morphology, mobility, growth and behaviour and that those found in natural nests had improved fitness and vigour (Booth, 2006).
- Incubating eggs in ground nests has been used as a successful recovery action in other chelonians (e.g. Nagy et al. 2020).
- A trial in 2022/2023 investigated if the conditions at our facility are conducive for successful hatching, some of the results were surprising.

To develop methodology:

- Collaborated with research scientists from Parks and Wildlife Service & UWA students to develop methodology

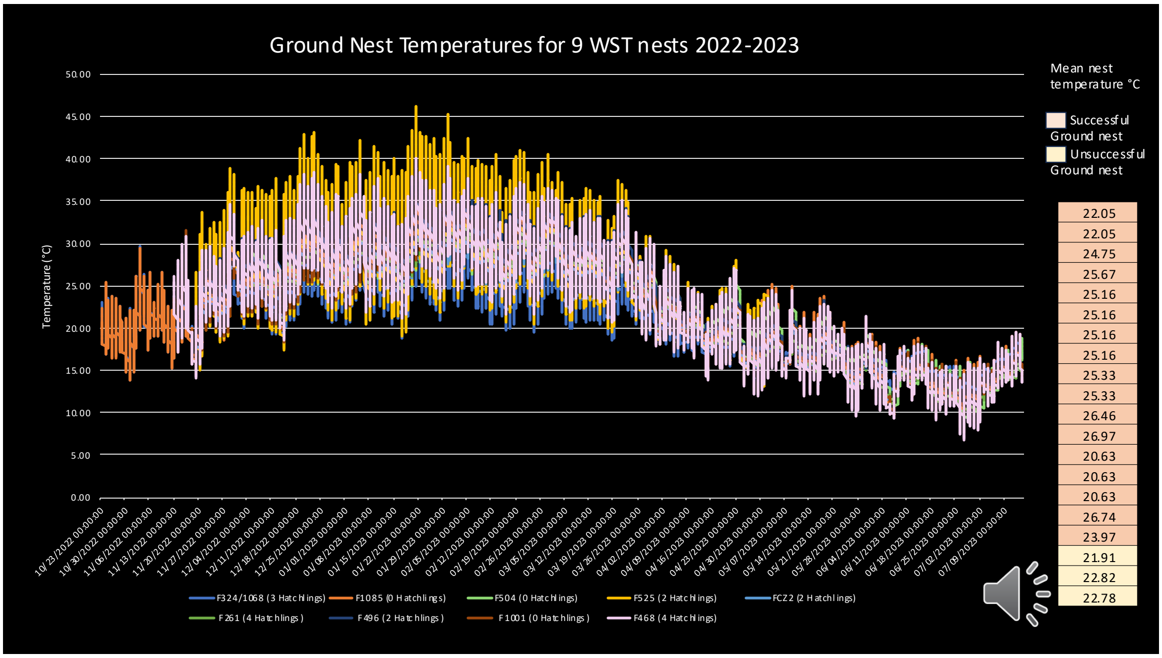
- Artificially incubated eggs from approximately half of nests according to current protocols.
- 9 nests had Bluetooth data loggers buried with the eggs, at the same depth of the shallowest egg.
- Guards were set up around nests to prevent other females digging in the same location & to track parentage of offspring.


Did it work..?
Photo: Perth Zoo



	Artificially Incubated	Ground Incubated
Total Hatchlings	35	20
Total Eggs	58	36
Hatch rate	60.3%	55.55%
Total Viable eggs	37	Unknown
Viable egg hatch rate	94.6%	Unknown
Total Nests	17	13

- 20 hatchlings. 55% hatching success. All still thriving.
- Compared with 60% hatching success for artificially incubated eggs



Maximum recorded temp from successful nest: 46.2 degrees
Minimum recorded temp from successful nest: 6.9 degrees

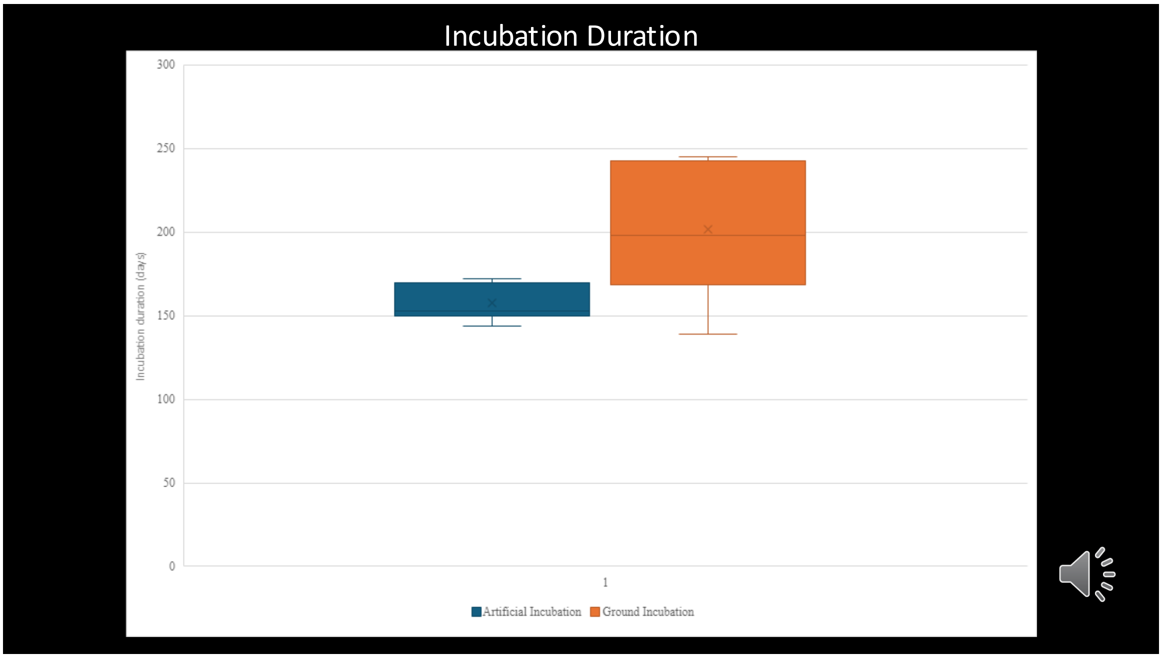
Compared with Artificial incubation strategy, fluctuations were much wider for all ground nests.

Some healthy hatchlings were exposed to temperatures over 34 degree for over 700 hours.

- Hatchlings were processed immediately after hatching or emergence
- Weight, plastron length, carapace length, width and height and were measured.
- Highly significant difference in carapace width to length ratio, with most ground hatchlings having higher carapace width to length ratios than artificially incubated hatchlings.

This appeared to have a positive impact on swimming ability, all were anecdotally observed to be strong and coordinated swimmers, whereas artificially incubated hatchlings were sometimes observed as uncoordinated when first introduced to the pond.

Allantois opening fully healed on ground hatchlings.



Most ground hatchlings had longer incubation durations than artificially incubated hatchlings

Nests were checked for unhatched eggs after 200-250 days.

- In one nest, checked at 245 days, 2 healthy hatchlings found sitting 4cm below the surface, in a nest that had the first hatchling emerge 70 days prior, and second hatchling emerge 53 days prior.
- Another nest checked at day 243, where no hatchlings had previously emerged - 4 healthy hatchlings were found sitting 8cm below the surface.
- Only 1 clutch where 2 individuals from same nest emerged on same day

- We don't know how much longer these hatchlings would have stayed underground if we hadn't excavated them.
- Lag periods between hatching and emergence have also been found in other species of marine and freshwater turtles under natural nest conditions (Bujes and Verrastro, 2009)
- The delayed emergence may be enough to change the shape of the carapace from spherical to the flat shape we observed, which was also found in the Hilaire's Side-necked turtle (Bujes and Verrastro, 2009).
- Found that the individuals who didn't dig out on their own had some of the highest carapace width/length ratios.

- Where to from here?
- Increasing our data set by repeating ground vs artificial incubation trial for third time this year.
- We want to better understand the influence of incubation conditions on morphology and fitness to optimise rearing conditions and increase survival likelihood.
- A research collaboration between Perth Zoo & UWA researcher, Ha Hoang to quantify fitness between the two groups & identify whether there are measurable differences.
- Achieved through swim and walking speed tests. Carried out 5 days from hatching and then repeated at ~6 months old.
- Data is still being processed, however Ha's preliminary analysis indicates a general trend towards ground hatchlings being less likely to participate in the walking & swimming test. Speed data analysis is not yet completed. There were 300 tests performed
- A review of artificial incubation treatments may be warranted.
- Other studies have indicated that captivity can cause behavioural changes in hatchling turtles (Meylan and Ehrenfeld 2000), including unfavourable habitat selection choice (Okuyama et al. 2010).

- Translocation of egg clutches, as opposed to juveniles, represents a potentially beneficial option for supplementing translocated populations that is also cost-effective. The first trial is planned for later in 2025.
- This research is likely to become increasingly relevant with continuing trial translocations at sites south of the species historical range in the face of climate change.
- We are working with DBCA Climate Adaptation Geneticists to establish a reference genome & ensure the most genetically diverse/reproductively fit offspring are bred for translocations, as well as establish a genetic sex marker.

- The program has been running for over 3 decades and we are still discovering new information about this species.
- Our learnings will contribute scientific knowledge to guide adaptive management and decision making to ensure the survival of this endearing species well into the future.

This project wouldn't have been possible without the expertise and dedication of all the swampy keepers that came before me, the support of Dr Harriet Mills, and the entire Perth Zoo Science team, who have all made contributions to enable this valuable work.

Thank you for listening!

Questions?

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