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TURTLE COOLING PHASE II: FIELD RESEARCH

PRELIMINARY RESULTS 2019-2020

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Keeping turtles cool

Marine turtles face a growing number of threats to their survival. **Climate change** is one of the greatest because for marine turtles and other reptiles, the sex of their incubating offspring is determined by the nest temperature of the sand. Warmer nests create more females, while cooler nests create more males. With sand temperatures rising across equatorial latitudes, turtle populations are feminising and on the northern Great Barrier Reef (nGBR), over 99% of juvenile green turtles are female (Jensen *et al*. 2018).

The *Turtle Cooling Project* started in November 2018 on nGBR's Milman Island. As an innovative and novel exploration, Phase I sought to find the best cooling method to combat the effects of feminisation. In collaboration with our partners, WWF compared known sand cooling methods (i.e. freshwater irrigation and shade cloth) with two under-studied management strategies: seawater irrigation and palm frond shade. The goal was to see if these strategies reduced nest temperatures enough to produce male-biased clutches.

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We found that, although confounded by heavy rainfall from a cyclone event, palm frond and artificial shade was effective in producing male-biased clutches (>90% male). Seawater irrigation also produced mostly males but had decreased hatching and cooling success. But as a result of the cyclone, we were unable to differentiate the cause of the decreased hatching success between the seawater irrigation and the cyclonic event. So, with no definitive answer as to whether seawater is an effective strategy, we knew more work needed to be done. Phase II of the *Turtle Cooling Project* was established. WWF-Australia and its collaborators have established a three-year program to answer three main questions:

1. How many male turtles do we need to maintain a healthy, genetically diverse population?
2. Which beaches are most at risk of feminisation?
3. How and when should we intervene with sand cooling methods to mitigate feminisation effectively?

During the 2019-2020 nesting season, our partnership began work on our third question – when and with what should we intervene. These experiments were undertaken at Heron Island, Mon Repos Turtle Rookery and the Conflict Islands, Papua New Guinea.

Heron Island: December 2019 – March 2020

Between 1 December 2019 and 8 February 2020, a team of researchers from the University of Queensland and WWF-Australia took off to the sunny paradise destination of Heron Island. Located 80 kilometres off the coast of Gladstone, Heron Island is a marine biodiversity hotspot of the southern GBR. The island is a vibrant coral cay, home to seabird breeding colonies, resident marine turtles, and an itinerant breeding population of green turtles (along with a few loggerhead turtles) that have been a part of the rigorous annual monitoring program of the Queensland Government for more than 50 years—making it a perfect site for the very first project of *Turtle Cooling* Phase II. Our time on Heron Island was split between two sub-projects: the *sand cooling* and the *seawater irrigation* trials.

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This first trip to Heron involved a lot of late nights due to the high tide being around 12 am for the majority of our trip. During this time a BBC film crew visited the project to record a segment on what we're actively doing to mitigate the effects of climate change.

The sand cooling trial

Rainfall has historically been the most natural and effective way of lowering sand temperatures and incubating clutches. But with climate induced warming conditions, rainfall events are becoming more unpredictable. Unfortunately, we can't call the rain at will, therefore as a part of *Turtle Cooling Phase II* we trialled replicating 'rainfall' by applying differing amounts of seawater on artificial nests. Our experiments ranged from pouring air temperature seawater equivalent to 20 mm of rain (a nice shower) to a whopping 200 mm of rain (a 1 in 10-year cyclone) onto the artificial nests over a set number of days. This will enable us to deduce how much 'seawater rain' is needed to cool the sand at sea turtle nest depth, and for how long? For this experiment, we didn't use turtle eggs, but buried temperature data loggers at nest depth to collect sand temperature throughout the experiment period.

The seawater irrigation trial

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Beaches that are high risk of producing female-only offspring are close to the equator and usually in remote areas or developing countries. This means that methods for cooling sand temperatures need to be inexpensive and easy to implement.

Some more well-known methods of cooling sand are shade cloth and freshwater irrigation. Although these might be ideal methods for areas close to towns and cities, they are not a viable option for remote areas where there is limited freshwater and not a hardware store in sight. Seawater, however, is plentiful. This experiment was designed to test whether seawater was lethal and reduced hatchling success. It consisted of three different treatments. A seawater treatment (eggs watered with seawater), a freshwater treatment (eggs watered with freshwater) and a control treatment (no water added). Water

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was only added once during the start of the first trimester and equivalent to 100 mm of rainfall.

We also tested the ‘watering’ response on turtle individuality. To do this, we collected multiple clutches from different nesting females. Why? Just like humans, turtles have variability in their fertility, meaning that one clutch of eggs might not be as viable as another. After collecting 12 clutches and splitting them between the three treatments, we relocated them to a fenced off area, where the experiment was performed.

Our second trip to Heron Island had one key objective: to dig up all 12 experimental nests after they had hatched in order to determine the hatching success of each of the three treatments.

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Papua New Guinea: November 2019 – February 2020

The Conflict islands is a coral atoll located 120 km southeast of Alotau, Papua New Guinea. Here, the Conflict Island Conservation Initiative established a turtle monitoring program in 2017, where more than 680 new turtles have been tagged so far. A replication of the *sand cooling experiment* was carried out on Panasesa by the Conflict Islands Conservation Initiative turtle rangers and volunteers. This study enabled us to investigate cooling strategies for other beaches that are at greater risk of feminisation due to their close proximity to the equator.

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Mon Repos Turtle Rookery:

November 2019 – March 2020

Research is still underway at Mon Repos Turtle Rookery (near Bundaberg) and the University of Queensland to pinpoint when turtle gonads differentiate into male and female during the second trimester of embryonic development. This project is being led by the University of Queensland with support from WWF-Australia and the Queensland Government.

Understanding this timing is an integral piece of our puzzle, as we need to know exactly when to intervene and implement cooling methods to ensure male hatchlings are being produced. In this experiment we set out to determine the 'temperature sensitive period' (TSP), simply put, the time in which our hatchlings turn into either male or female. At present the TSP has been identified to occur in the middle third of incubation (second trimester), this is approximately between 17-35 days of incubation (~ 20 days). Refining this from ~20 days down to say three to five days is imperative for sand cooling methods that mitigate feminisation, just like the ones we trialled on Heron Island. Our cooling methods trialled this season are extremely labour intensive and required manual water application by hand. Although ~20 days of carrying hundreds of kilos of water may be great for your biceps, it is not sustainable over a long period of time. We are hoping to save money, time and human effort by focussing methods over a smaller 3-5-day window.

This experiment was set up and carried out by University of Queensland honours student Ellen Porter. Ellen was required to collect close to 900 eggs from two different species, the loggerhead turtle and the green turtle. The loggerhead eggs were collected from Mon Repos and the green turtle eggs were collected from Heron Island. The green turtle eggs were transported to the University of Queensland. Before transportation the eggs were placed into insulated coolers at a temperature of (~10°C), which is shown to slow the development of the turtles in order to reduce movement induced mortality during transportation. The loggerhead eggs remained in incubators at Mon Repos. Once eggs were collected, they were randomly placed into 10 different treatments, then into incubators. Two of the treatments were controls for male and female hatchlings, meaning they remained at constant temperatures for the entire incubation period. The other eight treatments were tested at male producing temperatures (~27°C) at different times and for different durations during the sex-determining period.

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Preliminary results

Seawater irrigation

In the *seawater irrigation* trial the best hatching success was from the freshwater treatment (86.4 ± 3.3 %), followed by seawater (69.4 ± 7.3 %) and lastly the control treatment (65.4 ± 6.9%). This was surprising – the control (adding no water) had the worst hatchling success. We think that's because this was an exceptionally dry year on Heron Island ("the driest ever experienced" to quote people who have been working on the island for years). The control eggs became dehydrated resulting in higher than expected mortality. Therefore, we can suggest that seawater application is not as harmful as no water application in a very dry year. The results also confirmed that the irrigation event was successful in decreasing nest temperature by 1.5° C for a short period of time. The majority of hatchlings that did not make it through incubation died in the last stage of development not because of 'watering' applications, including for seawater. Looking at our temperature data, it seems that nest temperatures were above the safe range for incubation (>34°C) during this stage. This suggests that high temperature was probably the main cause of embryo death, and, that our sand cooling methods should include applying a second round of water towards the end of incubation to cool sand temperatures and reduce late stage embryonic death during hot years.

Sand cooling

The *sand cooling* trial gave us perplexing data because in some treatments the application of water increased sand temperatures where as others it decreased. This finding might be explained by the time of year that these trials were conducted and the temperature of the water being applied (i.e. warmer or warmed as it percolated to nest depth). Despite being an unexpected result, this information will be used to design more effective cooling strategies in the future, as we now know water needs to be several degrees cooler than the top sand layer to be an effective coolant.

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Papua New Guinea

In Papua New Guinea we collected integral data that we can directly compare to the *sand cooling trial* on Heron Island. Sand temperature at nest depth (no eggs used) decreased significantly when seawater was applied, contrary to the results we collected on Heron Island. Although the cooling effect was only significant for two to three hours when the sand had just been irrigated. This demonstrates that for sand cooling methods to work we need to consider the large variation between sand temperatures at nesting beaches at different latitudes and with different weather profiles. Secondly, results from PNG also showed that sand which received seawater irrigation had higher salt concentrations at nest depth, although we are unsure if this amount of salinity would be enough to kill eggs. Lastly, the trials in PNG also tested if the watering and shade combined increased the effectiveness of the cooling. The outcome showed that nests shaded by palm-fronds were cooler than unshaded areas, regardless of the amount or type of water applied. Combined with the Heron Island study, these projects highlight that beach temperature profiles vary greatly and that there is a need to carefully measure nest depth temperatures prior to implementing a cooling strategy.

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Mon Repos Turtle Rookery

The loggerhead turtles in the incubation trial at Mon Repos Turtle Rookery and the University of Queensland have since hatched. The green turtles, since the time of writing this report (~3 weeks) have hatched. Now they have hatched, we can test to see whether hatchlings are male or female, and therefore determine the point during incubation when sex determination occurred.

What's next for *Turtle Cooling Phase II*?

We aim to answer our other research questions - how many males should be produced to sustain the green turtle population and what beaches are most at risk of feminising across the equatorial latitude. Through our collaboration with the University of Queensland and Deakin University, we

FIELD SEASON STATISTICS

20 CLUTCHES RELOCATED

1,262 EGGS RELOCATED
892 EGGS IN INCUBATORS
39 EXPERIMENTAL PLOTS
120 DAYS IN THE FIELD
134 TEMPERATURE LOGGERS DEPLOYED

are already planning drone and boat surveys to help determine the sex ratio of male to female green turtles to sustain populations. And there are more experiments to come. The combination of all these projects will give us the necessary knowledge to implement efficient management strategies to mitigate the adverse consequences of global warming at sea turtle nesting beaches around the world.

This collaborative project included researchers and volunteers from the University of Queensland, Conflict Islands Conservation Initiative and WWF-Australia. The field trip was primarily supported by WWF (through *Koala* sponsorship) and the University of Queensland.

The Turtle Cooling Project was project managed by Dr David Booth, UQ, Ms Christine Madden Hof, WWF-Aus and Ms Caitlin Smith, WWF-Aus.

Special thanks and the utmost gratitude to Anne Crosby, Melissa Staines and Ellen Porter for their hard work and passion throughout this field season. A shout out to Liz Turner for helping Ellen Porter at Mon Repos, and Hayley Versace and all the turtle monitors at Conflict Islands Conservation Initiative for helping set up and facilitate the PNG experiment.

Until next time, I’m signing off!

Caitlin Smith, Marine Species Conservation Project Officer, WWF-Australia.

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