Turtle Cooling Phase II

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Turtle Cooling Phase II: Fieldwork Update II

All marine turtles experience temperature-dependent sex determination (TSD), a process that determines the turtles' sex based on the temperature of the sand in the nest. Specifically, it is the temperature during the middle third of embryonic development, called the sex-determining period (SDP), that determines sex. For marine turtles, an easy way to remember how temperature impacts the production of males and females is 'hot chicks and cool dudes'. Cooler incubation temperatures produce males, and warmer temperatures produce females. Unfortunately, rapid human-induced climate change is increasing global temperatures, including sand temperatures. This means that more and more female marine turtles are being produced as temperatures continue to warm. Currently, the green turtle hatchlings being produced in the northern Great Barrier Reef at Raine Island are 99.1% female. This increase in female production – or 'feminisation' – is potentially detrimental to the entire population if there are not enough males to reproduce. Hence, the conception of the *Turtle Cooling Project*. With funding from furniture company Koala, WWF-Australia, in partnership with the University of Queensland and the Conflict Island Conservation Initiative, have taken proactive measures to find effective ways to reduce nest temperatures and produce males to conserve the species.

Historically, rainfall events are the most effective way of cooling nest temperatures and producing males. However, as global warming continues, rainfall events will become few and far between at some locations, meaning nest temperatures won't be cooled enough to produce males. For this reason, during the 2019/20 nesting season, the *Turtle Cooling Project* mimicked rainfall by irrigating nests with watering cans full of freshwater or seawater to try to cool nests. Seawater was trialled for the first time as it is abundant at nesting sites and is more accessible and affordable than freshwater, particularly at remote nesting locations. The results showed that both freshwater and seawater successfully cooled nests by 1.3 °C, and hatching success was not significantly impacted as a result.

The research team experienced great successes with last season's research - a nest temperature reduction with artificial rainfall, and no decrease in hatching success from seawater irrigation compared to natural conditions.

But what about male production? Which irrigation treatment produces the most males?

The next step for the Turtle Cooling Project: How much seawater, how many males?

The promising results from last season prompted the team to revisit the use of seawater irrigation to confirm its success, but this time also determine if seawater irrigation could confidently produce males. We also wanted to test how much seawater irrigation was needed to produce the most males.

Heron Island

Fieldwork focussed on continued research at Heron Island, a small, picturesque island off the coast of Gladstone, in Queensland, Australia. The Southern Great Barrier Reef island is booming with terrestrial and marine wildlife, covered in pristine white sands and surrounded by crystal clear water. This natural haven is visited by 200-1,800 nesting green females every nesting season, from November to March.

Thanks to the Queensland Government's hard-working turtle monitoring team, this nesting population has been monitored and protected for more than 50 years.

The team from Heron Island Research Station

The Heron Island Research Station is home not only to an incredible diversity of flora and fauna, but to a very passionate and hard-working team who have supported this project since its inception.

Working in remote locations like this is a challenge, but the stations scientific staff assisted with many field and laboratory needs.

The Administration and Scientific Officer, Kirsten Slemint, has worked with many of the research team before and offered to help wherever she could. While the researchers were between visits, Kirsten helped maintain the barricades around the monitored nests, and collected data from temperature loggers, which helped the team determine embryonic development stages.

Speaking about her experience working on the project, Kirsten said she was "proud to facilitate and work with such a wonderful group", and that her team were happy to have "played even a small part in this profoundly important work".

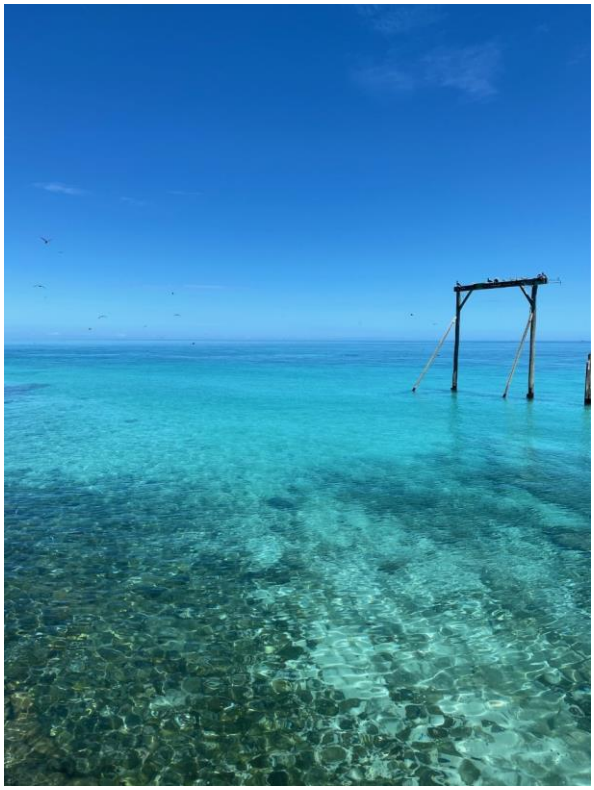
11-14 December 2020: Relocating eggs from nesting females

In December 2020, WWF-Australia and the University of Queensland researchers travelled to Heron Island in the Southern Great Barrier Reef to begin *fieldwork II of the Turtle Cooling Project*. Over four consecutive nights, the research team patrolled the beaches of Heron Island in search of 16 females ready to lay their eggs.

As each female began laying, the researchers collected her eggs – a process that does not impact the turtle or the eggs – and relocated them to a barricaded section of the beach at Shark Bay, on the eastern side of Heron Island. Each clutch of eggs was placed in a 70 cm deep man-made nest, similar to what the nesting females dig. Additionally, two temperature data loggers were placed in the nest with the eggs to monitor the developing embryos' temperatures throughout incubation.

Once all 16 clutches were relocated, the nests were marked and left to naturally develop until they reached 45-50% of development, which is within the sex-determining period during the middle-third of embryonic development.

6-15 January 2021: Irrigating nests



In January 2021, when the turtle embryos reached the middle third of embryonic development (the sex-determining period), researchers returned to Heron Island to begin irrigating the nests by mimicking rainfall with freshwater and seawater. This season the researchers trialled four irrigation treatments in comparison to the controls: 100 mm of 'rainfall' (52 L of water) with freshwater, seawater and chilled seawater; and 200 mm of 'rainfall' (104 L of water) with seawater.

New to the irrigation trials this season were the chilled seawater treatment and the 200 mm seawater treatment. Chilled seawater (between ~18-20 °C) should theoretically reduce nest temperatures even more than non-chilled seawater irrigation treatments (which were ~24 °C). Trialling 200 mm of seawater was also important to see if embryos could survive exposure to larger volumes of seawater, and to see if more seawater produced more males. Before sunrise across the 6-15 January 2021, all 16 nests were watered with their assigned treatments (four each morning). We used watering cans to evenly apply water across each nest. It was then time to wait for the embryos to continue to develop and return to Heron Island in February when the hatchlings were expected to emerge.

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8-18 February 2021: Hatchling time!

Samples to identify hatchling sex:

From the first hour that researchers arrived back on Heron Island on 8 February 2021, hatchlings began to emerge from their nests. As the hatchlings emerged, 20 were collected from each clutch and taken to Heron Island Research Station to be measured, weighed and sampled.

Although the data loggers recorded nest temperatures and helped us to predict the *likely* sex of the hatchlings (based on predicted pivotal temperatures), unless we quantify the hatchling sex, it's hard to know whether the irrigation treatments were truly effective at producing males. Currently, the most accurate way to identify their gender is to sacrifice the hatchlings and dissect their gonads to analyse under a microscope because there are no physical differences between male and female sea turtle hatchlings to visually determine their sex. However, green turtles are listed as endangered on the IUCN Red List of Threatened Species, so it's critical that we find a way to identify sex without sacrificing hatchlings. That's why our *fieldwork II* at Heron included testing a new technique of identifying gender that only involved taking a small blood sample from the hatchlings. Just like when you and I get a blood test, this process of taking a small blood sample from each hatchling (0.2 mL) is harmless, and the hatchlings can be immediately released.

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Hatching success:

In the same way that hatching success was determined last field season, each nest was excavated two days after the first emergence of hatchlings. The researchers counted the number of eggshells as successful hatchings, the number of live and dead hatchlings in the nest, and the number of unhatched eggs. Each unhatched egg was then opened to identify the stage of embryonic development when the embryo died.

From these counts, the researchers will determine the hatching success from each clutch, and consequently each treatment.

Still to come: Does seawater irrigation produce males?

With all the blood samples safely stored at the University of Queensland, the lengthy process of identifying gender is about to begin. Firstly, sex will be identified from the gonad samples. This is the tried-and-true method that uses a microscope to look at thin sections of the gonad tissue. Researchers can then identify sex from the differences between male and female gonads. We will use this method to validate the second method - identifying sex with the small blood samples.

Just like human blood, turtle blood carries many different hormones that have different functions within the body. Specifically, it is the plasma of the blood where hormones can be found. For this analysis, the University of Queensland researchers will look for the presence of Anti-Müllerian Hormone (AMH) in the plasma samples, as AMH should only be found in male hatchlings, not females. This method was first trialled in loggerhead sea turtle hatchlings with 100% success rate but has never been tested in green turtle hatchlings. In male hatchlings, when AMH is present, the precursor to female sex organs (the Müllerian/paramesonephric ducts) deteriorate, and male sex organs begin to develop. Conversely, when AMH is absent in the hatchling's blood, female sex organs develop. If successful, this minimally invasive method of sex identification can be used to check the effectiveness of turtle cooling strategies in the future, not just in our projects but many others globally. Researchers can then better monitor the number of males and females (sex ratios) being produced at each nesting location. With many months in the University of Queensland laboratory to come, the research team will be able to determine if seawater irrigation can produce males. This is imperative for green turtle conservation in Australia's Great Barrier Reef and beyond.

2020/21 Field Season Statistics

- 16 clutches relocated
- 1,800 eggs relocated
- 4 water irrigation treatments trialled
- 2,400 L of seawater used
- 320 small blood samples from hatchlings
- 32 days in the field

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Acknowledgements

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We would like to acknowledge the time, effort and passion given from our partners from the University of Queensland throughout this field season. We would specifically like to thank Larissa Young, Dr David Booth and Melissa Staines for their unwavering support, wisdom and motivation to complete this season's work.

We would like to thank John Preston for his support and financial contribution to hatchling sex identification.

We would also like to extend our wholehearted thanks to the team at the Heron Island Research Station, in particular, Kirsten Slemint, Megan Skelton and Adriana Campili.

We had a brilliant season this year and are excited for what's to come! Until next field season, this is me signing off.

- Caitlin Smith, Marine Species Conservation Project Officer

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