THE VIABILITY OF IRRIGATING GREEN SEA TURTLE NESTS TO COMBAT THE FEMINISATION OF HATCHLINGS ON RAINE ISLAND

Report to Raine Island Recovery Project

September, 2021

FINAL REPORT

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Front cover photo: Nesting green turtles on Raine Island © Queensland Parks and Wildlife Service (2017)

Cite this report as:
Gatto CR, Williamson SA & Reina RD (2021) The viability of irrigating green sea turtle nests to combat the feminisation of hatchlings on Raine Island. Report to the Raine Island Recovery Project. School of Biological Sciences, Monash University

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1. Executive summary

The green turtle population nesting on Raine Island faces numerous threats including sea level rise and high mortality of nesting females (Limpus et al. 2003, Dunstan & Robertson 2019, GBRMPA 2020). These threats have resulted in large investments in infrastructure and beach re-profiling on Raine Island (Dunstan & Roberston 2019). However, the largest threat facing this population is likely to be climate change, because nest temperatures are warming and the adult population is becoming increasingly feminised (Laloë et al. 2017, Booth et al. 2020c). Imbalanced primary and adult sex ratios along with elevated embryonic mortality from lethal nest temperatures could cause a significant and irreversible decline in this nesting population in the near future. Nest irrigation has been identified as a possible mechanism for reducing nest temperatures and ensuring that this population continues to produce male hatchlings. This report reviews the current understanding of how irrigation influences embryonic development and reproductive success. We also review the role of moisture concentrations during development and identify current knowledge gaps. Lastly, we discuss whether irrigation is a viable option for managing the Raine Island nesting green turtle population.

Moisture is an important factor influencing embryonic development and reproductive output in sea turtles. Moisture concentrations during incubation can indirectly and directly influence primary sex ratios (PSR) (Lolavar & Wyneken 2015), hatching success (Caut et al. 2010, Limpus et al. 2020), hatchling morphology (Reece et al. 2002, Tezak et al. 2020a) and locomotor performance (Miller 1993, Finkler 1999). Hatching success is generally high between sand water contents of about 2% and 8% w/w (Packard 1991, Patino-Martinez et al. 2014), and moisture concentrations outside this range can interfere with nest construction (Chen et al. 2007) and hatchling emergence (Rusli & Booth 2017, Swiggs et al. 2018). High sand water content limits oxygen availability to embryos, altering hatchling traits and reducing hatching success (Kraemer & Bell 1980, Foley et al. 2006). Tidal over-wash increases sand moisture but can deposit salts, unless rainfall periodically flushes these salts from the sand (Mazzotti 1989, Foley et al. 2006). The response of the nest environment and hatchling traits to moisture can be modulated by other environmental factors and by sand grain size and chemical composition (Ackerman et al. 1997, Hillel 2003). Overall, moisture is an important factor that influences embryonic development both directly and indirectly.

Artificial sea turtle nest irrigation reported to date has almost exclusively involved surface irrigation, generally using freshwater. Irrigation usually resulted in 1-2°C of cooling, which was similar to the cooling provided by shading nests (Wood et al. 2014, Esteban et al. 2018, Staines et al. 2019, Reboul et al. 2021), but less than rainfall. Only one study irrigated nests with both seawater and freshwater, reporting that both water types provided similar cooling effect (Smith et al. 2021). Irrigation provided more cooling at shallower depths and in nests exposed to the sun compared to shaded nests (Hill et al. 2015). Occasionally irrigation warmed nests, probably because hot surface sand warmed water as it percolated to the nest (Smith et al. 2021). The volume of water applied during irrigation varied substantially and larger volumes generally resulted in more cooling. Clutches can tolerate large volumes (500 mm) of water from natural rainfall over a few days (Laloë et al. 2020), but too much water for a prolonged period can significantly reduce hatching success (Kraemer & Bell 1980, Limpus et al. 2020). Sea turtle embryos are more sensitive to seawater inundation than freshwater during the early stages of development, but are generally resistant to inundation from seawater or freshwater during the middle of development (Limpus et al. 2020). Reported irrigation frequency in the literature was either daily for all or part of the incubation period, or just a single application, and nest temperatures generally took 4-10 days to return to pre-irrigation values (Hill et al. 2015, Smith et al. 2021). Longer durations of irrigation allow for the continued maintenance of cooler nest temperatures, but single applications of water can alter PSRs, if specifically applied during the thermosensitive period of the irrigated nest (Porter et al. 2021, Smith et al. 2021). However, targeting specific points during development is not feasible *in situ* unless it is possible to locate, identify and target individual nests of a known age. The large number and close proximity of nests at Raine Island make this option highly unachievable.

Raine Island nest temperatures during the thermosensitive period need to be cooled by 1-2°C to produce balanced PSRs and cooled by 2-3°C to produce 100% males during the period of irrigation (Booth et al. 2020b, Booth et al. 2020c). The amount of cooling needed will vary with inter-annual variation in weather conditions and as conditions change during the course of a nesting season. Although it is difficult to identify clear patterns among studies because of variation in irrigation regimes and beach characteristics, we suggest that irrigation at Raine Island starts with 200 mm of seawater applied to the sand surface every 4 days, with the aim to produce 100% male hatchlings for a limited period. Irrigation should occur during October and November when conditions are dry and nest temperatures during the thermosensitive period are lowest, but nesting density remains relatively high. This will ensure male hatchlings are produced for at least part of the nesting season. Nest temperatures are lower in April than November, but historically only about 0.2% of nests are laid during this time. If irrigation occurs in November, the PSRs will be more female-biased because nest temperatures are warmer than April, but 19% of nests are laid in November, leading to more males being produced overall. Irrigation should start with small trials, building up to large-scale irrigation. Application of 100 mm of water has regularly achieved cooling of over 1°C and is unlikely to result in nests becoming too wet (Jourdan & Fuentes 2015, Smith et al. 2021). However, 200 mm of water is more likely to achieve the 2-3°C of cooling required to produce 100% males, but is also more likely to significantly increase nest moisture concentrations with potential negative consequences and so, nest moisture should be monitored closely. Irrigating nests every 4 days maintains nest cooling while reducing the risk of inundation.

The application of any sea turtle nest irrigation regime on Raine Island may be limited by water availability. A single 100 mm irrigation of only the re-profiled beach sections on Raine Island will require around 5.5 million litres of water in order to achieve a temperature reduction of about 1.3°C. Thus, we recommend seawater irrigation because it requires minimal infrastructure and is unlimited in supply, allowing for regular irrigation. Additionally, cool water can be pumped from deeper parts of the ocean, increasing the cooling effect. However, salt accumulation in the sand will need to be carefully monitored and managed. Other potential options include the transportation of freshwater from the mainland, desalination and rainfall capture. These options are limited by the amount of rainfall, the storage required, large infrastructure needs, expenses, and possible negative environmental effects.

Overall, we conclude that nest irrigation is a viable means for cooling sea turtle nests on Raine Island. Nests will need to be regularly monitored to ensure that the required amount of cooling is achieved without negatively impacting hatching success and other hatchling traits. We suggest that trials start at a relatively small scale and are then increased, with adjustments made to compensate for changes in weather conditions, nesting density and any potential effects of irrigating larger areas.

2. Summary of threats to incubating eggs on Raine island

Raine Island is a 27-hectare coral cay, located on the northern tip of the Great Barrier Reef. The island is significant for Indigenous Australians, is listed on the Queensland Heritage Register, is the most important seabird rookery in the Great Barrier Reef World Heritage Area and is home to the world's largest nesting population of green turtles. Approximately 90% of the northern Great Barrier Reef green turtle population nests on Raine Island from November to March, peaking in December and January, with about 50,000 females nesting there during peak seasons (Limpus et al. 2003, Fuentes et al. 2010a, Booth et al. 2020c). Weather conditions are generally warm and wet during the nesting season, and become cooler and drier from June to August (Booth et al. 2020c). During the 1970s and 1980s, hatching success and hatchling production on Raine Island was generally high, but turtles nesting on the island had experienced dramatic decreases in hatching success and overall hatchling production by the mid-1990s (Limpus et al. 2003).

Tidal inundation on the island was identified as a major cause of clutch mortality, leading to significant beach re-profiling (Limpus et al. 2003, Dunstan & Robertson 2019, GBRMPA 2020). Sea level rise remains a threat to incubating clutches on Raine island, as inundated nests often experience increased or complete mortality (Fuentes et al. 2010c). Beach re-profiling works decreased the number of nests being inundated per season and had the added benefit of providing a greater area for females to nest, reducing the destruction of previously laid nests by other females (Dunstan & Robertson 2019, GBRMPA 2020).

The high density of nests still results in many clutches being dug up, or if undisturbed, being located close to other nests, resulting in elevated PCO_2, reduced PO_2 and high incubation temperatures (Booth & Dunstan 2018). It is possible that the high nesting density may lead to elevated microbial loads in the sand and thus to reduced hatching success, as observed on olive ridley mass-nesting 'arribada' beaches (Bézy et al. 2015). However, this effect does not appear to be consistent within or among nesting seasons, and is most likely to impact hatching success late in the nesting season (Booth & Dunstan 2018, Booth et al. 2020c).

Like many other sea turtle nesting grounds, high incubation temperatures and anthropogenic climate change are major concerns for the Raine Island nesting population, particularly as the island has no trees to provide shade (Limpus et al. 2003). Increasing sand and nest temperatures are likely to reduce hatching success, result in the increased feminisation of hatchlings and produce smaller hatchlings with decreased thrust production during swimming (Howard et al. 2014, Santidrián Tomillo et al. 2015, Booth 2017, Laloë et al. 2017). Climate change may also lead to the loss of Foraminifera that comprise the majority of Raine Island's sand budget (Fuentes et al. 2010b, Dawson & Smithers 2014). Initially, Raine Island's sediment budget may increase as Foraminifera die, but the budget will become negative once Foraminifera are locally extirpated, leading to erosion, higher rates of nest inundation and eventual nesting habitat loss (Hopley 2008).

Reductions in hatchling production are exacerbated by high adult mortality on the island, with many turtles overturning or becoming entrapped on low cliffs. This has led to the installation of cliff-top fencing and considerable efforts to rescue stranded and overturned females (Dunstan & Robertson 2019). Despite the extensive works to limit adult mortality and to increase hatchling production, the low reproductive success of green turtles at Raine Island remains a concern and indicates that this population is in the early stages of a major decline (Dunstan & Robertson 2019).

Hatching success at Raine Island ranges from 10% to 50%, compared to 75% to 85% at other green turtle rookeries in the Great Barrier Reef (Limpus 2008, Dunstan & Robertson 2019). In some years, hatching success can be as high as 80% early in the nesting season, but even in good years, generally decreases to below 50% by February (Booth et al. 2020c). During the 2016/17 and 2018/19 nesting seasons, female hatchling production was close to 100% throughout the nesting season, except in April when female hatchling production dropped to about 90% (Booth et al. 2020c). While nest inundation and sea level rise remain serious threats to hatchling production, elevated incubation temperatures threaten to both reduce hatchling survival and further feminise primary sex ratios (PSR).

Traditional methods to combat elevated sand temperatures, such as shading nests, are not feasible on Raine island, considering the lack of trees and the island’s remote location. One potential alternative is to irrigate or water sea turtle nests, directly cooling the nests with water and increasing their capacity for evaporative cooling. This report reviews the current understanding of how moisture concentrations in sea turtle nests influence other environmental variables, the factors that determine how much water is available to developing embryos and the effects of moisture concentrations on sea turtle hatchling traits and developmental success. We review studies that have watered nests, identifying current knowledge gaps and opportunities for future research. Lastly, we discuss whether irrigation is a viable option for managing the Raine island nesting green turtle population. We discuss the temperature reductions required, potential water sources, the appropriate watering frequencies, durations and volumes, and the risks associated with watering nests.

3. Definitions

% v/v: volumetric water content, determined as the volume of water in a sample divided by the volume of the substrate in a sample.

% w/w: gravimetric water content, determined as the weight of water in a sample divided by the weight of the substrate in a sample.

Emergence success: the proportion of eggs within a clutch that successfully hatch and produce hatchlings that successfully emerge from the nest.

Hatching success: the proportion of eggs within a clutch that successfully hatch and produce live hatchlings.

Inundation/flooding: when nests are saturated with water and eggs are fully or partially submerged in water.

Irrigation: the process of applying water to a substrate. Can be applied from the surface (e.g., sprinklers) or from underground (e.g., drip system from underground piping). We report the volume of water applied in mm rainfall equivalent to account for differences in irrigation area.

Irrigation duration: The length of time that nests were regularly irrigated, e.g., nests were irrigated from January to March. Can also be expressed in terms of development, e.g., nests were irrigated throughout development or just during the thermosensitive period (Figure 1).

Irrigation frequency: How often nests are irrigated over the duration of irrigation, e.g., daily, weekly (Figure 1).

Locomotor performance: the ability of hatchlings to crawl, swim and self-right.

Moisture concentration: the amount of water present in a sample. Can be presented as % w/w, % v/v or as water potential.

Timing of irrigation: At what time during the day were nest irrigated, e.g., morning, afternoon.

Water potential: a measurement in Kilopascals (kPa) of the potential energy of water under a given set of conditions. The maximum water potential, of pure water in a saturated substrate, is 0 kPa. Water potential decreases as a substrate becomes less saturated (e.g., as it dries) or the water source becomes less pure (e.g., more saline). Water moves from areas of high-water potential to low water potential, e.g., from wet substrates (0 kPa) to dry substrates (-500 kPa).

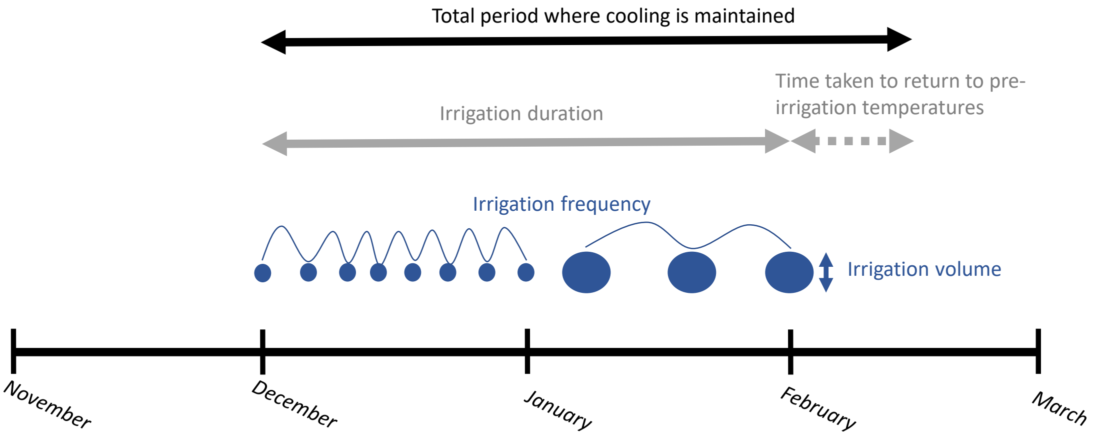

Figure 1: Visual representation of common descriptions of irrigation used in this report. Circles represent applications of water at different points during the nesting season, and the size of each circle represents the irrigation volume. The terms used in this figure are defined in Section 3.

4. Effects of moisture

In this section, we discuss how moisture variation influences nest site selection and nesting success in adults, and how moisture influences embryonic development and hatchling traits. We focus on hatching and emergence success, PSRs, hatchling size and locomotor performance. Studies on the effect of moisture during incubation are limited in comparison to those on the effect of temperature, so we include data on sea turtles where possible, but also include data on freshwater turtles where appropriate or necessary. We provide an overview of the research on how variation in moisture concentrations during incubation influence sea turtle nesting, embryonic development and hatchling traits. Thus, we do not draw any major conclusions on the viability of nest irrigation in this section.

4.1. Adult nest site selection and nesting success

Nesting females likely utilise multiple cues when selecting nest sites, including slope, vegetative cover, moisture, and light (Miller et al. 2003, Price et al. 2018). Beach slope has consistently been found to influence sea turtle nest site selection, likely because females tend to nest above the high tide line where nests are less likely to experience tidal inundation (Wood et al. 2000, Miller et al. 2003, Pfaller et al. 2009). While sand moisture and salt concentrations are not correlated with female nest site selection as consistently as slope, it is possible that females use moisture and salinity as cues to select nest sites that will not be inundated but still contain enough moisture for successful embryonic development (Wood et al. 2000, Miller et al. 2003). Both moisture and salinity are likely to be unreliable indicators of optimal incubation environments because of the spatial (e.g., moisture changes with nest depth) and temporal (e.g., both moisture and salinity can vary with rainfall) variation observed in both of these environmental factors (Wood et al. 2000). There are no studies to our knowledge that manipulate beach conditions (i.e., irrigation) to assess the effects on nest site selection. Moisture also plays an important role in determining whether nesting attempts by females are successful or are aborted. Sand that is too dry and not compacted enough is difficult for nesting females to build a nest in, resulting in higher rates of nest failure (Chen et al. 2007). Similarly, as the depth of the 'drying front' (the depth of dry surface sand) increases, fewer hatchlings successfully emerge from the nest because they are unable to dig their way out of the loose and hot sand (Swiggs et al. 2018). Thus, moisture concentrations, and to a lesser extent, salinity, play an important role in nest site selection by adult females and in the successful construction of nests.

Key points

- **Beach slope is a key indicator for nesting females when selecting nest sites.**
- **Moisture and salinity are likely unreliable indicators of suitable nest sites because of spatial and temporal variation in both factors.**
- **Dry sand results in more unsuccessful nesting attempts by females and reduces emergence success of hatchlings.**

4.2. Sex ratio

Differences in nest substrate moisture and humidity during incubation may account for some of the observed variation in hatchling PSRs in species with temperature sex determination. Moisture indirectly alters nest temperatures (Lolavar & Wyneken 2015, Sifuentes-Romero et al. 2017) and restricts oxygen availability (Cedillo-Leal et al. 2017), with potentially other direct, unknown mechanisms (Lolavar & Wyneken 2017). There is also some evidence that elevated carbon dioxide concentrations during incubation, potentially resulting from high moisture concentrations limiting gas diffusion, may result in more female-biased sex ratios (Etchberger et al. 1992, Etchberger et al. 2002).

Sea turtles exhibit the MF pattern of sex determination where higher incubation temperatures produce more females and lower temperatures produce more males (Standora & Spotila 1985). Studies of both freshwater turtles (Gutzke & Paukstis 1983, LeBlanc & Wibbels 2009, Sifuentes-Romero et al. 2017) and sea turtles (Lolavar & Wyneken 2015, Wyneken & Lolavar 2015, Lolavar & Wyneken 2017) have shown that increased moisture during incubation results in increased production of male hatchlings. However, other studies have found that moisture played no role in determining PSRs in certain Testudines (Packard et al. 1991, Bobyn & Brooks 1994, Hewavisenthi & Parmenter 2000) and one study in painted turtle hatchlings (*Chrysemys picta*) found that drier substrates produced more males than clutches incubated in wetter substrates (Paukstis et al. 1984). Comparisons among these studies can be difficult because substrate type and egg arrangement differed. Additionally, deciphering the role of moisture in sea turtle sex determination is complicated by the strong interactions between temperature and moisture (Hill et al. 2015, Lolavar & Wyneken 2017), making it difficult to isolate their individual effects.

Overall, the role of moisture in directly influencing reptile PSRs is not clearly defined. Further research is required to identify whether moisture can directly influence sex determination and if so, to identify the mechanism. It is currently thought that the interaction between moisture and temperature has the largest effect on sex determination (Sifuentes-Romero et al. 2017), highlighting the importance of considering multiple environmental variables when investigating the effects of incubation conditions on hatchling phenotypes.

Key points

- **Higher moisture generally results in cooler nests and the production of more males.**
- **Moisture may directly influence sex determination but strong interactions between moisture and temperature make separating their effects difficult.**

4.3. Hatching success and developmental rate

Excess moisture or inundation during incubation can result in decreased hatching success or even loss of the entire clutch (Kraemer & Bell 1980, Van Houtan & Bass 2007, Caut et al. 2010). While reptile eggs can be quite resistant to brief or intermittent inundation from rainfall, river flooding or unusually high tides (Caut et al. 2010, Pike et al. 2015), repeated stress due to excessive moisture almost always leads to embryonic mortality (Foley et al. 2006). Hatching success after rainfall or flooding varies depending on the elevation of egg clutches within a landscape (Kraemer & Bell 1980) and the stage of embryonic development (Rafferty et al. 2017, Limpus et al. 2020).

Inundation appears to limit oxygen supply to the developing embryos such that late-stage embryos, with higher metabolic demands, are more sensitive to oxygen deprivation than early stage embryos, even if hypoxia only lasts for a few hours (Ackerman 1981, Rafferty et al. 2013, Pike et al. 2015). Sea turtle eggs are capable of arresting development if re-exposed to hypoxic conditions within 6-12 hours of oviposition, but embryos require a relatively constant supply of oxygen and cannot re-arrest if exposed to hypoxic conditions more than 12 hours post-oviposition (Williamson et al. 2017). Inundation may interfere with water exchange in early-stage embryos (<20% of development), resulting in increased mortality rates (Limpus et al. 2020). Early-stage embryos were more sensitive to seawater inundation than freshwater (Table 1), supporting this hypothesis and suggesting that saltwater may have further negative effects on development than freshwater flooding alone (Bustard & Greenham 1968, Foley et al. 2006, Bower et al. 2013).

Typical hatching success varies significantly within sea turtles but is generally greatest at intermediate moisture levels (Packard et al. 1991, Foley et al. 2006). Egg size, specifically surface area to volume ratio, and egg water content, can influence the response of developing embryos to moisture concentrations (Ackerman et al. 1985, Gutzke & Packard 1985, Packard 1999). Large eggs exchange water more slowly relative to small eggs (Ackerman et al. 1985), although under normal nest conditions females generally allocate enough water to eggs for successful development via the albumen (Packard et al. 1979, Ackerman et al. 1985, Packard 1999). Overall, eggs incubated in dry conditions generally hatch earlier than those in wet conditions (Packard et al. 1981, Packard et al. 1985, McGehee 1990, Packard et al. 1991, Miller 1993).

Key points

- **Excess moisture or inundation results in increased embryonic mortality and reduced hatching success, in part because it limits oxygen supply to developing embryos.**
- **Embryos are relatively resistant to brief (<6 hours) or intermittent inundation.**
- **Embryos are most sensitive to inundation early (first 20%) or late (last 20%) in development.**
- **Saltwater inundation results in greater increases in mortality than freshwater inundation, possibly because it interferes with water exchange between the egg and the nest environment.**

4.4. Locomotor performance

Most research on possible effects of moisture during incubation on locomotor performance has involved snapping turtles. Turtle hatchlings incubated in wet conditions are generally faster swimmers and crawlers (Miller et al. 1987, Miller 1993, Finkler 1999) and also show a smaller reduction in crawling speed after desiccation compared to hatchlings incubated in dry conditions (Finkler 1999). Moisture concentrations during incubation may also influence hatchling thermal tolerance, but it is unclear whether moisture influences thermal tolerance directly or indirectly via temperature (Gatto et al. 2021).

There are several possible explanations for improved locomotor performance of some turtle hatchlings incubated in wet conditions. The first is that better performance is a result of the hatchling’s larger size (Miller 1993), although this is not always the case (Gatto & Reina 2020). Another possibility is that hatchlings incubated in wetter conditions accumulate lactate more slowly than hatchlings incubated on or within dry substrates (Miller et al. 1987) or may emerge more hydrated than hatchlings from dry nests (Gatto & Reina 2020). Hatchlings incubated in dry environments have larger residual yolk mass relative to their body mass (Packard et al. 1988), and may require increased anaerobic energy expenditure to carry this additional yolk mass that is not contributing to locomotion (Miller et al. 1987). However, hatchlings with larger yolk reserves will have access to greater energy reserves when moving this mass (Radder et al. 2004). Indeed, green turtle hatchlings incubated at high moistures exhibited reduced swimming performance after 24 hours of continuous swimming compared to hatchlings from low moisture nests, despite there being no difference in swimming performance between moisture treatments during initial swimming tests (Matthews 2018). Last, it is possible that moisture may directly or indirectly influence embryonic muscle development, but the mechanisms behind these potential effects are unknown and further investigation is required.

Key points

- **Higher moisture concentrations during incubation generally result in hatchlings that are faster crawlers and swimmers.**

- **The mechanism behind this effect is unknown but may relate to moisture influencing hatchling size, muscle development, hydration or lactate accumulation during exercise.**

4.5. Body size

Increases in moisture during incubation generally result in the production of heavier and longer sea turtle hatchlings (Tracy et al. 1978, Hewavisenthi et al. 2001, Reece et al. 2002, Tezak et al. 2020a). Some of this effect is likely explained by eggs in wet substrates absorbing more water than eggs in dry substrates (Tracy et al. 1978). Thus, embryos that develop on wet substrates have greater access to water, are more hydrated and are therefore heavier than embryos that develop on dry substrates (Packard et al. 1988).

Increased moisture levels in sea turtle nests during incubation results in hatchlings converting more yolk mass into body mass, thus hatching at a larger size (Miller et al. 1987, Hewavisenthi et al. 2001). Similarly, freshwater snapping turtle embryos incubated on wet substrates mobilised more protein and lipids from the yolk and had higher tissue hydration levels than embryos incubated on dry substrates, resulting in larger body size at hatching (Packard et al. 1988). However, the mechanisms behind this remain unknown. One possible explanation is that drier incubation conditions lead to higher blood viscosity in the developing embryo, reducing the rate at which nutrients can be converted into body tissues (Packard & Packard 1986, 1989). However, Bilinski et al. (2001) found that calcium mobilisation from eggshell to embryo in leatherback turtle embryos was higher in drier incubation conditions. Incubation moisture levels do not generally influence post-hatching growth rates in Testudines (Brooks et al. 1991, McKnight & Gutzke 1993, Bobyn & Brooks 1994). However, some studies have observed faster post-hatching growth rates in sea turtle hatchlings incubated in wetter conditions (Erb et al. 2018).

Embryos are generally less sensitive to moisture than they are to temperature (Packard et al. 1989), but in circumstances where moisture concentrations are very high or low, moisture can play a larger role than temperature in determining embryonic growth and survival (Cagle et al. 1993). Higher moisture levels appear to produce larger and heavier hatchlings within a certain range, but extreme moisture levels outside this range can have negative effects on body size and growth. Low moisture levels potentially increase embryo blood viscosity to levels that limit the mobilisation of nutrients and oxygen and thus reduce hatchling body size (Packard 1991).

Key points

- **Higher moisture concentrations during incubation generally produced larger and heavier hatchlings.**
- **This may result from hatchlings absorbing more water during development when in wet environments, but the mechanisms are unknown.**
- **Variation in temperature generally has a greater effect on size and development than similar changes in moisture.**

Table 1: Hatching success of nests inundated with seawater and freshwater from Limpus et al. (2020). Nests were inundated with water in plastic containers that were buried in a hatchery and each clutch of eggs was inundated only once. The amount of shade provided to nests and the temperature of the water applied was not reported. Inundations occurred over two years, hence the differences developmental rates after the same number of weeks post-oviposition between the two years. Data from 1981/82 is reported in bold and non-bold data is from 1982/83.

Duration of inundation	When during development did inundation occur? Weeks since oviposition, % of development					
	0 weeks 0%	1 week **81/82- 14%** 82/83- 12%	2 weeks 82/83- 24%	4 weeks **81/82- 54%** 82/83- 48%	6 weeks **81/82- 81%** 82/83- 72%	7 weeks **81/82- 94%**
Control	**50%** 100%	**85%** 95%	90%	**90%** 100%	**90%** 95%	**80%**
1 hour	Seawater: 0% Freshwater: 0%	Seawater: 37% Freshwater: 50%	Seawater: 75% Freshwater: 80%	Seawater: 95% Freshwater: 95%	Seawater: 90% Freshwater: 75%	
2 hours	**Seawater: 0%**	**Seawater: 30%**		**Seawater: 90%**	**Seawater: 50%**	**Seawater: 0%**
3 hours	Seawater: 0% Freshwater: 0%	Seawater: 15% Freshwater: 85%	Seawater: 65% Freshwater: 89%	Seawater: 70% Freshwater: 90%	Seawater: 90% Freshwater: 84%	
6 hours	**Seawater: 0%** Seawater: 0% Freshwater: 0%	**Seawater: 0%** Seawater: 15% Freshwater: 95%	Seawater: 67% Freshwater: 90%	**Seawater: 70%** Seawater: 80% Freshwater: 10%	**Seawater: 0%** Seawater: 75% Freshwater: 60%	**Seawater: 0%**
24 hours	**Seawater: 0%**	**Seawater: 0%**		**Seawater: 0%**	**Seawater: 0%**	**Seawater: 0%**
48 hours	**Seawater: 0%**	**Seawater: 0%**		**Seawater: 0%**	**Seawater: 0%**	**Seawater: 0%**

5. Effects of moisture on other environmental variables

Assessing the potential consequences of watering sea turtle nests requires an understanding of how changes in moisture both influences developing embryos directly, and also indirectly by altering other environmental variables. The interconnectedness of environmental variables means that changes in moisture concentrations are likely to produce multiple alterations to incubation conditions and these changes will vary among nests depending on the pre-existing conditions within those nests (Table 2).

Temperature and moisture are generally considered the main determinants of incubation conditions within clutches of eggs, and accordingly the interactions between these two factors are most studied. Elevated moisture concentrations resulting from precipitation or tides generally reduce incubation temperatures (Webb et al. 1977, Houghton et al. 2007, Warner & Shine 2008, Charruau 2012, Tezak et al. 2018). However, the amount of cooling in the nest depends on the temperature of the water and depending on the volume applied, even cool water may be heated to above nest temperature by the hot surface layers of sand that it percolates through (Smith et al. 2021). Elevated moisture concentrations also allow for greater evaporative cooling, although ambient temperatures generally have greater influence on evaporative cooling rates and higher evaporative rates result in faster depletion of moisture from nests (Ackerman et al. 1997, Shine et al. 2002).

Moisture concentrations strongly influence oxygen availability within nests, as water fills spaces between grains of sand, reducing the available space for oxygen (Ackerman et al. 1997, Hillel 2003). This not only limits oxygen availability but can also trap carbon dioxide within the nest. Substrate type and the metabolic needs of developing embryos both influence oxygen availability and would likely influence the response of embryos to changes in moisture concentration (Ackerman et al. 1997). Recent research has suggested that low oxygen availability in green sea turtle nests is not impacting developmental success on Raine Island (Booth & Dunstan 2018).

Water flowing through the nest substrate can mobilise salts and other water-soluble minerals (Mazzotti et al. 1988, Ackerman et al. 1997), while saline water sources (e.g., tidal over-wash, cooling nests with seawater) can deposit salts around underground clutches as the water evaporates (Foley et al. 2006) and deposited salts can influence the availability of water within nests (Ackerman et al. 1997). Deeper sand samples tend to be more saline, potentially because of seawater incursion from below or because rainfall washes salts down through the substrate, leading to salt accumulation further down (Foley et al. 2006).

The addition of seawater can reduce microbial loads in the sand surrounding sea turtle nests, decreasing oxygen consumption and carbon dioxide production by microbes, thereby resulting in more oxygen being available for developing embryos (Bézy et al. 2014, Bézy et al. 2015). Microbes are not the only biotic factors that can influence environmental conditions within nests. Shade created by vegetation can result in significantly cooler nest temperatures (Kamel & Mrosovsky 2006, Reboul et al. 2021) but roots can draw moisture from nests and trap hatchlings (Bustard & Greenham 1968, Conrad et al. 2011, Read et al. 2019). In some cases, vegetation that grows directly on the surface of the sand can increase nest temperatures, possibly because some plants have lower albedo than the surrounding white sand (Conrad et al. 2011).

Key points

- **Higher moisture concentrations generally reduce nest temperatures by directly cooling nests or increasing evaporative rates.**
- **Higher moisture concentrations can limit oxygen availability for developing embryos by filling pore spaces between grains of sand. This can also result in increase in carbon dioxide concentrations within nests.**
- **Water flowing through nest substrates can mobilise and 'flush' salt from the substrate. Frequent tidal inundations and greater evaporative rates can increase the concentration of salts within the substrate.**
- **Higher salt concentrations and the addition of saltwater to substrates can limit or reduce microbial growth, resulting in lower substrate temperatures, higher oxygen availability and reduced carbon dioxide accumulation.**

6. Factors that influence the response of clutches to environmental variation

It is important to note that not all the water in the sand surrounding eggs is available for uptake by embryos, and nests that are watered with identical volumes of water can respond differently depending on the pre-existing conditions within those nests. In this section, we discuss how variation in the nest environment, sand composition, (e.g., grain size and porosity), and the arrangement and size of clutches within nests can cause clutches to respond differently to the same changes in ambient conditions.

Large grain sizes with large spaces between grains allow water and gases to flow more easily than substrates with small grain sizes (Foley et al. 2006). However, depending on shape, larger particle diameters generally result in decreased total pore space compared to fine-grained substrates and the resulting decrease in total pore space leads to decreased water content around nests in coarse sands (Hillel 2003). Particle size also may affect the diffusion of gases, the conduction of heat around the nest and the mobility of salts within the substrate and nest.

Substrates with greater moisture content are generally better conductors of heat than dry substrates (Ackerman et al. 1985). Eggs that incubate in substrates with greater thermal conductivity generally absorb more water in wet conditions and less water in dry conditions, compared to substrates with lower thermal conductivity (Ackerman et al. 1985). Differences among substrate types therefore alter the nest microclimate relative to the broader external environment and can alter the responses of developing embryos to the incubation environment. It is important to note that although substrate type can alter the nest microclimate, this does not guarantee that hatchling traits will also change (Stewart et al. 2019).

The uptake and loss of water by sea turtle eggs is influenced by several factors. The structure of the eggshell, the water potential of the surrounding substrate and the egg albumin, nest temperature and the fraction of the eggshell in contact with the surrounding substrate all influence water exchange (Tracy et al. 1978, Packard 1999). Sea turtle eggs have a water potential of approximately -900 kPa, while the surrounding sand is generally -5 to -50 kPa (Ackerman et al. 1997). Thus, eggs typically absorb water and increase in mass during development, but changes in substrate water potential can influence the rate at which this occurs. Generally, eggs absorb water across the eggshell from the substrate, and lose water by transpiration into the gaseous atmosphere of the nest chamber (Tracy et al. 1978, Packard 1999). Thus, eggs on the periphery of the nest that have large surfaces in contact with the surrounding substrate will typically absorb more water than eggs in the centre of the nest (Ackerman et al. 1997, Packard 1999). Eggs tend to lose water in warmer, drier environments and gain water in cooler, wetter environments (Packard 1999). Higher temperatures increase rates of evaporative water loss from both the substrate and eggs (Packard et al. 1977), but may also result in higher humidity within the nest, reducing evaporative water loss into the nest atmosphere (Lolavar & Wyneken 2017).

While the position of individual eggs in a single clutch can influence their response to moisture and other environmental factors, the size of the clutch can also impact its response to environmental variation. Entire clutches of eggs can be thought of as a single large egg, where larger clutches have fewer eggs and less surface area in contact with the surrounding substrate, relative to its volume (i.e., smaller surface area to volume ratio), compared to smaller clutches. Thus, larger clutches, like larger eggs, are likely to be more resistant to changes in moisture concentration in the surrounding substrate (Ackerman et al. 1997). Larger clutches of eggs produce more metabolic heat and may therefore be more susceptible to increases in temperature (Van De Merwe et al. 2006, Chen et al. 2010). They also consume more oxygen and produce more carbon dioxide than smaller clutches, making them more susceptible to hypoxic and hypercapnic conditions (Ackerman 1980, Chen et al. 2010).

Increases in salinity can influence embryonic responses to moisture in a couple of ways. Seawater has a water potential of approximately -2000 kPa, significantly lower than that of eggs (approximately -900 kPa) (Ackerman et al. 1997). Thus, the presence of seawater in nests may osmotically draw water out of the egg. Additionally, regulating and removing excess salts by the developing embryo requires considerable energy, potentially altering metabolic rates and reducing growth rates (Holliday et al. 2009).

Key points

- **The response of clutches to the same changes in ambient conditions varies because of several factors.**
- **Eggs absorb more water when surrounded by moist sand, when nest temperatures are cooler and when more of their eggshell is in contact with the surrounding substrate rather than air or with other eggs.**
- **Substrates with large grain sizes allow water and gases to flow more easily, but also hold less water between grains. Large grain sizes allow salts to be ‘flushed’ from the substrate more easily.**
- **Higher substrate water content results in greater thermal conductance within that substrate.**
- **Seawater has a lower water potential than that of eggs. Thus, the presence of seawater in nests can result in water being drawn from out of the egg.**

Table 2: The interacting effects of environmental variables within sea turtle nests. For salinity and oxygen concentration, we also list how they can modulate the response of developing embryos to other environmental variables.

	Temperature	Moisture	Oxygen concentration	Salinity
Increased temperature		Increased evaporative rates resulting in reduced nest moisture levels [A]	Nest temperature generally increases during incubation due to metabolic heat production of the embryos. Both the increased temperatures and the increased development and size of the hatchlings results in increased oxygen demands for the embryos and results in decreased oxygen availability within the nest [B] Temperature can also influence diffusion rates and gas densities within clutches [C]	Increased temperatures do not directly influence salt concentration within nests, but increased temperatures can increase evaporative rates resulting in increased salt concentration within nests [A]
Increased moisture	Decreased temperature either via direct cooling or increased evaporative cooling [A, E, F]		Water displaces air in-between substrate particles resulting in reduced oxygen availability within the nest [A, I, J]	Depends on the salinity of the water. Seawater can deposit salts while rainfall can rinse the nest thereby reducing salinity [A, K]
Increased oxygen concentration	Oxygen concentration does not directly influence nest temperatures, but higher oxygen levels can help embryos be more resistant to thermal stress compared to embryos developing in low oxygen environments [D, L]	Oxygen concentration does not directly influence nest moisture but caiman embryos that had access to oxygen via air bubbles trapped on their rough shell had increased resilience to inundation compared to embryos with smooth shells [G]		Oxygen concentration does not influence salt concentration
Increased salinity	Salinity does not influence nest temperatures.	Salt concentrations can influence water gradients and potential within nests. However, the effects of salt on the movement of water within nests is minimal [A]	Salinity does not directly influence oxygen concentrations within nests. However, increased salinity can result in increased metabolic stress for developing embryos. This can impact embryonic metabolic rates and the availability of oxygen within the nest [H]	

[A] Ackerman et al. (1997)
[B] Chen et al. (2010)
[C] Ackerman (1980)
[D] Liang et al. (2015)
[E] Houghton et al. (2007)
[F] Tezak et al. (2018)
[G] Cedillo-Leal et al. (2017)
[H] Bustard and Greenham (1968)
[I] Caut et al. (2010)
[J] Kam (1994)
[K] Foley et al. (2006)
[L] Smith et al. (2015)

7. Artificial irrigation of turtle nests

There have been several studies reporting the effects of additional moisture on the incubation environment, development and hatchling traits of turtles. These studies reported deliberate nest irrigation as well as the effects of rainfall events, tidal inundation and the impact of maintaining different moisture concentrations during incubation (Table 3). Here, we focus on the deliberate irrigation of sea turtle nests and the effects of rainfall to assess the overall effects of adding water to nests on incubation temperature, developmental success and hatchling traits. To account for differences in plot sizes among studies, i.e., the area around each individual nest that was irrigated, we report irrigation volumes in mm of water (rainfall equivalent) applied to each plot. This also allows for direct comparisons with the effects of rainfall. Studies that report on nest irrigation with seawater or saltwater are limited, so we also include information on the tolerance of eggs to inundation from high tides and storm surges. Data on inundation was typically collected from *in situ* nests and from nests artificially inundated with seawater. Thus, they provide further insight into the potential implications of irrigating nests with seawater and also give a greater understanding of how much seawater and freshwater sea turtle eggs can tolerate and for how long.

The irrigation of turtle nests typically applied water from the surface, either using a watering can (Bodensteiner et al. 2015, Matthews 2018, Gatto et al. 2021, Smith et al. 2021) or using a sprinkler (Jourdan & Fuentes 2015, Erb et al. 2018, Lolavar & Wyneken 2021). We did not find any studies that reported the underground irrigation of nests, presumably because of cost or difficulty compared to simply watering the surface. Underground irrigation may be limited to nests in hatcheries where underground pipes will not increase the number of aborted nests or be damaged by nesting females (Hays & Speakman 1993, Kamel & Mrosovsky 2005). Regardless of the method used to irrigate nests, investigators typically used freshwater, normally sourced from taps or rainfall (Table 3). Only Smith et al. (2021) directly compared the effects of seawater and freshwater irrigation under various watering regimes. Only Limpus et al. (2020) directly compared seawater and freshwater inundation for different durations of time at various stages of development.

The volume of water used to irrigate nests, the duration of irrigation and the frequency of irrigation varied among studies (Table 3), making direct comparisons difficult. Additionally, irrigation was only applied to clutches of eggs in some studies, the timing of irrigation varied and, in some cases, the volume and frequency of irrigation were adjusted as necessary to maintain certain moisture concentrations (Table 3). Here, we discuss trends among studies and highlight key findings in individual studies. We refer to the application of water to incubating eggs as ‘watering nests’, and to the application of water to sand only as ‘watering sand’.

Key points

- **Nest temperatures were not measured as often as sand temperatures.**
- **Irrigation regimes, (frequency, duration and volume) varied among studies, so directly comparisons are difficult.**

Table 3: Summary of irrigation regimes, and the responses of the nest environment and hatchling traits in key studies used in this report. For studies presenting the effects of rainfall, we only include data for rainfall events where the amount of cooling is reported for a specific volume of rain in a single event, rather than for the total amount of rain over an entire nesting season.

Study type	Study	Were clutches of eggs or just sand irrigated? (Depth, cm)	Level of shade	Water source	Timing of irrigation		Irrigation regime			Water content	Amount of cooling, °C	Sex ratio, % females	Hatching success, %
					When during the day (water temp., °C)	When during dev.	Frequency	Duration, days	Volume, mm/d				
Irrigation	Smith et al. (2021)	Sand & eggs, green (70 cm)	Sun & shade	Freshwater & seawater	Dawn (24.2-25.1)	Day 18, before thermosensitive period	Single & daily	1, 4 & 7	20, 50, 100 & 200	Not reported	+0.1-0.5 to -1.3	100	74.9-92.4
	Hill et al. (2015)	Sand (45 cm & 75 cm)	Sun & shade	Freshwater	Afternoon	No eggs	Daily	31	3.2, 10.4 & 23.3	1.3-8.5 % g/g	-0.6 to -4	No eggs	No eggs
	Jourdan and Fuentes (2015)	Sand in plastic containers (50 cm)	Sun & shade	Freshwater	Noon (34) & night (25)	No eggs	Daily	12	50 (noon) & 100 (night)	Not reported	+0.83 to -2.23	No eggs	No eggs
	Lolavar and Wyneken (2021)	Eggs, loggerhead (60 cm)	Sun	Freshwater	Morning	Entirety of dev.	Daily	Entirety of dev.	40, 80 & 140	~5 to 8 % v/v	-0.2 to -1.5	71 to 100	45 to 90
	Erb et al. (2018)	Eggs, loggerhead (60 cm)	Sun	Freshwater	Morning	Entirety of dev.	Daily	Entirety of dev.	40	5.3 & 6.8 % v/v	-0.16	Not reported	Not reported
	Matthews (2018)	Eggs, loggerhead (70 cm)	Shade	Freshwater	Not reported	Entirety of dev.	As required	Entirety of dev.	As required, 4-9 mm	4.89 & 7.89 % v/v	-0.83	~70 & ~90	94.4 & 95.6
	Bodensteiner et al. (2015)	Eggs, painted turtle (7 cm)	Not reported	Freshwater	Not reported	Majority of dev. (not the last few days)	Twice weekly	Majority of dev. (not the last few days)	30	11 to 32 % v/v	-0.1 to -0.9	39 & 44	52 to 82
Rainfall	Laloë et al. (2020)	Eggs, green (70 cm)	Not reported	Rain	Not reported	Not reported	Single	4	129.8 (519 total)	Not reported	-2.3 & -4.3	Not reported	Not reported
	Staines et al. (2020)	Eggs, hawksbill	Sun & shade	Rain	Not reported	Not reported	Single	2	62.5 (125 total)	Not reported	-3.1 to -4.1	Not reported	Not reported

		(50 cm) & green (65 cm)											
	Lolavar and Wyneken (2015)	Sand & eggs, (30 cm & 45 cm)	Sun	Rain	Not reported	Some during thermosensitive period	Single	Not reported	60 to 143	Not reported	As high as -7	0 to 100	4 to 99
Inundation	Limpus et al. (2020)	Eggs, loggerhead (50 cm)	Not reported	Freshwater & seawater	Not reported	0, 1, 2, 4, 6 & 7 weeks	Single	0, 1, 2, 3, 6, 24 & 48 hours of inundation	Complete inundation	Not reported	Not reported	Not reported	0 to 100%
	Patino-Martinez et al. (2014)	Eggs, leatherback (no depth)	Not reported	Freshwater	Not reported	Start of dev.	Single	Moisture set and then not adjusted for the rest of dev.	Volume added to create specific water content	1, 2, 6, 10 & 12 % g/g	Not reported	Not reported	0 to 65%
	Pike et al. (2015)	Eggs, green (no depth)	Not reported	Saltwater, 28 ppt	Not reported	0, 33, 50 & 66% of dev.	Single	1, 3 & 6 hours of inundation	Complete inundation	Not reported	Not reported	Not reported	30 to 70%
	Foley et al. (2006)	Eggs, loggerhead (15 cm, 30 cm & 45 cm)	Not reported	Saltwater, water salinity: 26.9 ppt, nest: 16.8 ppt (15.7-67.9)	Not reported	Not reported	No inundations, single & multiple	Not reported	No inundation, part inundation & full inundation	1 to 19 % w/w	Not reported	Not reported	20.5 to 84.4%
	Ware and Fuentes (2018)	Eggs, loggerhead (no depth)	Not reported	Seawater	Not reported	Not reported	Relocated clutches: 1.5 inundation & 0.7 wash overs *In-situ:* 1.9 inundations & 1 wash over	Not reported	Wash over & complete inundation	2.66 & 2.9 % w/w	Relocated clutches were 0.4 cooler	No sex ratios. Mean temp. of the middle third of dev.: 29.6 & 29.7.	65.8 & 66.3%

7.1. The effect of nest depth

Surface irrigation of sand and nests was less effective as depth increased (Table 4). Under three watering regimes, sun-exposed sand at 45 cm depth decreased in temperature by 2.2°C on average, compared to an average decrease of 0.7°C at 75 cm (Table 5). Similarly, relocated hawksbill clutches buried at 50 cm decreased by 4.1°C after a rainfall event, compared to a decrease of 3.6°C in relocated green clutches, buried at 65 cm (Staines et al. 2020).

7.2. Irrigating nests in the shade and exposed to the sun

Surface irrigation was generally less effective at reducing temperature when performed in the shade, compared to nests exposed to the sun (Table 4). For example, sun-exposed sand (hereafter 'exposed sand' or 'exposed nests') at 45 cm depth generally cooled by 0.5°C more than shaded nests when irrigated with various volumes of water (Table 5). Differences between exposed and shaded nests may result from the already lower temperatures of shaded nests limiting their ability to be further cooled or it is possible that evaporative cooling rates are reduced in shaded nests. Shade structures may also limit the cooling benefits of rainfall as they can keep nests dry (Jourdan & Fuentes 2015, Staines et al. 2020).

7.3. Method of application

As mentioned above, we did not find any studies that reported on underground irrigation and thus, this section focuses on surface irrigation. We also discuss the effects of tidal inundation and rainfall events, as a comparison to surface irrigation. It is important to note that surface irrigation does not always result in reduced nest temperatures at nest depth (Table 6). Increases in temperature are likely the result of warmer surface sand heating the water to temperatures above those at nest depth. Only a once-off application of 200mm reduced nest temperatures by 1.5°C (Smith et al. 2021).

While we will discuss the effects of different watering frequencies, durations and intensities in more detail below (see Sections 7.5, 7.7 and Table 4), it appears that generally, rainfall provides the greatest cooling effect when compared to tidal inundation or irrigation (Figure 2). Artificial nest irrigation generally decreased temperatures below 2°C, while rainfall events consistently produced cooling effects of 2°C or more. The highest cooling effects observed when irrigating sand or nests was 2.4°C but this effect was observed at shallow depths of 45 cm, where irrigation is likely to have the greatest effect (Tables 3 & 5). A similar cooling effect (2.3°C) was observed by Jourdan and Fuentes (2015), where sand was watered in plastic containers (Table 7). It is likely that cooling was enhanced by the plastic containers ensuring that water did not drain from the soil, which could have maximised evaporative cooling. In comparison, rainfall events in Laloë et al. (2020) and Staines et al. (2020) led to cooling effects of 2.3°C, and 3.6°C and 4.1°C, respectively (Table 3). Nests in the study conducted by Lolavar and Wyneken (2021) were watered with similar volumes (140 mm/d) as Laloë et al. (2020). However, nests in Laloë et al. (2020) decreased in temperature by 2.3°C, compared to 1.5°C in nests in the Lolavar and Wyneken (2021) study, despite only experiencing 4 days of rain compared to being watered for the entirety of incubation. This difference between irrigation and rainfall may result from rain being a cooler temperature than water sitting in underground pipes or in buckets, or rain may water entire beaches rather than smaller sections, resulting in greater evaporative cooling potential and reducing the loss of water from the nest to the surrounding sand. Inundation had the smallest effect (0.4°C) on nest temperatures (Ware & Fuentes 2018), likely because inundation and tidal washing are sporadic and ocean temperatures are likely to be warmer than rain or freshwater sources. Initial conditions within nests before irrigation were rarely reported, making it difficult to account for the effect of initial temperature or moisture on cooling.

Key points

- **The cooling effect of irrigation decreases with increasing nest depth.**
- **Temperature reductions are greatest in exposed/unshaded nests.**
- **There appear to be no published reports of underground irrigation.**
- **Rainfall generally had a greater cooling effect than artificial irrigation.**

7.4. Water source

When irrigating sand and nests, most researchers used freshwater, from rainfall or mains water supply (Table 3). Only Smith et al. (2021) irrigated sand and nests with both seawater and freshwater, finding no difference in the cooling capacity of freshwater compared to seawater when irrigating sand or nests (Table 6). Hatching success is generally higher in nests irrigated with freshwater than seawater, but they do not always differ statistically (Booth 2021, Smith et al. 2021, Young 2021). Early-stage embryos are more sensitive to seawater inundation, but embryos at intermediate and late-stages of development do not differ in their sensitivity to seawater and freshwater (Table 1). Bézy et al. (2015) found that on arribada beaches with high microbial abundance a single treatment of 480 mm of seawater resulted in a hatching success of ~35%, which was slightly higher than freshwater treatment (~27%), but similar to untreated control nests (~30%). However, the higher hatching success of nests irrigated with seawater likely relates to the seawater's negative effect on microbial abundance, resulting in higher hatching success, rather than directly improving hatching success through an effect of moisture or temperature.

To our knowledge, only Bézy et al. (2015) presented data on the consequences of nests irrigated or flooded with seawater for sea turtle hatchling morphology, and none have examined the effects on locomotor performance. Generally, higher salt concentrations during incubation produce hatchlings that are smaller and exhibit similar traits to hatchlings incubated under low moisture conditions (Bower et al. 2013, Bézy et al. 2015). It seems unlikely that the salt concentration will directly influence hatchling sex.

Key points

- **Freshwater was the most common water source for irrigation.**
- **The cooling effect of freshwater and seawater did not differ.**
- **Hatching success was generally higher in nests irrigated with freshwater than seawater.**
- **Early-stage embryos are more sensitive to seawater than freshwater.**

Table 4: How likely various factors are to influence the response of nests and embryos to irrigation. For example, how the level of shade influences how nest salinity responds to irrigation. We do not include information on rainfall events or tidal inundation in this table.

	Nest temperature	**Duration of temperature change**		**Nest moisture**	**Nest salinity**	**Incubation period**	**Hatching success**	**Primary sex ratio**	**Hatchling morphology**	**Hatchling locomotor performance**
		From the start of irrigation	**Post-irrigation only**							
Nest depth	Usually	Unknown		Usually	Usually	Likely				
Shade	Usually	Unknown		Usually	Usually	Likely				
Method of application	Unknown- studies on irrigation exclusively report surface irrigation.									
Water source	No	No		No	Usually	Unlikely	Likely	Unlikely	Likely	Likely
Water temperature	Usually	Unlikely		No	No	Likely				
Time of day	Likely	Unlikely		Unlikely	Unlikely	Likely				
Time during incubation	Possible [A]	Unlikely		Unlikely	Unlikely	Likely				
Time during the nesting season	Likely	Unlikely		Likely	Likely	Likely				
Volume	Usually	Unlikely	Unlikely	Usually	Possible [B]	Likely				
Frequency	Usually	Usually	Unlikely	Usually	Possible [B]	Likely				
Duration	Usually	Usually	Unlikely	Usually	Possible [B]	Likely				

[A] Depends on the amount of metabolic heating

[B] Depends on the salinity of the water source and/or the amount of salt already present in the nest

Table 5: Summary of results from Hill et al. (2015). Irrigation was conducted in a hatchery at Playa Grande, Costa Rica. Sand was irrigated with freshwater using watering cans. The temperature of the water being applied was not reported.

Nest depth, cm	**Level of shade**	**When during the day did irrigation occur?**	**Irrigation regime**			**Initial water content, % w/w**	**Final water content, % w/w**	**Mean nest temperature, °C (change compared to control)**
			Frequency	**Duration, days**	**Volume, mm total over the duration**			
45cm, olive ridley nest depth	Sun	Control- no irrigation				3.7	1.3	31.7
		Afternoon	Daily	31	100 (3.2 mm/d)		3	(-1.8)
					323 (10.4 mm/d)		6.9	(-2.3)
					721 (23.3 mm/d)		7.8	(-2.4)
	Shade	Control- no irrigation					Sand too dry to take samples	29.5
		Afternoon	Daily	31	100 (3.2 mm/d)		2.8	(-1.3)
					323 (10.4 mm/d)		5.8	(-1.8)
					721 (23.3 mm/d)		7	(-1.8)
75cm, leatherback nest depth	Sun	Control- no irrigation				4	Sand too dry to take samples	30.8
		Afternoon	Daily	31	100 (3.2 mm/d)		3.2	(-0.6)
					323 (10.4 mm/d)		8	(-0.7)
					721 (23.3 mm/d)		8.5	(-0.7)
	Shade	Control- no irrigation					Sand too dry to take samples	29.5
		Afternoon	Daily	31	100 (3.2 mm/d)		2.5	(-0.2)
					323 (10.4 mm/d)		6.6	(-0.6)
					721 (23.3 mm/d)		7.4	(-0.8)

Table 6: Summary of results from Smith et al. (2021). Sand and nest temperatures were measured at 70cm depth (green sea turtle nest depth) on Heron and Panasesa Islands. Sand and nests were irrigated from the surface by watering can and nest water content was not reported.

Location	**Level of shading**	**Water source**	**Water temperature, °C**	**Timing of irrigation**		**Irrigation regime**			**Mean nest temperature, °C (change compared to control)**	**Temperature during sex determination, °C (mean temperature or CTE)**	**Primary sex ratio, %females (predicted or measured)**	**Hatching success, %**
				When during the day did irrigation occur?	**When during development did irrigation occur?**	**Frequency**	**Duration, days**	**Volume, mm/d**				
Heron Island	Sun	Control- no irrigation							27.4	No eggs		
		Seawater	25.1	Dawn	No eggs	Daily	4	20	(0.5 warmer over 4 days, 0.1-0.3 per application)			
						Daily	7	20	(0.5, 0.2-0.3)			
						Single	1	50	(0.5)			
						Daily	4	50	(-0.5)			
						Daily	7	50	(0.5, 0.1-0.5)			
						Single	1	100	(0.5)			
						Daily	4	100	(0.3, 0.1-0.2)			
						Single	1	200	(-1.5)			
Panasesa Island	Sun	Control- no irrigation							Not reported	No eggs		
		Seawater	Not reported	Dawn	No eggs	Daily	4	50	(-0.8)			
		Freshwater				Daily	4	50	(-0.8)			
	Shade	Control- no irrigation							28.1			
		Seawater	Not reported	Dawn	No eggs	Daily	4	50	(-0.9)			
		Freshwater				Daily	4	50	(-0.9)			
Heron Island	Sun	Control- no irrigation							28.2	29 (CTE)	100% (predicted-temperature)	74.9
		Seawater	24.2	Dawn	Day 18- before thermosensitive period	Single	1	100	(-1.3)	28.7 (CTE)	100% (predicted-temperature)	79.3
		Freshwater	24.2			Single	1	100	(-1.3)	28.7 (CTE)	100% (predicted-temperature)	92.4

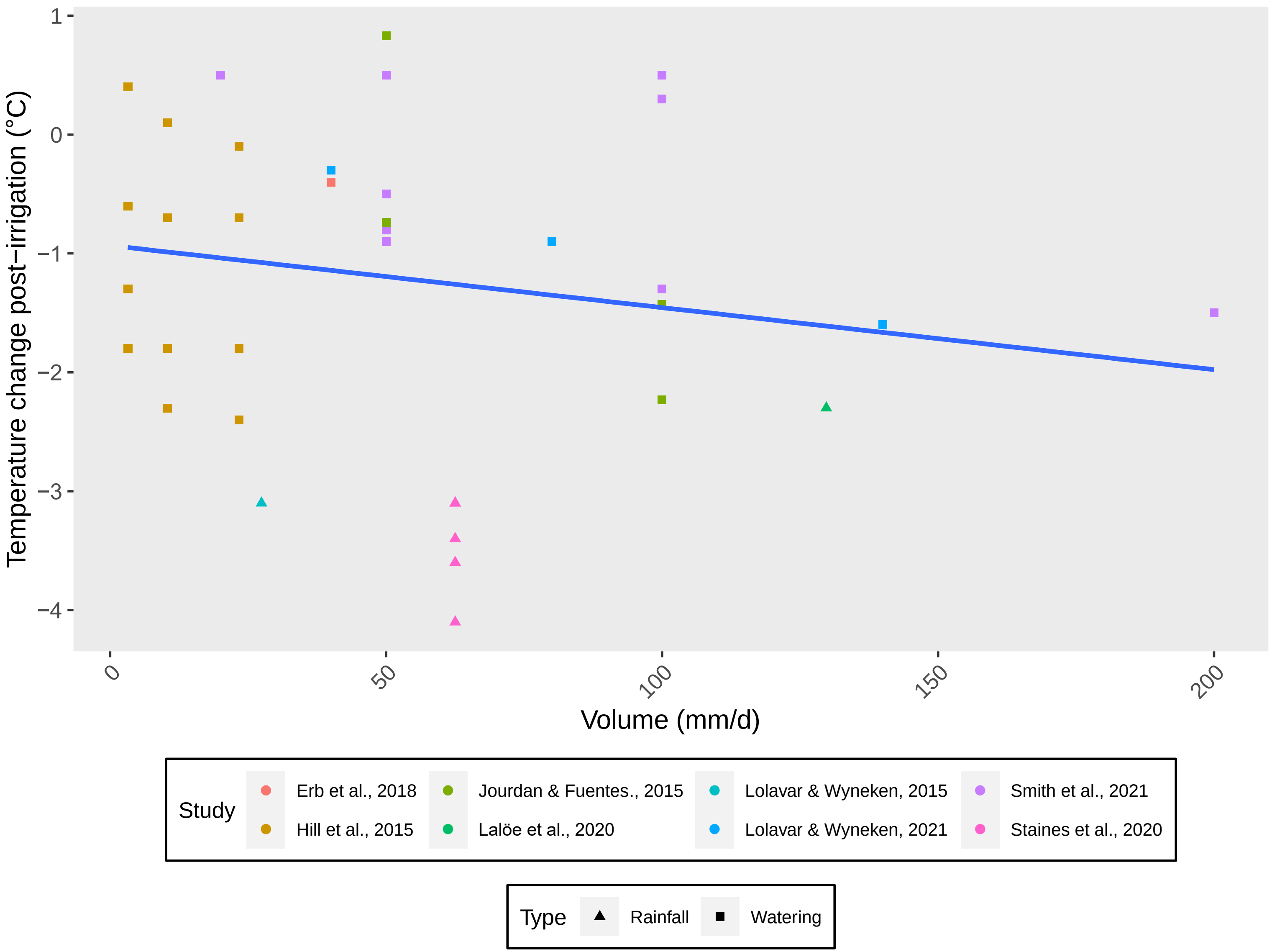

*Figure 2: The relationship between the volume of water used to irrigate a nest and the amount of cooling achieved. We include data from the irrigation of sand only and of clutches of eggs. For nests that were watered, we report irrigation volume in mm/day rainfall equivalent. The shaded area represents the 95% confidence intervals of the line of best fit. The relationship between irrigation volume and the amount of cooling achieved is described by the equation Temperature change post-irrigation (°C) = -0.93 – 0.005 * Irrigation volume (mm/d)*

Table 7: Summary of results from Jourdan and Fuentes (2015), Lolavar and Wyneken (2021), Erb et al. (2018) and Matthews (2018). Jourdan and Fuentes (2015) measured sand temperatures at 50 cm depth in plastic containers located in Townsville, Australia. Sand was irrigated with freshwater using sprinklers. Lolavar and Wyneken (2021) and Erb et al. (2018) both irrigated loggerhead clutches at 60 cm depth with freshwater using sprinklers. Nests were located in Boca Raton, Florida. Matthews (2018) irrigated green turtle clutches at 70 cm depth with freshwater. Clutches were buried in a shaded hatchery located in Terengganu, Malaysia and irrigated with a watering can.

Study	**Level of shading**	**Water temperature, °C**	**When during the day did irrigation occur?**	**Irrigation regime**			**Final water content, % v/v**	**Mean nest temperature, °C (change compared to control)**	**Temperature during sex determination, °C (mean temperature or CTE)**	**Primary sex ratio, %females (predicted or measured)**	**Hatching success, %**	**Morphology, mass (g) SCL (mm) SCW (mm)**
				Frequency	**Duration, days**	**Volume, mm/d**						
Jourdan and Fuentes (2015)	Sun	Control					Not reported	Not reported	No eggs			
		34	Noon	Daily	12	50		(+0.83) ^{A}				
		25	Night			100		(-2.23) ^{A}				
	Shade	Control						Non-rainy days: -0.68^{A} Rainy days: +0.96^{A}				
		34	Noon	Daily	12	50		(-0.74) ^{A}				
		25	Night			100		(-1.43) ^{A}				
Lolavar and Wyneken (2021)	Sun	Control					~5	~32.8	Not reported	~100 (laparoscopy, 10 hatchlings/nest)	~50	Not reported
		Not reported	Morning	Daily	Entirety of development	40	~8	~32.6 (-0.19)		~100 (laparoscopy, 10 hatchlings/nest)	~70	
						80	~5.1	~31.6 (-1.2)		~97 (laparoscopy, 10 hatchlings/nest)	~90	
						140	~7	~31.3 (-1.5)		~71 (laparoscopy, 10 hatchlings/nest)	~45	

Erb et al. (2018)	Sun	Control					5.3	32.46	Not reported	Not reported	Not reported	Mass: 16.1 SCL: 40.1 SCW: 32.2
		Not reported	Morning	Daily	Entirety of development	40	6.8	32.3 (-0.16)				Mass: 16.5 SCL: 42.4 SCW: 33.3
Matthews (2018)	Shade	Control					4.89	30.13	30.99 (CTE)	~90% (predicted-temperature)	94.4	Mass: 21.1 SCL: 46.3 SCW: 35.5
		Not reported	Not reported	As required	Entirety of development	As required, 4-9 mm	7.89	29.3 (-0.83)	30.16 (CTE)	~70% (predicted-temperature)	95.6	Mass: 21.6 SCL: 46.1 SCW: 36.2

[A] Compared to sun-exposed control

7.5. Irrigation volume

Determining how the volume of water applied to nests influenced nest temperatures is complicated by the wide range of watering regimes presented by different studies (Table 3). However, more water being applied to sand and nests generally resulted in larger cooling effects within and among studies (Figure 2; Table 4), but the amount of additional cooling achieved with larger volumes varied (Table 5, 6 & 7). Therefore, the amount of water to apply to sea turtle nests will vary depending on the characteristics of the nesting beach, the ambient climatic conditions at the time of irrigation and the amount of cooling required. We discuss the timing of irrigation below (Section 7.6).

Generally, the volume of water applied to clutches of eggs within each study did not differ. Thus, it is difficult to determine the effects of irrigation volume on hatching success. However, nest irrigation is likely to have a negative effect on hatching success if nest moisture becomes too high (see table 1 & Lolavar & Wyneken 2021 in table 7). Thus, it is vital to understand how irrigation regimes influence sand moisture content and the capacity of clutches to tolerate elevated moisture concentrations. As expected, irrigating with higher volumes of water resulted in higher sand moisture concentrations (Table 4 & 6). Deeper nests generally increased in moisture concentration more than shallower nests during irrigation (Table 5). Interestingly in Smith et al. (2021), sand exposed to the sun had higher moisture concentrations than shaded plots at two nest depths and under all irrigation regimes (Table 6). In comparison, loggerhead clutches watered with 40 mm per day for the entirety of incubation were ~3% v/v wetter than unwatered control clutches (Erb et al. 2018, Lolavar & Wyneken 2021). Although, clutches watered with larger volumes of water had lower moisture concentrations (see Lolavar & Wyneken 2021 in table 7). Thus, sand in the study by Lolavar and Wyneken (2021) may have had greater drainage than the sand reported by Hill et al. (2015), where the addition of more water did not result in higher sand moisture concentrations. Other factors including evaporative rates, groundwater and vegetation are likely to influence how the amount of water applied to nests influences moisture concentrations.

The amount of water applied to nests and thus, the amount of cooling achieved has been shown to influence PSRs when nests were irrigated daily throughout incubation. Greater volumes of water during irrigation generally resulted in more cooling and thus, the production of more male hatchlings (see Matthews 2018, Lolavar & Wyneken 2021 in table 7). The effects of single or intermittent irrigation are less consistent than daily irrigation and may depend on the timing of irrigation. We discuss the importance of the timing of nest irrigation below (Section 7.6), but for single applications of water, the amount of cooling achieved becomes irrelevant for sex determination if nest temperatures return to previous levels before the thermosensitive period begins or if the thermosensitive period has passed.

Generally, watering turtle nests results in longer incubation periods (Bodensteiner et al. 2015, Matthews 2018), but effects on morphology and locomotor performance vary. Nests with higher moisture concentrations generally produce larger and heavier hatchlings that are faster crawlers and swimmers than hatchlings from dry nests, but this effect is not consistent (Finkler 1999, Erb et al. 2018, Gatto & Reina 2020, Tezak et al. 2020a). The increased performance of hatchlings initially may also come at the cost of reduced endurance (Matthews 2018) and lower thermal tolerance (Gatto et al. 2021). Variation in both size and locomotor performance with moisture concentrations during incubation is generally small, and it is unknown if this variation significantly impacts hatchling survival. Lastly, the addition of seawater to nests is less likely to increase hatchling body size, as higher salinities during incubation generally result in smaller hatchlings (Bower et al. 2013, Bézy et al. 2015). This may depend on the initial salinity of the nest sand, salt concentrations of the water added to nests, the amount of salt retained in the nest after watering and evaporative rates.

Key points

- **Greater irrigation volumes generally resulted in more cooling, higher nest water content and the production of more male hatchlings.**
- **Irrigating with too much water can reduce hatching success.**
- **During irrigation, water content in deeper nests increases more than in shallower nests.**

7.6. Timing of irrigation

Potentially, the temperature of the water used for irrigation and the time of application may be just as important as the volume of water used. Water was most often applied in the morning, but irrigation also occurred at noon, in the late afternoon and at night. Results from two studies suggest that watering nests in the morning achieves the greatest cooling effect. First, as mentioned above (Section 7.5), Smith et al. (2021) found that applying lower volumes of water warmed nests rather than cooling them. Sand at nest depth was cooler than sand on the surface because it was still warming from winter temperatures at the start of the nesting season on Heron Island (Booth & Freeman 2006). Thus, water applied on the surface, even when applied in the morning, warmed to temperatures higher than that of the sand at nest depth, resulting in a rise in temperature. Presumably, irrigating nests during the middle of the day or the afternoon would have a greater effect, when the hot surface sand heats up the water as it percolates to the nest or deeper sand. Second, in remote locations or where large quantities of water are required, water will need to be stored, most likely in large tanks. Water stored in direct sunlight or in hot air temperatures will inevitably warm up, decreasing its cooling potential for nests. Irrigating during the day can lead to warmer water being applied to nests and reduced cooling, or even warming, of those nests (see Jourdan & Fuentes 2015 in table 7). Interestingly in Jourdan and Fuentes (2015), shaded sand did not show an increase in temperature when watered during the day, but sand exposed to the sun did, despite being irrigated with the same temperature water. Additionally, in Young (2021), clutches of green turtle eggs irrigated with a single application of 100 mm of 20°C and 25°C seawater respectively, both had a 0.2°C decrease in temperature during the thermosensitive period. This suggests that the temperature of the surface sand may play a larger role in determining the amount of cooling than the temperature of the water itself or water temperature becomes more important as irrigation duration and frequency increases.

Key points

- **High surface sand temperatures can warm water as it percolates to lower depths.**
- **Water stored in tanks or pumped through pipes can heat up before being applied to nests, reducing its cooling capacity.**

7.7. Irrigation frequency, duration and the effect of developmental stage

This section reviews three factors that influence the amount of cooling provided by nest irrigation and the effect this has on development and hatchling traits. We consider how often are nests irrigated, how long are they are irrigated for, and at which point during development are they irrigated. We also consider how long cooling effects last once various irrigation regimes end before nest temperatures return to previous levels or to control temperatures.

The frequency of nest irrigation among studies can be grouped in three ways: single applications, daily irrigation and irrigation as required. All three frequencies produced cooling effects (Hill et al. 2015, Matthews 2018, Staines et al. 2020) and in Smith et al. (2021), both single applications and daily applications resulted in warmer sand temperatures (Table 6). Only a single application of 200 mm resulted in nest cooling, suggesting that the volume of water may be more important for cooling than the frequency of irrigation. However, smaller volumes may have eventually cooled nests if applied for more than 7 days. It typically took 4 to 10 days after irrigation stopped until nest and sand temperatures returned to pre-irrigation levels, but effects lasted for 20 days in some cases (Hill et al. 2015, Lolavar & Wyneken 2015, Laloë et al. 2020, Staines et al. 2020, Smith et al. 2021). Most likely, the characteristics of the nesting beach such as grain size, pore spacing, and ambient climatic conditions including solar radiation and air temperatures, determined how long cooling effects lasted once irrigation ceased. Thus, the benefit of daily or regular irrigation is that lower sand and nest temperatures can be maintained. Single applications cool nests for 4 to 7 days, while daily irrigation maintains cooler nest temperatures for the duration of irrigation plus 4 to 7 days post-irrigation (Figure 1).

Targeted irrigation is where the frequency or volume of irrigation is varied to maintain pre-determined incubation conditions. Few studies report the use of targeted irrigation, most likely because it would require a detailed understanding of the relationship between irrigation frequency and volume, and incubation temperature and moisture concentrations. However, Matthews (2018) aimed to maintain nest moisture concentration at 8% v/v and were able to maintain nests on a natural beach at 7.9 ± 0.41% v/v with only a short pilot study, compared with unwatered nests that had a mean nest moisture concentration of 4.89 ± 0.29% v/v. Thus, targeted irrigation could be used to maintain not only specific moisture concentrations, but also predetermined incubation temperatures throughout development.

One potential consequence of daily irrigation is that irrigation can increase the size of daily temperature fluctuations within nests (Naro-Maciel et al. 1999, Jourdan & Fuentes 2015), resulting in more females being produced than expected based on mean nest temperatures (Georges et al. 1994). Sex determination is dependent on the proportion of embryonic development that occurs at each temperature, not the proportion of time spent at each temperature. Embryonic development rates are faster at higher temperatures and thus, if the same amount of time is spent at a warm and a cool temperature, a greater proportion of development will occur at the warm temperature. Therefore, larger fluctuations in incubation temperature will result in a greater proportion of development occurring at higher temperatures, and the production of more female hatchlings compared to stable incubation temperatures (Georges et al. 1994). However, this may only be problematic for irrigation regimes that only shift nest temperatures slightly and in populations that already produce both males and females. In locations where most nests already produce 100% females then the effect of greater temperature fluctuations is moot, and if the irrigation regime significantly decreases nest temperature, then male production will increase, although the effect will not be as large as expected. Greater diel thermal variation can result in reduced hatchling size (Horne et al. 2014) and if fluctuations exceed critical limits then embryonic survival can be reduced (Howard et al. 2014).

The duration of nest irrigation is potentially of more importance than the frequency. Reported irrigation durations ranged from single applications to daily applications for the entirety of incubation. While the duration of irrigation does not appear to influence how long cooling effects last once irrigation ceases, regular irrigation ensures that reductions in sand and nest temperatures are maintained until irrigation ends (Figure 1). Thus, regular irrigation throughout incubation ensures cooling effects are maintained for the entirety of development, while shorter irrigation durations may need to be targeted to the thermosensitive period to reduce female hatchling production. For example, Smith et al. (2021) found that a single application of 100mm of water on day 18 of incubation did not alter PSRs in green turtles because nest temperatures were still too warm (Table 6). However, if the water had been applied to nests laid two weeks earlier, when ambient temperatures were cooler, then irrigated nests would have produced 36% females, compared to control nests that would have produced 98% females. If nests had been watered daily throughout the thermosensitive period or for the entirety of incubation, then it is possible that reductions in the proportion of female hatchlings being produced would have been observed irrespective of when the nests were laid.

In circumstances where increased hatching success is the objective rather than changed hatchling sex ratios, single applications of water will still need to be targeted. Greatest benefit will be gained by irrigating nests on days that are particularly hot or at times during development when embryos are most at risk to thermal stress, usually early during development when embryos are more sensitive to temperature and later during development when nest temperatures are warmer because of metabolic heating (Howard et al. 2014). However, this needs to be balanced with the increased risk of over irrigation, because hatching success can be reduced by nest inundation during early and late stages of development (Table 1). During wet nesting seasons or during the early, cool portions of the nesting season, irrigation may be required less often or not at all (Lolavar & Wyneken 2015)

Fortunately, sex is generally determined during the middle third of development (Girondot et al. 2018), when embryos are least sensitive to temperature and moisture concentrations. Thus, in situations where the stage of development is known for each nest, it is possible to irrigate those nests during the middle third of incubation, increasing male hatchling production while minimising the risk of decreasing hatching success. However, *in situ*, this is unlikely to be possible, particularly on Raine Island, because of the sheer number of nests and because adjacent nests are not at the same stage of development.

Key points

- **Single applications of water or daily irrigation were the most common irrigation frequencies.**
- **Nest temperatures generally return to pre-irrigation levels in 4-7 days.**
- **Irrigation likely increases temperature fluctuations, potentially resulting in the production of more females because differences in embryonic development rates lead to a greater proportion of development occurring at warm temperatures compared to cool temperatures.**
- **Single applications of water should be targeted at specific points during development or during the nesting season to maximise effect.**

7.8. Shading versus irrigating nests

Shading typically reduces sea turtle nest temperatures by up to 2°C (Wood et al. 2014, Esteban et al. 2018, Staines et al. 2019, Reboul et al. 2021) and like irrigation, has stronger cooling effect at shallower depths (Hill et al. 2015, Vindas-Picado et al. 2020). Unlike irrigation, shading has minimal effect on moisture concentrations at nest depth even though shading probably reduces evaporation. Possibly this is because the water content is equilibrated with exposed sand surrounding the shaded plots (Vindas-Picado et al. 2020). In comparison to shading, irrigation is generally less effective at reducing nest and sand temperatures (Table 3), but this is not always the case. Rainfall in Staines et al. (2020) cooled exposed hawksbill nests by 4.1°C and exposed green turtle nests by 3.6°C. In comparison, shaded hawksbill and green turtle nests were initially 0.9°C and 1.3°C cooler than exposed nests, respectively.

Non-porous shade structures can prevent rain from reaching nests, resulting in shaded nests being cooler during dry periods, but being up to 1°C warmer during rainy periods (Jourdan & Fuentes 2015), although a more typical example is Hill et al. (2015) where, at 45 cm depth, shaded plots of sand were 2.2°C lower than exposed plots (Table 5). Thus, both shading and irrigation are viable options for reducing nest temperatures during incubation. The preferred method may depend on logistics, costs and water availability. The one advantage of irrigation is that it can simultaneously cool nests and increase moisture concentrations, which may be beneficial on particularly dry beaches.

Key points

- **Shading generally cools nests more than irrigation.**
- **Non-permeable shade structures can prevent rain from reaching nests, resulting in shaded nests being warmer than exposed nests.**

8. Current knowledge gaps and future research

Here we consider knowledge gaps and opportunities for future research. We discuss knowledge gaps and research options that are specifically required for Raine island and potential adaptive management actions to improve nesting and hatching success for green turtles on Raine Island in later sections.

Overall, there are few studies that specifically report nest irrigation in sea turtles. Others report on rainfall, storm and inundation events, but purely present data on clutch loss rather than specifically examining how the volume, duration and frequency of these events impacts nest temperature, hatching success and other traits. Additionally, many studies report sand temperatures, rather than nest temperatures. Studies that do present the response of actual clutches of eggs tend to manipulate either one irrigation factor, typically volume of water added, or two factors, typically duration and volume. Ideally, future research will manipulate multiple factors including irrigation frequency and duration, water volume and the timing of watering. These studies should aim to report initial nest or sand conditions and as many responses as possible. The pre-existing state of the sand is likely to an important determining its ability to absorb more water, which in turn may impact its ability to be cooled. Of particular importance is the amount of rain before irrigation begins which can influence nest water content and the thermal conductivity of the sand. Additionally, variation in air temperatures and solar radiation can alter the difference in temperature between the surface of the sand and the temperature at nest depth, impacting the heating of water as it percolates to the nest during irrigation. Thus, sand and nest temperatures should be measured at multiple depths.

Although modelling PSRs based on fluctuating nest temperatures is becoming more accurate (Girondot et al. 2018), development rates and pivotal temperatures can differ substantially among and within species and populations (Bentley et al. 2020). Thus, new, more accessible methods to directly measure PSRs (see Tezak et al. 2020b) will help improve our understanding of how changes in temperature, moisture and how different irrigation regimes influence sex determination. Temperature-based estimations will remain important tools, particularly on high-density nesting beaches such as Raine Island but managers should be aware of the limitations of temperature-based sex ratio estimates and studies should clearly report how sex ratios were calculated.

Of particular importance is understanding how saltwater and freshwater irrigation differ in their impact on development. Seawater is a readily available source of water on every nesting beach, and its use for irrigation considerably simplifies logistics on remote beaches. However, few studies report on nest irrigation with seawater, and these have limited the duration of irrigation to single applications (Smith et al. 2021). Key questions on the use of seawater to irrigate nests include 1) can seawater be used to regularly irrigate clutches without negatively impacting hatching success, 2) how often, for how long and at what volumes can seawater be applied to nesting beaches without impacting salt concentration in the nest, 3) what impact does extended or regular seawater irrigation have on other traits, such as sex determination and hatchling size, compared to freshwater and control nests, and 4) how long does it take for the added salt to be washed from the sand once saltwater irrigation ends? It is possible that seawater can be used sporadically to irrigate nests during heat waves to cool nests but would then require freshwater irrigation or rainfall to wash salt from the sand.

Another often overlooked factor that influences the effectiveness of nest cooling is the temperature of water applied to nests. Only a few studies report water temperatures or evaluate the effects of water temperature on nest cooling. Water temperature may be one reason why rainfall is generally more effective at cooling nests than artificial irrigation, another is that rainfall irrigates entire beaches rather than localised areas such as single nests or hatcheries. Understandably, most research focuses on the effects of irrigation to inform the management of defined areas such as hatcheries. However, it is possible that irrigating larger areas is more effective than irrigating smaller zones because it cools the surrounding sand and limits the loss of water to the surrounding sand. On a smaller scale, cooling in the middle of the clutch may be greater and last longer than on the periphery of the clutch.

One other avenue for further exploration is the use of underground irrigation, either at or above nest depth, and targeted irrigation. No studies, to our knowledge, have used underground irrigation to cool nests. Some projects do have surface grids of hoses in their hatcheries that spray and leak water onto the surface of the sand (Marcovaldi, Pers. Comm.), but these systems are typically in hatcheries where females will not crawl over them or destroy them. Underground irrigation would likely be the most water efficient way of cooling nests because water could be supplied directly above the nest, but on natural beaches, would need to be implemented in a way that does not interfere with female turtles digging a nest. Further investigations are also required for targeted irrigation, to irrigate nests during heat waves or at specific points during development. The amount of water could also be varied to ensure that nests remain in targeted temperature ranges. Targeted irrigation systems would require real-time monitoring of ambient and nest conditions, and potentially determining the stage of development of each clutch, if irrigating only during the thermosensitive period.

Key points

- **Currently, studies only report the manipulation of one or two variables, typically water volume and irrigation frequency. In future, multiple variables need to be manipulated to truly understand how irrigation influences embryonic development.**
- **Future research on irrigation should aim to establish standard protocols, ensuring that the same units for water content are used and that all relevant information is included:**
    - **Frequency, duration and volume**
    - **Water temperature**
    - **Initial nest conditions**
    - **Timing of irrigation, including time of day and at what point during development.**

  - **Nest depth**
  - **Shaded or exposed nests**
  - **Water source**
- **There is a need to directly measure sex ratios whenever possible, rather than relying solely on estimations based on temperature or incubation duration.**
- **More focus is required on the effects of regular irrigation using seawater is required.**

Table 8: The benefits and drawbacks of various water sources for irrigation on Raine Island.

Water source	Pros	Cons	Volume provided	Logistic considerations	Relative costs
Seawater	• Readily available • No storage required • Minimal infrastructure	• Saltwater • Negative effects on development?	Unlimited	Minor. Requires pumps and pipes. Will depend on depth of water	Low
Desalinated water-powered	• Can produce large volumes • Can be powered by solar or wave action	• Takes up space • Environmental impacts • Energetically intensive	High. Only limited by amount of water that can be processed	Challenging. Requires power and expensive infrastructure	High
Desalinated water-unpowered	• More environmentally friendly than powered alternatives • Less maintenance than powered alternatives • Often transportable • Longer lasting than powered alternatives • Less energetically intense	• Produce limited volumes of water • Still produces concentrated brine • Water may require cooling before use on nests	Low	Intermediate. Requires some infrastructure but maintenance costs are significantly lower than powered alternatives.	Intermediate. Much less than powered alternatives

Atmospheric extraction	• Low energy requirements • Environmentally friendly	• Rely on atmospheric and climatic conditions	Low	Intermediate- can require considerable space	Intermediate. Relatively low maintenance costs
Rainwater	• Generally, a source of cool water • Freshwater • Free • Capture structure could provide shade to nests	• Will require considerable storage • Dependent on rainfall • Do not get the water when you need it i.e., during dry months • Depends on the area used to capture the rain • May impact nesting birds	Variable- depends on capture area	Low	Low
Transportation from the mainland	• Ship may be able to apply water directly to the beach via hoses • Freshwater	• Potential environmental effects of the ship • Will likely need multiple trips to Raine Island per nesting season • May require storage	High	Intermediate to high. Will depend on storage, size of the ship, access to the island by the ship etc.	Likely high
Groundwater	• Less salt than seawater	• Brackish water, not fresh • Potential impacts on plants and animals on Raine island	Unknown. Likely limited.	Intermediate to high. Depends on ability to access to groundwater and impacts on the island.	Intermediate

9. Potential water sources for Raine Island

In this section, we describe potential water sources for irrigating nests on Raine Island (Table 8). We consider the amount of water needed (Table 9), the salinity of the water source and the logistics involved with supplying each source. We also consider the relative costs of each water source. There is considerable variation in the amount of water that may be needed to irrigate nests on Raine Island. Clutches laid when conditions are cooler and wetter may not need to be watered as often, or at all, so that clutches laid in the hotter, drier part of the nesting season may be the priority. Sections of the island that we have included as nesting areas in our rough estimation may also be rocky or unsuitable for nesting and will not need to be irrigated. Lastly, it may be decided that only specific areas such as the highest density nesting areas need to be irrigated.

The most accessible source of water for Raine Island is seawater because it is readily available and would not require storage or extensive infrastructure. Thus, long-term, it is likely to be an inexpensive option. Additionally, seawater could be pumped from lower depths some distance offshore, where temperatures are cooler, to maximise the cooling potential of irrigation. However, seawater may have negative consequences on hatching success, hatchling locomotor performance and morphology. Seawater irrigation will require a thorough understanding of how quickly sand salt concentrations accumulate when irrigated with seawater (Section 10.2) and at what salt concentration hatchling development is compromised (Sections 4.1, 4.2, 4.3 & 4.4). For Raine Island, the benefits of cooler nests and higher male production may outweigh reductions in locomotor performance and size. Thus, we focus on hatching success and PSRs here. Salt concentrations in nests on Raine Island ranged from 0 to 34 ppt (Dunstan & Roberston 2019, Booth et al. 2020a). There did not appear to be a relationship between emergence success and initial salt concentration (r^2=0.03, n=123), but both nests over 30 ppt had an emergence success of 41% or less (Dunstan & Roberston 2019, Booth et al. 2020a). Thus, sea turtle clutches may be relatively resistant to salinity during incubation, at least below salt concentrations 30 ppt. Further research on how salt builds up in the sand and the factors that determine salt build up is needed to identify how much and how often seawater can be used to irrigate nests. Alternatively, seawater could be mixed with freshwater to reduce salt accumulation.

Seawater could be used to produce freshwater via desalination. To produce the quantities of water needed (Table 9), it is likely that a generator powered or solar powered desalination plant would be required. However, this is likely to be expensive and logistically challenging. The plant would take up considerable space on Raine island and desalination plants have been shown to have extensive impacts on the local environment. Desalination is energetically intensive and produces large amounts of greenhouse gases if not powered by renewable sources (Elsaid et al. 2020). Numerous chemicals are used during the process and can leach into groundwater or into the ocean. Additionally, the brine can alter the stratification of the water column, increase salinity and lower oxygen if deposited in the ocean, resulting in the mortality of organisms and changes to community structures (Elsaid et al. 2020). Lastly, intake and outflow systems can trap organisms and can alter water circulation and seafloor bathymetry (Elsaid et al. 2020). Thus, the negative effects of desalination may outweigh the benefits. However, new technologies (e.g., Aquanet Power) that utilise wave motion and solar power are emerging that may make desalination a more appealing option.

A more environmentally friendly alternative is unpowered desalination, such as solar stills. Typically, these systems utilise solar power to heat seawater with the water vapour collected and the leftover brine removed. They have fewer moving parts, are often transportable, produce limited greenhouse gases, last longer than powered plants and are cheaper, requiring lower initial investments and less maintenance (Mohan et al. 2019). However, these systems typically can only produce limited volumes of 30 litres per m^2 per day (Beysens & Milimouk 2000). It is also possible to extract moisture from the atmosphere for to use for nest irrigation. These systems do not typically produce enough water to irrigate just the re-profiled sections of Raine Island (Table 9), although some systems can produce over 10,000 L per day (Abualhamayel & Gandhidasan 1997, Beysens & Milimouk 2000, Ferwati 2019). They are most effective in locations with high humidity, where air temperatures regularly drop below dew points and where fog is common (Beysens & Milimouk 2000).

The second most obvious water source on Raine Island is rainwater. Relying on rainfall to irrigate nests requires 1) sufficient rain, 2) storage and 3) a large area to capture rain. Even in the wettest months, based on historical averages from Lockhart River Airport (~150km from Raine Island), extremely large areas would be needed to capture enough water to irrigate nests in the re-profiled beach areas just once (Table 9). Such a structure could have significant effects on one of the largest seabird nesting colonies on the Great Barrier Reef (Queensland Government 2021). The amount of water that needs to be captured and stored could be reduced by mixing freshwater with seawater or to alternate freshwater and seawater irrigation of nests.

Freshwater could also be transported to Raine Island from mainland Australia and stored on the island. Potentially, tanker ships could anchor offshore and act as temporary storage units, removing the need to build storage infrastructure. Additionally, water could be sprayed from the ship, removing the need for pipes and sprinklers. This would depend on whether the ship can get close enough to the island to spray nests. Ships would have to make repeat trips to Raine Island. One potential compromise is to capture rain and then top up tanks with freshwater from the mainland. Tanks or bladders could be stored underground to minimise heating from solar radiation.

The last water source that we considered is the use of groundwater on Raine island, which is considerably less saline than seawater (Guard et al. 2008). It could be accessed and used for irrigation, but it is unlikely that enough water is present for regular irrigation (Booth et al. 2020c). Additionally, the removal of groundwater could have negative consequences for local flora and fauna. Groundwater could be used as a last resort or could be used to top up other water stores when they become depleted.

Overall, irrigating nests with seawater appears to be the most cost effective and environmentally friendly option (Table 8). However, it does bring some risk of decreasing sea turtle hatching success. If seawater is not a viable option, then a combination of rainwater, groundwater and transported water from the mainland could supply enough water to irrigate nests. Reliance on each method could depend on conditions at the time, the ability to capture and store water (Table 9) and the potential environmental consequences including air pollution and damage to the seabed and reefs. Desalination is another option that could supply enough water to irrigate nests but comes with considerable costs, is energy intense and has environmental consequences. New and future technologies may limit these negatives but would still require a large investment.

Table 9: Potential target sections for irrigation and the volume of water, capture area and storage required.

Irrigating:	Dimensions	Total area (m^2)	Water required for 100 mm rainfall equivalent of irrigation (L)	Capture area required [D] (m^2)	Size of a storage unit (m) [E]
All non-vegetated areas	40% of Raine Island [A]	~108,000	10,800,000	~27,000	Diameter: ~30 Height: ~15
Re-profiled areas (2014 & 2017)	1: 150 x 100m 2: 250 x 100m [B]	40,000	4,000,000	~10,000	Diameter: ~22 Height: ~11.4
Re-profiled areas (2014, 2017 & 2019)	Sections 1 & 2 plus: 3. 160 x 65m 4: 160 x 40m [C]	56,800	5,680,000	~14,000	Diameter: ~22 Height: ~14.5

[A] Hopley (2008)

[B] Dunstan and Roberston (2019)

[C] Dunstan et al. (2020)

[D] Area required to capture enough water for a single 100mm application of water based on the average rainfall for January 15 to the end of February (404mm; Dunstan & Roberston 2018). This period is likely to have the highest number of clutches incubating, lowest male production and coincides with the warmest and wettest part of the nesting season (Limpus et al. 2003, Booth et al. 2020c)

[E] Dimensions of a cylindrical tank large enough to hold enough water for a single 100mm application of water

10.The viability of irrigation

Irrigation has been used to cool nests in most sea turtle species, on multiple beaches, all over the world. However, the irrigation regimes utilised by each study, the amount of cooling achieved and the consequences for embryos and hatchlings have differed substantially. Here, we synthesise and integrate the information that we have discussed above to answer key questions on whether irrigation is a viable option for cooling nests on Raine Island. Then, in the following section, we recommend management actions and potential research specifically designed to inform management decisions for nesting green turtles on Raine Island.

One important factor that determines the response of sand and sea turtle clutches to irrigation are sand characteristics and composition. Not all sand is the same and thus, responses can vary significantly among beaches. Raine Island sand is largely (36-63%) comprised of Foraminifera that is susceptible to abrasion (Dawson et al. 2012, Dawson & Smithers 2014), compared to Heron Island that is comprised of coral sand (60%) and little Foraminifera (7%) (Schueth & Frank 2008). Thus, despite having similar grain sizes (Dunstan & Roberston 2019, Booth et al. 2020a), sand on Heron and Raine islands may differ significantly in their thermal conductivity and water potential (Fuentes et al. 2010b, Booth et al. 2020a). Thus, we make recommendations based on the available data but this data is site-specific and trials will need to be conducted on Raine Island to ensure irrigation achieves the desired amount of cooling.

10.1. The required temperature reduction

Nest temperatures (constant temperature equivalents) during the thermosensitive period are typically 30-31°C from October to February but decrease to slightly below 30°C by April (Table 10). These temperatures are 1-2°C above the pivotal temperature for northern Great Barrier Reef green turtles of ~29.1°C (Booth et al. 2020c). Thus, to produce balanced PSRs, nests on Raine Island will need to be cooled by at least 1°C. To produce almost 100% males in a single nest, nest temperatures during the thermosensitive period would need to be reduced by 2-3°C to ~28°C (Booth et al. 2020c).

Mean nest temperatures during the first week of incubation were generally below 32°C, but during the last week of incubation nest temperatures frequently reached 34-36°C, and occasionally as high as 37°C (Booth et al. 2020a). Early-stage embryonic mortality increases at temperatures above 33°C (Booth et al. 2020a) and thus, newly laid nests in high density nesting years are likely to be exposed to fatally high temperatures, if laid close to mature nests (Booth et al. 2020a). A 1-2°C cooling effect, like that needed during the thermosensitive period, would reduce total mortality in many nests but cooling would likely need to reach 2-3°C below ambient to ensure that total embryonic mortality is avoided. Late-stage embryos can generally tolerate temperatures above 35°C for multiple days, only experiencing significant reductions in hatching success when temperatures reach 36-37°C (Howard et al. 2014, Dunstan & Roberston 2019). Thus, 1-2°C of cooling would reduce late-stage mortality and focus should be on cooling early-stage embryos, particularly in high-density areas and years.

Overall, ensuring that incubation temperatures are male-producing during the thermosensitive period should be the priority, followed by cooling early-stage embryos and then late-stage embryos. If 2°C of cooling is achieved throughout development, then PSRs are likely to be balanced or at least close to 50:50, and both early- and late-stage embryonic mortality will decrease. Thus, reducing incubation temperatures by 2°C is a strong initial target for nest irrigation. However, the transitional range of temperatures in sea turtles can be quite narrow (1-3°C; Booth et al. 2020c, Porter et al. 2021) and therefore, maintaining balanced or mixed PSRs will be difficult. Instead, it may be more feasible to ensure that PSRs are 100% male for some nests and that other nests are irrigated as necessary to minimise embryonic mortality without modifying the PSR. It is important to note that sea turtle populations may be naturally female-biased because females do not nest as frequently as males (Hays et al. 2010). Creating balanced PSRs may therefore, result in reduced population viability (Santidrián Tomillo et al. 2021). Thus, aiming for balanced PSRs may not be appropriate, except in populations where adult sex ratios are already highly feminised. Males may only need to be produced for short periods of the nesting season to maintain population viability, but the irrigation of sea turtle nests should be conducted with regular monitoring of adult and operational sex ratios, and with population viability analyses to ensure that population viability is being maintained.

Table 10: Nest temperatures at various stages of development throughout the Raine Island nesting season.

	October	November	December	January	February	April
Mean nest temperature (°C)	31.5 ± 0.2 [A] ~31.5 [B]	~30.5 [B]	31.6 ± 0.1 [A] ~31.3 [B]	~31 [B]	30.3 ± 0.1 [A] ~30.5 [B]	~30 [B]
Mean first week temperature (°C)	29.4 ± 0.2 [A] ~29.5 [B]	~28.5 [B]	32.1 ± 0.1 [A] ~31 [B]	~30 [B]	29.9 ± 0.1 [A] ~30 [B]	~30 [B]
Mean temperature during the thermosensitive period (°C)	31.0 ± 0.2 [A] ~31 [B]	~30 [B]	30.9 ± 0.1 [A] ~30.8 [B]	~30.8 [B]	30.6 ± 0.1 [A] ~30.8 [B]	~29.7 [B]
Mean last week temperature (°C)	34.4 ± 0.2 [A] ~35 [B]	~32 [B]	33.0 ± 0.1 [A] ~33 [B]	~32.5 [B]	30.9 ± 0.2 [A] ~32 [B]	~30.5 [B]

[A] Booth et al. (2020a)

[B] Constant temperature equivalents estimated from graphs in Booth et al. (2020c)

10.2. Managing nest salinity

Ideally, freshwater would be used to irrigate sea turtle nests whenever possible. The cooling effects of seawater and freshwater do not differ (Smith et al. 2021), but increased salt concentrations in sea turtle nests can have negative effects on hatching success, locomotor performance and morphology (Bustard & Greenham 1968, Foley et al. 2006, Bower et al. 2013, Bézy et al. 2015). However, on Raine Island, nest salinity ranged from 0 to 34 ppt and did not significantly alter hatching success (Dunstan & Roberston 2019, Booth et al. 2020a). Although, the two highest salinity levels, 32 and 34 ppt, only had a hatching success of 14% and 41%, respectively (Dunstan & Roberston 2019). Thus, sea turtle embryos can develop successfully at salinity levels close to seawater. Re-profiling of beach sections with sand saturated with seawater initially reduced hatching success compared to unmodified control areas, but quickly returned to control levels within a couple of months (Dunstan & Roberston 2018). This may have resulted from increased salinity, moisture or a combination of both. Early-stage embryos are sensitive to seawater inundation (Limpus et al. 2020) and thus, clutches laid early in the nesting season produced fewer hatchlings, but those laid later in the season, when the sand had dried, had similar hatching success to controls. Overall, the tolerance of sea turtle embryos to high salinity levels and seawater suggests that seawater could be a viable alternative to freshwater for nest irrigation. Further research, tailored to identify how sand and nests on Raine Island respond to seawater irrigation, will be required to determine how much and how often seawater can be applied to nests. Nest salt concentrations should not be allowed to increase above that of seawater (~35 ppt) and ideally, would be kept below 15-20 ppt (Bustard & Greenham 1968).

In summary, there are three main benefits to using seawater over freshwater for nest irrigation on Raine Island. First, the ocean is an unlimited supply of water for irrigation and second, the use of seawater requires minimal infrastructure, reducing costs (Table 8). The third benefit is that seawater is likely to be the coolest source of water because 25°C can be pumped from depths of ~100 m and 20°C water can be pumped from ~200 m deep (Andrews & Gentien 1982, Englebert et al. 2017, Frade et al. 2018). In comparison, freshwater will need to be stored in tanks which will heat during the day and may not cool enough even if nests are irrigated at night. Limiting salt build-up in the sand on Raine island could be achieved by diluting seawater or alternating seawater and freshwater applications. Further research would be required to determine how often freshwater applications would need to be applied and how much seawater would need to be diluted to minimise the effects of increased salinity. Future re-profiling efforts would provide an ideal opportunity to study how long salts remain in the sand and how much rain/freshwater is required to flush the sand of salts.

10.3. Selecting an appropriate irrigation regime

10.3.1. Irrigation frequency

In this section, we discuss the appropriate irrigation frequency if aiming to produce balanced PSRs throughout the nesting season. If aiming to produce fewer or more males, then the frequency of irrigation would need to be adjusted to maintain the appropriate temperature.

Post-irrigation, it typically takes 4-10 days for temperatures to return to pre-irrigation levels (Hill et al. 2015, Lolavar & Wyneken 2021, Smith et al. 2021). Considering that sand grain size on Heron Island is similar to that on Raine Island (Booth et al. 2020a) and that it took 4 days for temperatures to return to pre-irrigation levels on Heron Island (Smith et al. 2021), irrigating nests every 4 days is a good starting point when developing an irrigation regime for Raine Island. It is also important to consider how long it takes for water to drain or evaporate from the nest post-irrigation. This will influence how fast water accumulates and will determine the frequency and volume of nest irrigation before eggs become inundated. Moisture content (% w/w dry mass basis) on Raine Island ranged from almost 0% to just over 8% (Dunstan & Roberston 2019, Booth et al. 2020a). Nest water contents in February, one of the wettest months (~9 mm of rain/day on average; Dunstan & Roberston 2018), ranged from 5-8% w/w. If roughly 9 mm of rainfall per day can result in nest water contents of 5-8% w/w then applications of 100-200 mm every 4 days (25-50 mm/day on average) may result in nest water contents that inhibit embryonic development. Further research and monitoring of irrigated nests would be required to assess whether irrigating nests every 4 days would lead to reduced hatching success or whether nests would reach a ‘steady state’ where the sand drains quickly enough such that nest water contents never rise too far above 8-10% w/w, or do not remain above this for too long.

It is unlikely that single applications of water to nests will be sufficient to significantly increase male production. A single application of 100mm did not result in significantly more males being produced in green turtle clutches on Heron Island but this was, in part, because of the timing of irrigation (Smith et al. 2021). If irrigation had occurred during cooler weather or during the thermosensitive period, rather than just before, then it is likely that irrigation would have had a greater effect on PSRs (Porter et al. 2021). Thus, applying a single application of water would only impact the PSRs of clutches in their thermosensitive period at the time of irrigation and only if ambient temperatures were not too high, i.e., below 32-33°C. This is unlikely to significantly impact sex ratios at a population level. It may also be appropriate to reduce irrigation frequency, particularly if using seawater, when many nests have been laid recently. Sea turtle nests are most sensitive to inundation during the early-stages of development and therefore, frequent irrigation during this stage of development could result in increased mortality (Limpus et al. 2020).

10.3.2. Irrigation duration

Regarding the duration of irrigation, there are two main tactics that could be used. The first is to irrigate nests for the entire nesting season and aim to produce a particular PSR in all nests (see Santidrián Tomillo et al. 2021). The second tactic is to only irrigate during specific periods of the nesting season. This may involve targeting the hottest or driest months to limit mortality, targeting cooler months to maximise the production of male hatchlings, or targeting the peak nesting months so that overall male hatchling production is maximised. We discuss the benefits and drawbacks of targeting specific months in the nesting season below (Section 10.4).

We discussed above the possibility that increasing the frequency of irrigation may result in the inundation of developing embryos and increased mortality (Section 7.7). Altering the duration of irrigation may be one way to mitigate these effects without altering frequency. Nests could be irrigated for short periods of time such as two weeks, then have a break from irrigation before irrigation recommences. This would allow not only the sand to dry but also embryos to develop in low moisture conditions for a short period. Limiting the duration of irrigation would depend on how sand and nest water contents respond to irrigation and would require further investigation on Raine Island. Lastly, irrigation with seawater may require shorter irrigation durations, depending on how developing embryos respond to seawater and how fast salts accumulate in the sand.

10.3.3. Irrigation volume

Mean nest temperatures on Heron Island generally range from 28-31°C (Booth et al. 2013) compared to 30-31.5°C on Raine Island (Table 10). This difference will impact how effective cooling will be on Raine Island relative to Heron Island. The hotter sand on the surface may warm up water applied to nests resulting in reduced cooling or even heating of the nest (Smith et al. 2021) but the higher initial nest temperature means that irrigation may have a larger cooling effect because of larger differences between water and nest temperatures. A single 100mm application of both seawater and freshwater resulted in 1.3°C of cooling for green turtle clutches on Heron Island (Smith et al. 2021). While this did not cool nests by the 2-3°C required on Raine Island to produce 100% male PSRs, the higher nest temperatures on Raine Island may result in greater cooling and regular irrigation may reduce nest temperatures more than a single application. Additionally, if cool seawater is pumped from ocean depths, then more cooling will be achieved.

100mm of water per irrigation is a good starting point for irrigating nests on Raine Island, if aiming to produce balanced PSRs. 100 mm irrigations have consistently achieved cooling effects in other studies, without resulting in extremely high nest water content (Jourdan & Fuentes 2015, Lolavar & Wyneken 2021, Smith et al. 2021). However, the amount of cooling achieved with 100mm is not likely to be sufficient to produce 100% male PSRs. Thus, 200 mm is a good starting point if aiming to produce 100% males, provided that nest water content remains below ~8% w/w and if using seawater, that salt concentrations do not increase to levels that unreasonably jeopardise hatchling survival and/or fitness. Although similar, grain sizes on Raine Island are slightly smaller than Heron Island, suggesting that nests will maintain slightly higher water contents and salts may take slightly longer to wash out than on Heron Island (Hillel 2003, Foley et al. 2006, Booth et al. 2020a). This may lead to greater salt accumulation on Raine Island than Heron Island.

10.4. Timing of irrigation during incubation

On a nesting beach where thousands of turtles may nest in a single night, it is unlikely that each individual nest can be located, monitored and irrigated at specific points during development. Additionally, nests at different stages of development are mixed spatially meaning that nests would need to be watered individually to target specific stages of development, making this an inefficient and time-consuming task.

If possible, it is best to avoid irrigating early-stage clutches with seawater because seawater inundation has a greater risk of a negative effect on embryonic survival than freshwater inundation (Limpus et al. 2020). Additionally, nest water contents should be kept below ~8% w/w in early- and late-stage clutches that are more sensitive to high sand water content than mid-stage clutches. In comparison, late-stage clutches may require additional irrigation because they generally experience the highest temperatures as a result of metabolic heating (Van De Merwe et al. 2006, Howard et al. 2014). If nests are not irrigated for the entirety of development, then irrigation should target clutches in their thermosensitive period. This would have the largest effect on PSRs and male hatchling production but would likely have less benefit for hatching success than irrigating throughout development.

Overall, the sheer number of nests and their spatial and temporal variation makes targeted irrigation an almost impossible task. One alternative is to utilise targeted irrigation on specific sections of Raine Island, while the rest of the beach is irrigated uniformly. If targeted irrigation is possible then we would recommend irrigating as necessary with freshwater during early stages of development to maximise hatching success, irrigating most intensively during the thermosensitive period to maximise male hatchling production and irrigating as necessary during the later stages of development to maximise hatching success.

10.5. Timing of irrigation during the nesting season

Sea turtle nesting on Raine Island runs from October to March, with hatchlings emerging from December to May (Booth et al. 2020c). Mean nest temperatures are consistently 30-31°C during the nesting season and rainfall is highest from January to March with nest water content lowest in October (0.72% w/w) and peaking in February (5.86% w/w) (Limpus et al. 2003, Booth et al. 2020a, Booth et al. 2020c). Peak nesting occurs in December and January (Booth et al. 2020c). Nest irrigation will be most important early in the season when beach conditions are driest and warmest, and irrigation frequency can be reduced during the wettest and coolest periods of the nesting season (Table 11). As we have suggested above (Section 10.3), the most suitable frequency, volume and duration of irrigation will be affected by the ability to monitor incubation conditions for multiple nests on Raine island, the accumulation of water and salts in nests and the ability of developing embryos to tolerate various nest water and salt concentrations.

If irrigating nests with seawater, the accumulation of salts should be monitored most closely during the driest months when evaporative rates are highest and nest salt concentrations are most likely to exceed 20 ppt and approach those of seawater (~35 ppt) leading to reduced hatching success (Bustard & Greenham 1968, Booth et al. 2020a). In comparison, less salt is likely to accumulate when rainfall is highest because salts are washed from the sand by rain. Microbial loads in nests on Raine Island do not appear to be having a significant effect on hatching success (Booth & Dunstan 2018), but application of seawater later in the nesting season when microbial loads are highest could help limit the effects of microbial accumulation on hatching success (Bézy et al. 2015).

If aiming to produce 100% males during a certain period, with 100% females probably being produced the rest of the nesting season, then irrigation should occur when nest temperatures are already lowest to minimise the cooling needed to produce males, or during the peak of nesting to maximise male hatchling production relative to the amount of irrigation undertaken (Table 11). As the number of males in the population increases, the period during which male production is 100% may be reduced or shifted to periods where nesting density is lower.

Accidental nest destruction by conspecifics is possible on any beach, with nests most likely to be destroyed if they are laid before or during the peak of the nesting season. Depending on local nesting densities, nest destruction therefore has the potential to reduce the benefits of irrigation if irrigated nests are destroyed before they hatch. This may need to be considered as a potential factor influencing the timing and effectiveness of irrigation in producing male hatchlings. Consequently, the potential for nest destruction may result in irrigation being more effective later in the nesting season when nest destruction is lower, or some additional irrigation being required at that time, even if more water is needed to achieve desired nest temperatures during those periods. The risk of nest destruction will also vary inter-annually and will need to be monitored to determine its potential effect and whether there is a need to modify planned irrigation schedules.

10.6. Additional risks associated with nest irrigation

There are a number of risks associated with nest irrigation that need to be considered. Generally, these risks are manageable if nest conditions are monitored regularly and irrigation water sources, frequencies, durations and volumes are adjusted to manage these risks.

10.6.1. Saltwater irrigation during early stages of development

Early-stage embryos (<20% of development) are particularly sensitive to saltwater inundation because it may interfere with water exchange between the egg and its nest environment (Limpus et al. 2020). The irrigation of early-stage clutches will need to ensure that inundation does not occur and will need to monitor the effect of seawater irrigation on embryonic development. Inundation becomes a risk when moisture concentrations exceed ~8% w/w (Patino-Martinez et al. 2014).

10.6.2. Nest inundation during early- and late-stages of development

Nest inundation during early stages of development, particularly by seawater as noted above, but also by freshwater, can result in embryonic mortality. Similarly, inundation during later stages of development can decrease hatching success because inundation limits oxygen supply to embryos when their oxygen demands are highest (Limpus et al. 2020). Thus, over-irrigation should be monitored most closely for clutches less than 20% of development and greater than 80% of development.

10.6.3. High beach surface temperatures

Surface irrigation when sand surface temperatures are hot may result in the heating of the applied water and thus, heating, rather than cooling, of nests (Smith et al. 2021). Irrigation should occur when the temperature of water, as it reaches the nest, is as low as possible. This needs to consider daily variation in sand temperatures as well as annual variation. Irrigating nests are night, once surface sand has cooled to at least nest temperatures, would minimise this problem.

10.6.4. Water temperature

Similar to above, water temperatures should be as low as possible to ensure that the maximum amount of cooling occurs during irrigation. Water applied during the day or stored in exposed tanks can experience significant warming, limiting the effectiveness of irrigation (Jourdan & Fuentes 2015). Thus, irrigation should occur at night or just before dawn when water temperatures are coolest or using cool seawater pumped from deep below the ocean surface.

10.6.5. Producing too many males may negatively impact population viability

Sea turtle populations are naturally female-biased and populations may be resilient to further feminisation because males remigrate to nesting grounds to breed more frequently than females (Hays et al. 2014, Hays et al. 2017). Thus, producing PSRs that lead to balanced adult sex ratios may reduce population viability by decreasing the number of females and therefore, decreasing the number of eggs being laid and hatchlings being produced each nesting season (Santidrián Tomillo et al. 2021).

10.6.6. Impacts of irrigation on traits other than sex or on hatching success

This report has largely focused on the effects of irrigation on PSRs because this has been identified as the key threat to the northern Great Barrier Reef green turtle population (Booth et al. 2020c, Dunstan et al. 2020). It has also considered the consequences of irrigation for hatching success because of its role determining overall hatchling production. However, irrigation may also impact other hatchling traits such as morphology, size, locomotor performance and thermal tolerance (Erb et al. 2018, Gatto & Reina 2020, Gatto et al. 2021). Variation in these traits may have important consequences for the survival of hatchlings as they disperse from the nesting beach (Booth & Evans 2011, Cavallo et al. 2015). Unfortunately, it is difficult to determine exactly how changes in these traits will impact survival because multiple phenotypes can be beneficial, and the most advantageous phenotype may vary depending on environmental conditions at the time.

10.6.7. Trade-offs between hatching success and primary sex ratios

When deciding between maximising male hatchling production or hatching success, this report has generally chosen to maximise male hatchling production because it is, currently, a more pressing threat than low hatching success (Booth et al. 2020c, Dunstan et al. 2020). There may be times during irrigation where greater volumes or more frequent irrigation is required to achieve the desired PSR, but simultaneously, may decrease hatching success by creating hypoxic nest conditions or interfering with embryonic water exchange. This trade-off between hatching success and male hatchling production will need to be carefully monitored to ensure that the production of higher proportions of male does not come at the expense of significant decreases in hatchling production, leading to further reductions in population viability.

10.6.8. Promoting ‘poor’ mothers

If the decision is made to only irrigate nests during certain times of the nesting season, then it is possible that this will have negative impacts on the population gene pool. More experienced leatherback turtle females have been shown to nest earlier, produce more clutches and have higher hatching success than less experienced females (Rafferty et al. 2011). Thus, if targeted irrigation focuses on producing males during the later months of the nesting season, then this may result in males being produced only from the nests of potentially ‘poorer’ mothers. This could eventually lead to most males in the population being of ‘poorer’ genetic quality. Fortunately, studies have suggested that this is unlikely to be problematic (Pfaller et al. 2009) but is a concern worth noting and monitoring. If irrigation occurs throughout the nesting season, then this problem is not a concern.

11. Potential adaptive management actions to improve nesting and hatching success for green turtles nesting at Raine Island

Previous studies on sea turtle nest irrigation have reported a wide range of irrigation regimes on beaches with vastly different characteristics. These factors make identifying clear patterns and recommending a specific irrigation regime for Raine Island difficult. Therefore, we recommend a starting point from which a dynamic irrigation regime, specifically tailored for Raine Island, can be developed as further, site-specific information is obtained (Figure 3).

We suggest that irrigation on Raine Island starts with 200 mm of seawater every 4 days throughout October and November, with the aim to create 100% male PSRs. From December to April, we suggest that 100 mm of irrigation occurs when nest temperatures reach critical thermal maxima of ~33°C for early-stage clutches and ~36°C for late-stage clutches. If aiming to produce balanced or mixed PSRs, then irrigation volume can be decreased to 100 mm, but irrigation will need to occur for longer durations (Table 11). However, we recommend creating 100% male PSRs because the transitional range of temperatures in many sea turtle populations is narrow, meaning that slight deviations in nest temperature can have large effects on PSRs. Aiming for 100% male production allows for greater fluctuations in nest temperature and increases the likelihood that some males will be produced in every nest during the irrigated period. Additionally, when producing 100% males, irrigation can be restricted to specific periods of the nesting season or specific areas of the nesting beach, ensuring that some nests are allowed to develop without intervention (Table 11).

Nest temperatures during the thermosensitive period are lowest in April, but consistently between 30°C and 31°C for most the nesting season (Booth et al. 2020c). Thus, irrigating nests in April will require the least amount of cooling, but only 0.2% of nesting occurs during this time (Table 11). If aiming to produce as many males as possible, then nests should be irrigated during the peak of the nesting season in December and January, but temperatures are warmest during this time. November may, therefore, be the best month to irrigate nests because temperatures are low and 19% of nesting occurs during this month (Table 11). Additionally, producing 20% males may maximise population viability, compared to producing balanced PSRs (Santidrián Tomillo et al. 2021). Nest temperatures during the first week of development are highest in December and during the last week of development are highest in October (Booth et al. 2020c). Thus, regular irrigation is likely to occur from October until late December to produce males and to limit embryonic mortality from lethal temperatures.

Targeting 100% male production in some nests does run the risk of losing male producing nests to weather events or nest destruction by conspecifics, may advantage females that nest during irrigation because they produce the rare sex and producing 100% males will require greater irrigation volumes, increasing the risk of over-irrigation and salt accumulation. Based on nest water contents measured in Booth et al. (2020a) and mean monthly rainfall in Lockhart River (Bureau of Meteorology 2021b), 538 mm of rainfall in a month would result in nest water contents of 8% w/w and 676.9 mm of rainfall increases nest water content to

10% w/w. While this is a rough estimate and nest water content likely plateaus eventually, nests on Raine Island may only be able to be irrigated with 200 mm two or three times per month before hatching success is affected. Measuring how nest water content responds to irrigation is a critical first step to establishing an irrigation regime on Raine Island. Realistically, nests are likely able to handle more than 500 mm of irrigation per month because not all water reaches the nest and nests on Raine Island produce hatchlings even after extreme rainfall events of 500 mm over a few days (Laloë et al. 2020).

100 mm of water has consistently cooled nests by 1-2°C and 200 mm is likely to increase this cooling, potentially to 2-3°C, enough to produce close to 100% males on Raine Island. Irrigating nests every 4 days will ensure that cooling is maintained while minimising over-irrigation risks. Trials will need to establish how our recommended irrigation regime influences nest temperature, moisture and salinity, and how this impacts development. Monitoring salt accumulation will be particularly important. The volume of water can be adjusted to alter the amount of cooling, while frequency can be adjusted to vary nest moisture and limit salt accumulation. Irrigation volume may be able to be reduced as the area being irrigated increases. Irrigation should occur just before dawn when water and sand temperatures are likely to be lowest. Surface irrigation at night may interfere with nesting females or spray water directly onto eggs.

Once an overall irrigation regime has been established that achieves the desired nest temperatures and PSRs without negatively impacting hatchling production, then fine-tuning can begin to occur. Ultimately, the aim should be to adjust the frequency and volume of irrigation to compensate for changes in air temperatures, solar radiation, rain and other climatic variation. Additionally, nesting density, metabolic heating and the stage of development of the nests present on Raine Island could be incorporated into decisions determining irrigation frequency, volume and water source. Irrigation duration may extend into December and January during cool, wet years, and irrigation may focus on limiting embryonic mortality in hot, dry years, even in October and November.

Lastly, we recommend recording sand and nest temperatures at multiple depths and directly determining PSRs as often as possible. Not only will this ensure that irrigation is impacting nest temperatures uniformly throughout the nest but will also help identify how sand temperatures at various depths influences the heating of water as it percolates through the sand to the nest. Regularly sexing hatchlings will ensure that the targeted PSR is being achieved and population viability analyses will be critical in adjusting the irrigation regime as the proportion of males in the population increases.

Key points

- **We recommend that irrigation begins with 200 mm of seawater every 4 days throughout October and November to produce male hatchlings. Irrigation should continue from December to April but only when required to reduce overheating of clutches.**

- **This irrigation regime will result in approximately 20% of all hatchlings being male, if 2-3°C of cooling is achieved.**
- **The regime will need to be dynamic, adjusting volume, frequency and duration based on weather conditions, nest conditions and nesting density.**
- **Some risks of producing 100% males include the loss of male producing nests to weather events, salt accumulation and over-irrigation.**

11.1. Key questions that need be answered to develop an irrigation regime for Raine Island

- How do nest temperatures on Raine Island vary spatially and temporally?
    - How many days do early-stage clutches spend above 33°C and late-stage clutches above 36°C?
- How do nest temperatures respond to rainfall and irrigation on Raine Island?
- How does nest water content respond to various irrigation volumes, frequencies and durations?
    - How do ambient conditions influence this relationship?
    - What nest water content decreases hatching success?
    - How much water is needed to increase nest water content above 8-10% w/w?
- How do salt concentrations respond to various irrigation volumes, frequencies and durations?
    - How do ambient conditions influence this relationship?
    - What salt concentration decreases hatching success?
    - How much freshwater is needed to flush salts from the sand?
- How does irrigating with seawater early in the nesting season influence the hatching success of nests once irrigation is complete?
- How does the destruction of nests laid in October and November, by females nesting during the peak of the nesting season in December and January, influence the number of male hatchlings produced?

Table 11: The number of nesting females, nest conditions, hatching success and primary sex ratios on Raine Island throughout the nesting season. We also provide two scenarios where (A) irrigation aims to produce 100% males in certain months and (B) irrigation aims to produce 50% males throughout the nesting season. For each scenario, we calculate the amount of water required to irrigate nests and estimate nest water contents post-irrigation

	October	**November**	**December**	**January**	**February**	**March**	**April**	**Comments**
Number of females nesting	137	2801	4940	5697	1237	129	10	Averages calculated from Seasonal Technical Reports (Dunstan 2015, 2016, Dunstan & Robertson 2017, Dunstan & Roberston 2018, 2019, Dunstan et al. 2020) & Limpus et al. (2003)
Proportion of nests, %	1	19	33	38	8	0.8	0.2	
Mean nest temperature (1st week of development), °C	29.5	28.5	31.5	30	30	N/A	30	From table 10
Mean nest temperature (thermosensitive period), °C	31	30	30.9	30.8	30.7	N/A	29.7	
Mean nest temperature (last week of development), °C	34.7	32	33	32.5	31.5	N/A	30.5	
Hatching success, %	65	76	74	57	45	N/A	15	Booth et al. (2020c)
Primary sex ratios, % male	2.4	3	0.1	0.5	0.5	N/A	8	
Proportion of all hatchlings that are male, % male	0.02	0.57	0.03	0.19	0.04	N/A	0.02	Calculated by multiplying the proportion of nests for each month and the primary sex ratio
Cooling °C required to produce 50% males (~29.1°C)	1.9	0.9	1.7	1.7	1.7	N/A	0.6	
Cooling °C required to produce 100% males (~28°C)	3	2	2.8	2.8	2.8	N/A	1.7	
Mean monthly rainfall, mm (mm/day)	27.8 (0.9)	67.3 (2.24)	206.8 (6.67)	404.4 (13.05)	384.3 (13.73)	447 (14.42)	2919 (9.73)	Data from Lockhart River airport (Bureau of Meteorology 2021b)
Nest water content, % w/w	0.72	N/A	3.1	N/A	5.86	N/A	N/A	Booth et al. (2020a)
Estimated nest water content, % w/w	0.65	1.22	3.23	6.08	5.79	6.69	4.46	Calculated using linear regression of the rainfall and nest water content data listed here.

	October	November	December	January	February	March	April	Comments
Scenario A- 100% male production for certain months (200 mm irrigations for producing males, 100 mm for reducing embryonic mortality)								
Primary sex ratio, % male	100*	100*	0.1	0.5	0.5	N/A	0.02	*Irrigation occurred these months
Proportion of all hatchlings that are male, % male	1	19	0.03	0.19	0.04	N/A	0.02	Seasonal male production: 20.3%
Days of irrigation needed to produce males	8	8	0	0	0	0	0	16 days of irrigation
Water required to produce males, L	218,533	4,481,240	0	0	0	0	0	4,699,773 L of water required to irrigate 1 m^2 of sand around each nest with 200 mm rainfall equivalent
Days where nest temperatures may result in embryonic mortality	0	0	3	9	4	1	0	Estimated using the number of days that the maximum air temperature was above 35°C in 2020 (Bureau of Meteorology 2021a)
Days of irrigation needed to reduce embryonic mortality	0*	0*	2	4	2	1	0	
Water required to reduce embryonic mortality, L	0*	0*	987,969	2,278,800	247,400	12,888	0	3,527,057 L of water required to irrigate 1 m^2 of sand around each nest
Total water required, L	218,533	4,481,240	987,969	2,278,800	247,400	12,888	0	8,226,830 L of water required to produce males in October and November, and to limit embryonic mortality from December to April
Estimated nest water content, % w/w	23.3	23.3	3.1	6.0	3.1	1.7	0.3	Calculated using linear regression of the rainfall and nest water content data listed here.
Scenario B- 50% male production throughout the nesting season (100 mm irrigations)								
Primary sex ratio, % male	50*	50*	50*	50*	50*	50*	50*	*Irrigation occurred in every month
Proportion of all hatchlings that are male, % male	0.5	9.5	16.5	19	4	N/A	0.1	Seasonal male production: 50%
Days where irrigation was not required because of natural rainfall (maximum daily rainfall, mm)	0 (1)	1 (34.8)	6 (85.2)	3 (120.4)	2 (118.2)	5 (60.2)	1 (37.2)	Estimated using the number of days that daily rainfall was above 25 mm in 2020 (Bureau of Meteorology 2021b)
Days of irrigation needed to produce males	8	8	7	7	7	7	8	Irrigation occurred every 4 days except when rainfall
Water required to produce males, L	109,267	2,240,620	3,457,892	3,987,900	865,900	90,213	8,067	10,759,858 L of water required to irrigate 1 m^2 of sand around each nest
Estimated nest water content, % w/w	11.8	11.8	10.3	10.3	10.3	10.3	11.8	Calculated using linear regression of the rainfall and nest water content data listed here.

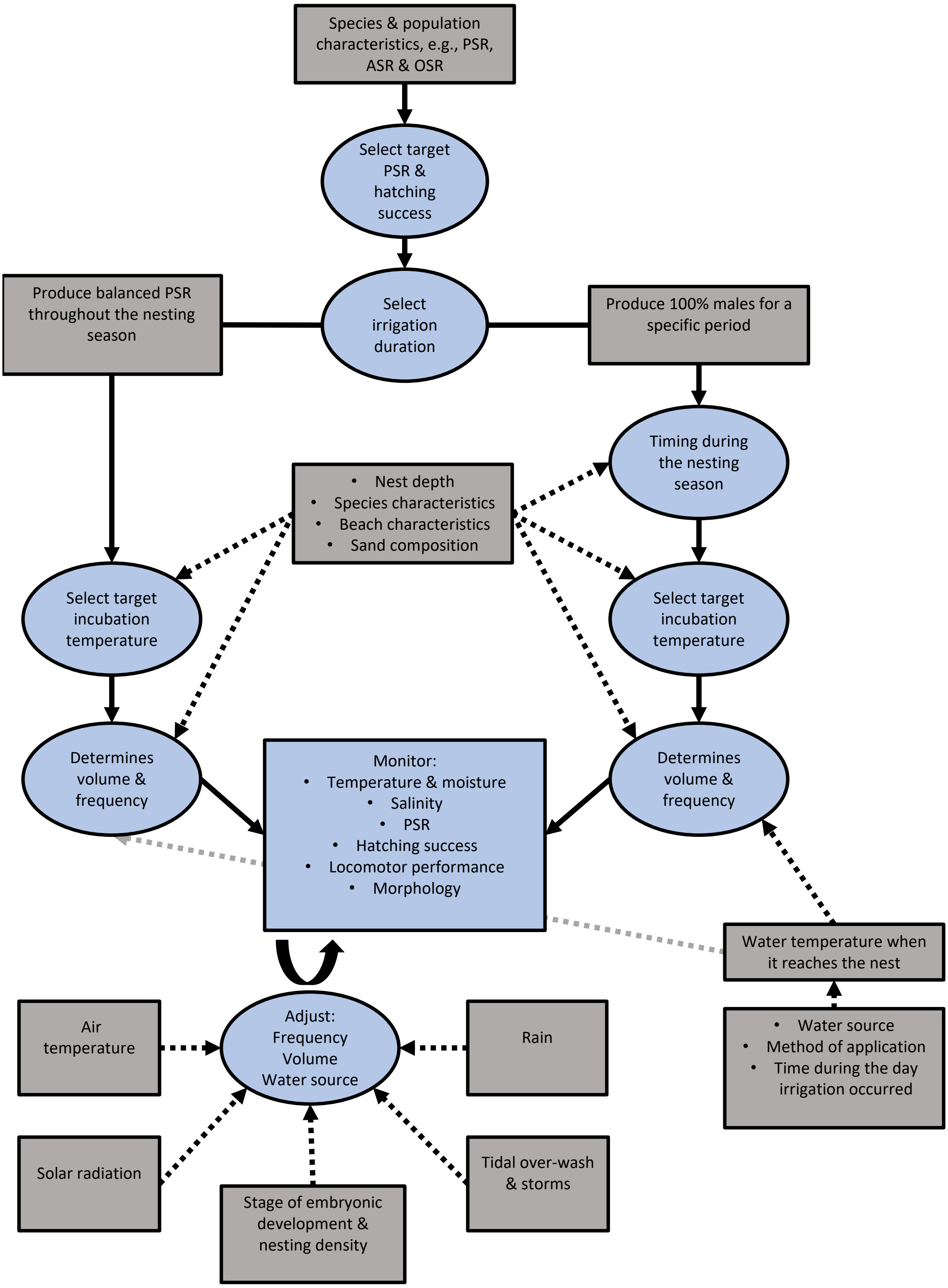

Figure 3: Decision-making flowchart for establishing and refining an irrigation regime. Blue boxes represent management and decision-making stages, and grey boxes represent factors that influence these decisions. PSR = primary sex ratio, ASR = adult sex ratio and OSR = operational sex ratio

12. Overall recommendations and conclusions

Previous studies have shown that irrigation can be a viable tool for cooling sea turtle nests. It can achieve cooling effects similar to shading, but it generally provides less cooling than natural rainfall events. Studies on nest irrigation are limited and often vary significantly in their approach, thus identifying clear patterns and making specific recommendations for Raine Island is difficult. Not only does irrigation frequency, duration and volume differ among studies, but beach and species characteristics such as sand composition and nest depth, strongly influence the amount of cooling. While we suggest starting irrigation trials on Raine Island with 200 mm of seawater every 4 days during October and November, when nest temperatures during the thermosensitive period are lowest, it is likely that adjustments will be needed. Trials will develop a baseline irrigation regime for achieving desired PSRs, but regular adjustments will be needed to account for environmental variation and changes in nesting density. Monitoring nest moisture and salinity will be critical to maximise hatchling production. Nest destruction by conspecifics will need to be monitored, particularly during high density nesting seasons. Nest irrigation can be extended into early December or recommenced in late February, March and April in nesting seasons if nest destruction is high.

We suggest initially irrigating nests with seawater because it provides similar cooling to freshwater but requires less infrastructure and no storage. Additionally, colder seawater can be pumped from ocean depths to maximise cooling. However, seawater irrigation will require regular monitoring of salt accumulation in nests. Freshwater could be used to irrigate nests but sourcing enough water will require desalination, rainfall collection or transportation from the mainland. These freshwater options require significant infrastructure and financial commitments and may negatively impact the local environment. Some dilution of seawater with freshwater or alternating between freshwater and seawater irrigations is possible, dependent on the availability of freshwater or the capacity to store it.

Lastly, sprinklers will probably be the most effective method for irrigating nests on Raine Island. Underground irrigation is likely to be destroyed by or disturb nesting females, limiting its use *in situ*. Watering cans will be inefficient considering the large number of nests to be irrigated on Raine Island. Permanent sprinklers in the sand are likely to be dug up by females, so permanent sprinklers would need to be fenced or in locations where they cannot be accessed by females. Pipes connecting water tanks and sprinklers can be buried below nest depth to prevent females from digging them up. Alternatively, transportable agricultural sprinklers could be towed around the beach, provided they do not crush nests or turtles. Irrigation should occur in the early morning or before dawn to ensure that the applied water is not warmed by hot surface sand and because fewer females will be on the beach than at night.

In summary, we conclude that nest irrigation is a viable means for cooling sea turtle nests on Raine Island. Nests will need to be regularly monitored to ensure that the required amount of cooling is achieved without negatively impacting hatching success and other hatchling traits. We suggest that trials start at a relatively small scale and are then increased, with adjustments made to compensate for changes in weather conditions, nesting density and any potential effects of irrigating larger areas.

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