Potential feminization of Red Sea turtle hatchlings as indicated by in situ sand temperature profiles

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Abstract
Climate change poses a serious threat to species that demonstrate temperature-dependent sex determination, including marine turtles. Increased temperatures can result in highly female-skewed sex ratios and decreased hatching success. The pivotal temperature that delineates hatching sex ratios is commonly considered to be 29.2°C, but whether this threshold applies to turtles in the Red Sea region has not been tested in situ. For all species of marine turtles, there is a supposed thermal range of 25–33°C in which egg incubation is successful, with prolonged temperatures above 33°C resulting in morphological abnormalities and hatchling mortality. Sand temperature data were collected from May–September 2018 from the average nesting depth of hawksbill (Eretmochelys imbricata) and green turtles (Chelonia mydas) at five study sites. We calculated the expected sex ratio based on a maximum likelihood model. The sand temperature profile at four of the sites exceeded the pivotal temperature (29.2°C) throughout the study duration, which suggests feminization of turtles could be occurring; however, the pivotal temperature in this region still needs to be empirically confirmed. The percentage of days with sand temperature exceeding the maximum thermal threshold between June 3, and September 16, 2018, was site-specific rather than determined by latitudinal temperature gradients, and ranged between 0 and 100% of days. Maximum temperature recordings were as high as 36.0 and 35.3°C at 30 and 50 cm depth, respectively. Nesting sites in the Red Sea region could already be exceeding the thermal limits and may be particularly vulnerable to rising temperatures. Sites with lower sand temperatures, such as Small Gobal Island, may represent priority areas for conservation efforts. Alternatively, local adaptation may be a reality under extremely warm conditions, thus, further research into the thermal tolerance of hatchlings in the region could provide insight on how they might adapt to future climate change.

KEYWORDS
climate change, hatching success, sand temperature, temperature-dependent sex determination
INTRODUCTION

There are seven extant species of marine turtles, which share common life-history traits that make them vulnerable to environmental change and anthropogenic impacts. These characteristics include a long lifespan, late sexual maturity, cyclic migrations between nesting and feeding grounds, fidelity to foraging habitats, and dependence on low-lying sandy beaches to lay their clutches. Thus, nearly all species of marine turtles are included in the International Union on the Conservation of Nature’s Red List of Endangered Species (IUCN, 2019) and are protected by Appendix I of the Convention on the International Trade of Endangered Species, which restricts international commercial trade (CITES, 2019). Despite recent global protection, turtles continue to be overexploited for their meat, oil content, and intricate shells (Humber, Godley, & Broderick, 2014; Mancini & Koch, 2009; Nada, 2001).

Marine turtles first appeared during the late Triassic period 220 million years ago (Li, Wu, Rieppel, Wang, & Zhao, 2008) and have since survived and adapted to climate changes throughout their evolutionary history (Harris, 1993). Despite survival through past climatic shifts, current rates of change are likely unprecedented for tens of millions of years (Zeebe, Ridgwell, & Zachos, 2016), leading to a rising concern of how present-day populations will respond to anthropogenic climate change, particularly as they face other human-induced threats (Root, Price, Hall, & Schneider, 2003). In the past century alone, the global mean air temperatures have risen 0.8°C and could increase by at least 1.8°C by 2100 under a carbon emission stabilization scenario (RCP4.5) (IPCC, 2014). Marine turtles demonstrate temperature-dependent sex determination, which is a common trait of some vertebrates including turtles, crocodiles, and some fish species (Valenzuela & Lance, 2004). While the sex of mammals is determined by sex chromosomes at the time of fertilization, the sex of marine turtles is determined by the nest temperature during the middle third of embryo development (Mrosovsky & Yntema, 1980). In addition to sex determination, incubation temperature influences growth rate (Gillooly, Brown, & West, 2001), incubation duration (Mrosovsky & Yntema, 1980), morphology of hatchling (Du & Ji, 2003), long-term physiology and behavioral traits (Sibly & Atkinson, 1994), and hatchling mortality (Howard, Bell, & Pike, 2014). Thus, there are significant implications to these species in the face of climate change (Hawkes, Broderick, Godfrey, & Godley, 2009).

For marine turtles, the pivotal temperature (defined as the constant incubation temperature in which eggs will produce a 50:50 sex ratio) is ~29°C, which differs slightly among species and geographic locations (Godfrey & Mrosovsky, 2006; King, Cheng, Tseng, Chen, & Cheng, 2013; Mrosovsky & Pieau, 1991). Temperatures above the pivotal temperature produce predominantly females and temperatures below the pivotal temperature produce predominantly males (Mrosovsky, 1994). The transitional range of temperature (TRT) is the range of incubation temperatures in which both female and males are produced (Mrosovsky & Pieau, 1991). Temperatures above or below the TRT result in 100% females or males, respectively (Mrosovsky & Pieau, 1991). Both pivotal temperature and TRT have been defined in constant incubation temperature lab experiments (Mrosovsky & Pieau, 1991; Mrosovsky & Yntema, 1980); therefore, the application of these parameters to field experiments should be approached with caution since natural nesting conditions have fluctuating temperatures throughout incubation (Bull, 1985; Stubbs, Kearney, Whiting, & Mitchell, 2014). Although, both pivotal temperature and TRT have been used in field experiments to infer sex ratios (e.g., Laloë, Cozens, Renom, Taxonera, & Hays, 2014; LeBlanc, Drake, et al., 2012; LeBlanc, Wibbels, Shaver, & Walker, 2012).

For all seven species of marine turtles, there is a suggested thermal range of 25–33°C in which egg incubation is successful (Howard et al., 2014; Miller, 1985; Spotila & Standora, 1985), and temperatures toward the higher threshold (prolonged exposure above 33°C) can result in morphological abnormalities, decreased emergence, smaller hatchlings, and hatchling mortality (Hawkes, Broderick, Godfrey, & Godley, 2007; Hays, Broderick, Glen, & Godley, 2003; Laloë, Cozens, Renom, Taxonera, & Hays, 2017; Maulany, Booth, & Baxter, 2012; Packard, Tracy, & Roth, 1977; Sim, Booth, & Limbus, 2015). Compared to sex-determining temperatures, the maximal upper limit to successful incubation is poorly constrained. Current estimates of both 33°C (Miller, 1997) and 35°C (Ackerman, 1997) are frequently cited as the maximum thermal threshold, while the minimum thermal threshold has been identified as 25°C (Howard et al., 2014). Presently, there is no research on pivotal temperatures or critical thermal thresholds for the Red Sea, a region of the world exhibiting high air and sand temperatures throughout the year.

Five of the seven extant species of marine turtles have been observed within the Red Sea: the green turtle (Chelonia mydas), hawksbill (Eretmochelys imbricata), loggerhead (Caretta caretta), olive-ridley (Lepidochelys olivacea), and leatherback (Dermochelys coriacea) (Frazier & Salas, 1984). The hawksbill and green sea turtle are the most common species and are known to nest along the coastline and on sandy islands throughout the
Red Sea region (Attum, Kramer, Mahmoud, & Fouda, 2014; Hanafy, 2012; Pilcher & Al-Merghani, 2000). All the countries surrounding the Red Sea legally protect marine turtles through national laws and international agreements, although enforcement at sea and around nesting sites is lacking (Mancini, Elsadek, & El-alwany, 2015). The political situations in Somalia, Eritrea, Sudan, and Yemen have made formal turtle surveys difficult or impractical for many years, and therefore the status of sea turtles in these areas is largely unknown (Mortimer & Donnelly, 2007). Current estimates predict that there are about 1,500 green turtles and 450–650 hawksbill turtles nesting per year in the Red Sea region (Hanafy, 2012; Mortimer & Donnelly, 2007). In Egypt, the nesting season is thought to occur between May and September (Hanafy & Sallam, 2003). The main green turtle nesting sites in Egypt are Zabargad Island and Wadi el Gemal National Park, with an estimated 250 nesting green turtle females for the entire Egyptian Red Sea coast (Hanafy, 2012). In Saudi Arabia, less is known about nesting sites and seasonality. The main green turtle nesting sites are Tiran Island and Ras Baridi (Al-Merghani, Miller, Pilcher, & Al-Mansi, 2000; Mancini et al., 2015). At Ras Baridi, the nesting season is between August and November and is estimated to host 150 nesting females (Pilcher & Al-Merghani, 2000). Nesting is thought to occur throughout coastal beaches and sandy islands of Saudi Arabia, but data on nesting occurrence is lacking, with published information only from Ras Baridi (Al-Merghani et al., 2000; Pilcher, 1999; Pilcher & Al-Merghani, 2000).

The main objective of this study was to evaluate the sand temperature at hawksbill and green turtle nesting sites in the Red Sea region. Using these in situ temperatures, sex ratios were determined based on the Girondot (1999) statistical model. There are limitations in using this model with temperatures from field experiments since the temperature input assumes constant incubation temperature, and pivotal temperature and TRT have been established in constant incubation experiments (e.g., Godfrey & Mrosovsky, 2006; Mrosovsky, Bass, Corliss, Richardson, & Richardson, 1992). We also assessed the percentage of days exceeding the possible maximum thermal thresholds of 33 and 35°C at each site. Thus, we were able to evaluate which sites might experience highly skewed primary sex ratios as well as extreme temperature exposures that could lead to low emergence success.

2 | METHODS

The Red Sea exhibits extreme high salinity and temperatures, which demonstrates environmental conditions which vary along a latitudinal gradient. Salinity is highest in the north due in part to evaporation processes while conversely, the temperature is highest in the southern Red Sea (Carvalho, Kürten, Krokos, Hoteit, & Ellis, 2018). Five study sites were selected to cover these strong temperature and environmental gradients (Figure 1). Each site showed some turtle nesting evidence from hawksbill and/or green turtles (Table 1). The northernmost site in this study was Small Gobal Island located in Egypt near the Gulf of Suez. The second Egyptian study site was located within the Wadi el Gemal National Park. At a similar latitude to Wadi el Gemal was the northernmost Saudi Arabian site, Ras Baridi. Ras Baridi is thought to be the main green sea turtle nesting area in Saudi Arabia (Al-Merghani et al., 2000; Pilcher, 1999). The next site in Saudi Arabia was Abu Gisha near Thuwal in the central Red Sea. The southernmost site in this study was located about 50 km offshore of Al Lith within the Farasan Banks, on Marmar Island.

HOB0 Pendant Temperature Data Loggers (henceforth referred to as “loggers”), UA-001-64, Onset Computers, were used to record sand temperature from May to September 2018. The accuracy reported by the manufacturer was ± 0.53°C and the resolution was 0.14°C. The accuracy of each data logger was verified as ≤ 0.3°C after deployment in a stepwise temperature bath (24 hr from...
Temperature measurements were recorded every 15 min. Four logger stations were selected at each site on the presumed nesting line of each beach (Table 2). Loggers were buried at 30 and 50 cm, the average depths of hawksbill and green turtle nests, respectively (Carr, Hirth, & Ogren, 1966; Jourdan & Fuentes, 2015; Spotila, Standora, Morreale, & Ruiz, 1987; Witzell & Banner, 1980). The nest depth from green turtles were verified at Ras Baridi, but we were unable to confirm nesting depth of hawksbills in the Red Sea region.

To facilitate the location and retrieval of the loggers, they were attached together on a string, which was secured to a rope buried just under the sand, and GPS coordinates and photos were taken at each site.

2.1 | Sand temperature

To compare the mean sand temperature at 30 and 50 cm depth at each study site, HOBOware software was used to visually interpret if loggers were dislodged from their deployed depth, upon confirmation of dislodgement the data were omitted from the analysis. HOBOware was also used to find the average daily temperature for each logger. At each of the five study sites, the mean daily temperature measurement was calculated for 30 and 50 cm depth. These mean values were plotted with the theoretical pivotal temperature (29.2°C) over time from May 30 to September 20, 2018. In addition to reporting daily means, the maximum temperatures recorded at each site were also determined.

2.2 | Maximum thermal threshold

There are no Red Sea region-specific values of maximum thermal threshold, so the commonly cited values of 33°C (Miller, 1997) and 35°C (Ackerman, 1997) were used in this study to assess the percentage of days that exceeded these thermal thresholds at 30 and 50 cm depth. To calculate this percentage, the number of days that recorded temperatures exceeding these values was counted for each site by depth, and then this value was divided by the total number of days (105). This analysis was done between June 3 and September 16, the time period in which temperature measurements overlap from each site.

2.3 | Sex ratio prediction

$$sr(t) = \frac{1}{1 + e^{(t)(\ln(P+K) - \ln(t+K))}}$$

To predict the sex ratios, a maximum likelihood mathematical model suggested by Girondot (1999) was used, referred to as the Hill equation. This equation
describes the relationship between pivotal temperature \( (P) \), the value that produces an equal number of each sex, the incubation temperature measured in the sand \( (t) \), and hatchling sex ratios \( (sr) \): where \( sr \ (t) \) is the ratio of females, at a specific temperature, \( t \). \( S = -0.0336281, p = 29.2, \) and \( K = 0.1 \). The mean weekly temperature was used as the \( t \) value. The \( S \) parameter defines the shape of transition from masculinizing to feminizing temperature. Since there have not been any turtle incubation experiments in the Red Sea region, \( S \) and \( K \) values were based on theoretical values recorded in laboratory studies (Laloë et al., 2014). This computation was conducted on R (R version 3.6.1, R Development Core Team (2019)). This equation has been used to estimate sex ratios at nesting sites in the Cape Verde Islands (Laloë et al., 2014), Georgia (LeBlanc, Drake, et al., 2012), Texas (LeBlanc, Wibbels, et al., 2012), and Ascension Island (Godley, Broderick, Glen, & Hays, 2002). We did not include metabolic heating as a factor in our calculation—as studies have shown that the amount of metabolic heating during the middle third of embryotic development, when sex is determined, is negligible (Zbinden, Margaritoulis, & Arlettaz, 2006). The pivotal temperature value of 29.2°C was used because it appears to be illustrative for all species of sea turtles (Ackerman, 1997). Thus, while these sex ratios are estimated based on theoretical values widely applied in the literature, they are not based on data from Red Sea region turtle populations.

3 | RESULTS

3.1 | Sand temperature

A comparison between the five study sites from May 30 to September 19, 2018 was conducted to assess the sand temperature patterns in the Red Sea region (Figure 2). Overall, temperatures recorded at 30 cm were warmer and more variable compared with the loggers buried at 50 cm in the same location. All study sites except Small Gobal Island (the northernmost site) had sand temperatures well above the pivotal temperature (29.2°C), which suggests that there could have been a female bias produced during the 2018 nesting season. Ras Baridi demonstrated the highest sand temperatures, with a maximum measurement of 36.0°C at 30 cm depth and 35.3°C at 50 cm. Ras Baridi also showed the greatest diel temperature fluctuation, with an average daily fluctuation of 0.84 ± 0.12°C and 0.24 ± 0.06°C at 30 and 50 cm depth, respectively. Marmar Island in Al Lith, the southernmost study site, had the next highest sand temperatures, with a maximum recording of 35.9°C at 30 cm and 34.5°C at 50 cm depth. Wadi El Gemal had similar temperatures to Al Lith, with a maximum temperature of 35.0°C at 30 cm and 34.1°C at 50 cm depth. Furthermore, the Thuwal study site had sand temperatures consistently cooler than Ras Baridi, Wadi el Gemal, and Al Lith, with a maximum temperature of 33.5°C at 30 cm depth and 33.0°C at 50 cm depth. This site also demonstrated the smallest diel temperature fluctuation, with an average daily fluctuation of 0.20 ± 0.06°C and 0.07 ± 0.04°C at 30 and 50 cm depth, respectively. Finally, the lowest sand temperatures were recorded at Small Gobal Island, which had a maximum of 31.0°C at 30 cm and 30.5°C at 50 cm depths.

The temperature increase throughout the nesting season was the largest at the Thuwal site, despite demonstrating the second coolest temperatures, with an increase of 2.2°C at 30 cm and 2.6°C at 50 cm. Similarly, Ras Baridi showed a relatively small increase throughout the nesting season despite having the highest temperature of all the sites, with an increase of 1.2°C at 30 cm and 1.5°C at 50 cm depth.

3.2 | Maximum thermal threshold

The period above the maximum thermal tolerance was calculated as the percentage of days between June 3 and September 16 that had temperature recordings exceeding 33 and 35°C (Figure 3). Small Gobal Island had 0 days crossing either the 33 or 35°C limit for the time period studied, whereas Ras Baridi exhibited 100 and 96% of days exceeding 33°C at 30 and 50 cm, respectively. The thermal threshold of 33°C is largely surpassed by the majority of sites at both 30 and 50 cm depth for at least 50% of the days studied (except for Small Gobal Island
and Thuwal at 50 cm depth). However, if 35°C is considered as the thermal threshold for successful incubation, then only Ras Baridi faces a high threat with nearly 60 and 25% of the days exceeding 35°C at 30 and 50 cm depth, respectively. Furthermore, both Al Lith and Thuwal exhibit a small percentage of days with temperature exceeding 35°C at 30 cm depth (11 and 19%, respectively).

### 3.3 | Sex ratio prediction

The maximum likelihood model resulted in highly female-skewed sex ratios at all sites except for Small Gobal Island, the northernmost study site, and Thuwal during May (Figure 4). Assuming that the pivotal temperature 29.2°C (Ackerman, 1997) is applicable to the Red Sea region nesting sites, at 30 cm depth, it is estimated that Small Gobal would have had the lowest female sex ratio (17.5%) in the end of May. This percentage of females increased with time until a maximum of 80% in early August. After August, the percent of predicted females decreased to about 50% in late September. The Thuwal study site had the next lowest predicted female sex ratios, with as little as 32% in May, which increased until to over 90% from July until September. Ras Baridi, Wadi el Gemal, and Al Lith had extremely high-predicted female sex ratios above 97% for the duration for deployment.

The trends from 30 cm depth were very similar to that of 50 cm, but the percentage of predicted females is slightly lower due to the cooler temperatures (Figure 5). For example, on Small Gobal Island the predicted percent female was as low as 8% in late May, with a maximum of 69% in August. The Thuwal study site had a predicted percent female as low as 24% in May, which increased to a maximum of 96% in August. Wadi el Gemal, Ras Baridi, and Al Lith had predicted percent females between 85 and 99%.

### 4 | DISCUSSION

We assessed sand temperatures at turtle nesting beaches in the Red Sea region in order to predict sex ratios of hatchlings throughout the nesting season. Sand temperature varied greatly between and within study sites. We measured sand temperatures as high as 36°C, illustrating a clear threat to both hawksbill and green sea turtles at our study sites. Based on the thermal profiles of Ras Baridi, Wadi el Gemal, Thuwal, and Al Lith, incubating embryos might exhibit mortality from the high sand temperatures. Due to the large latitudinal gradient of the study, patterns of increasing temperature from north to south were expected. However, latitude did not seem to be the only factor influencing the thermal profile recorded at each study site. Similar results have been observed in the Great Barrier Reef, where temperature of nesting sites did not change over four degrees of latitude (Fuentes, Hamann, & Limpus, 2010). The lack of predictable latitudinal variation in nest temperatures indicates the complexity of influencing factors, and demonstrates the need for careful in situ investigation into these patterns.

Many parameters contribute to the thermal profile of sand, including seasonal temperature changes (Mrosovsky, Hopkins-Murphy, & Richardson, 1984), shading by vegetation (Kamel, 2013), grain size, color of sand (albedo) (Hays et al., 2001), rainfall (Laloë, Esteban, Berkel, & Hays, 2016), and extreme high tides (Laloë et al., 2016). While these factors have been identified as causing thermal variations between nests, little is known...
about how exactly these aspects dictate nest temperatures. It is important to note that many of our study sites (i.e., Small Gobal Island, Wadi el Gemal, Ras Baridi, and Thuwal) have little to no vegetation, so shading is not a cooling factor. Furthermore, the turtle nesting season coincides with the summer in Saudi Arabia, which is the hottest time of the year, only receiving an average of about 2.50 mm of rain per month and air temperatures reaching highs of 39°C between June and September. Additionally, the Red Sea does not demonstrate a dramatic tidal range, which is an important factor of cooling in other parts of the world (Laloë et al., 2016). Thus, the turtle nesting sites in the Red Sea region do not exhibit cooling mechanisms that are crucial for regulating temperatures as reported in other turtle nesting sites (Houghton et al., 2007; Laloë et al., 2016; Patrício et al., 2017), potentially rendering Red Sea turtles more vulnerable to increasing temperature regimes.

Results from this study indicated that there are some behavioral changes that turtles could make in order to change the incubation temperature of their nests. For example, temperature recorded at 30 cm was generally warmer compared with 50 cm at a given logger station. Although nesting turtles could slightly adjust their nest depth to reach cooler temperatures, an individual turtle’s physical attributes and inherent nesting behavior may limit this flexibility. Additionally, there were seasonal differences up to 2.6°C between June and August, so females potentially could shift their nesting earlier in the season to adjust the incubation temperature of their clutch of eggs, but again, it is not known how flexible nesting behavior is at this time scale. Sea surface temperature has been found to correlate with nesting seasonality (Dalleau et al., 2012), but as documented shifts are on the order of days per year, such a shift is more likely to occur as a possible response to long term global warming than habitual seasonal temperature differences. Furthermore, results from this study indicate that sand temperature varied among logger stations within a single site, with a maximum of 1.3°C difference between logger stations on the same day at a depth of 50 cm. This highlights the importance of nest location selection by nesting females.

By nesting at sites with a high range of thermal profiles, turtle populations can produce both male and female hatchlings. Unfortunately, our findings suggest that many of the nesting sites exceed the presumed pivotal temperature for most of nesting season, and feminization could already be occurring if the assumed pivotal temperature is applicable to this region. To confirm this, further investigation should focus on measuring the sex ratio of turtles produced at the sites with the highest nesting activity, such as Ras Baridi and the offshore islands of Al Lith, as well as the coolest nesting sites, such as Small Gobal Island or Thuwal. Alternatively, unreported nesting may be occurring in cooler months of the year, which could lead to the production of more male hatchlings.

Several sites had temperature recordings in August and September (the end of nesting season as most sites) that exceeded 35°C. Thus, the Red Sea region sand temperatures are not only exceeding the presumed pivotal temperatures but are reaching the commonly cited maximum thermal tolerance. Thermal tolerance of developing embryos is not fully understood, but reviews on the topic cite a maximum thermal tolerance of either 33 or 35°C depending on the nesting beach (Ackerman, 1997; Miller, 1997). A review of thermal tolerances at different
nesting sites indicated that there is a range of maximum values that is dependent on the location (Howard et al., 2014). However, a recent study suggests that there might not be a local adaptation to thermal tolerance limits, thus, increased nest temperatures under predicted climate change scenarios will result in increased embryo mortality and female hatching production unless temporal or spatial range shifts occur (Tilley et al., 2019). In addition to mortality, high temperatures can cause reduced oxygen levels, which negatively affects muscle coordination, inhibiting the ability for turtles to crawl out of the nest (Matsuzawa, Sato, Sakamoto, & Bjørndal, 2002). High nest temperatures also lead to smaller hatchlings, which have decreased ability to emerge from the nest and an increased probability of predation (Booth & Evans, 2011; Wood, Booth, & Limpus, 2014). There is currently no information on the maximum temperature threshold of turtles in Saudi Arabia or anywhere in the Middle East, so future studies should assess the thermal maximum of incubating eggs from this region, which experiences relatively high temperatures compared to other turtle nesting sites.

Overall mean sand temperatures at the majority of our study sites were high compared with other turtle nesting sites around the world (DeGregorio & Williard, 2011; Godley et al., 2001, 2002; Horrocks & Scott, 1991; Kaska et al., 2006; Laloë et al., 2016). For example, mean hawksbill nest temperatures ranged between 30.3 and 32.4°C in Barbados (Horrocks & Scott, 1991) and mean green turtle nest temperatures ranged between 29.2 and 32.2°C on Ascension Island (Godley et al., 2002).

The results of the sex ratio prediction reported highly female-biased primary sex ratios, with the exception of the Small Gobal island site, being the coolest site. This finding is similar to sex ratios found in some other regions of the world. For example, a study conducted in the northern Great Barrier Reef in Australia found that 99.1% of juvenile green turtles were female (Jensen et al., 2018) and a study from Turkey found 64–94% of green hatchlings produced were female (Yalçin Özdilek, Sönmez, & Kaska, 2016). Similarly, studies found >90% female hawksbill hatchlings emerging from nests Brazil (Godfrey, Amato, Marcovaldi, & Mrosovsky, 1999) and 80% female juvenile hawksbill turtles in the U.S. Virgin Islands (Geis et al., 2003). Our sex ratios were determined using the Girondot (1999) statistical model, which has a limitation since the input (t) assumes constant incubation temperature. This is because many pivotal temperature and TRT studies are conducted in a laboratory setting, with one temperature throughout incubation. For in situ sand temperature, we found daily variation up to 1.6°C. This variation can complicate simple predictions of sex ratio from daily mean temperature values. To validate the results from the sex ratio prediction, studies on the sex ratio of hatchlings at nesting sites throughout the Red Sea region could be conducted. For hatching turtles, a lack of external sex characteristics precludes visual determination of sex and their small size does not allow for a safe observation of the gonads through laparoscopy (e.g., Mrosovsky & Yntema, 1980). Thus, a direct assessment of sex traditionally requires sacrificing hatchlings for histological examination of the gonads (Mrosovsky & Benabib, 1990). Promising alternatives exist, including using anti-Mullerian hormone from blood samples as a gender-indicative marker, since it is detected only in neonate male turtles (Tezak, Sifuentes-Romero, Milton, & Wyneken, 2020).

Upcoming mega-developments are planned in Saudi Arabia which could threaten both nesting and foraging behavior of turtles in the Red Sea (NEOM and the Red Sea Project). NEOM is proposed to span 26,000 km² across the northern Saudi Arabian Red Sea coast and across parts of Egypt and Jordan (Public Investment Fund, 2017). In addition, the Red Sea Project plans to develop 50 existing islands between the city of Umluj and Al Wajh. This construction has the potential to increase shipping traffic, which can cause injury and mortality in many marine species, including turtles. As the projects are all at very early stages of planning and development, there is an opportunity for the country to set a good example and proceed with these developments while preserving environmental conditions (including the critical nesting habitats for turtles). Careful planning and interaction with scientists will be necessary to achieve sustainability in the near future. For example, marine spatial planning efforts that allow for potential nesting refugia in cooler areas (more northern region) could provide an important conservation tool along with the implementation of long-term monitoring programs to assess movement patterns (Pendoley, Schofield, Whittock, Ierodiaconou, & Hays, 2014).

In conclusion, we found nesting beach sand temperatures that were amongst the hottest in the world. We measured temperatures far exceeding the presumed pivotal temperature, and reaching temperatures that could cause mortality and developmental abnormalities. Current studies suggest the potential for significant feminization and possible mortality and sublethal effects of high incubation temperatures, which could be occurring at some of our Red Sea region nesting sites. It is important to note that temperatures have been historically high in this region, so these turtles might be especially adapted to withstand the hot environment. Thus, these turtle populations might provide insight on how other turtles might react to increasing temperatures from climate change, and should be a conservation priority. Moreover,
climate change poses significant conservation concerns since the current rates of change are unprecedented (Brohan, Kennedy, Harris, Tett, & Jones, 2006; IPCC, 2014) and it is unclear if turtles are capable of evolving fast enough to counter the negative impacts of climate change (Janzen, 1994).

There are several management strategies that could be used to artificially lower the incubation temperature of turtle nests if it was determined that intervention was needed. At rookeries with extreme female-biased hatching production, artificial shading can be used to decrease temperatures (Esteban et al., 2018). Shade can be created with simple materials, such as white sheets or white sand, which make it a low-cost and low-technology conservation strategy to minimize the impacts of climate change (Esteban et al., 2018). Another strategy used as a mitigation of climate warming impacts is the relocation of sea turtle eggs (Tuttle & Rostal, 2010). Clutches can be relocated into the shade or to a greater depth to decrease incubation temperature. Relocation should occur within 12 hr of deposition to reduce the risks of egg mortality (Limpus, Baker, & Miller, 1979); this duration can be prolonged if the eggs are kept in hypoxic conditions (Williamson et al., 2017). Some studies suggest that nest relocation may impact hatching morphology and fitness of hatchlings (Sönmez, Turan, & Yalçın Özçilek, 2011). An additional method utilized is cooling nests with water (Hill, Paladino, Spotila, & Tomillo, 2015). Out of these strategies, shading has been found to have the largest impact on sand temperature, followed by watering and increasing the nest depth (Hill et al., 2015). The applicability of these methods in the Red Sea region is currently limited due to the inaccessibility of some of the nesting sites, but they could be feasible options in the future to protect turtles in the Red Sea region from climate change warming, and should be prioritized on the beaches with the greatest hatching output, such as Ras Baridi.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
Lyndsey K. Tanabe led the study and wrote the original manuscript; Lyndsey K. Tanabe, Joanne Ellis, and Michael L. Berumen designed the study and contributed to the editing of the manuscript. Lyndsey K. Tanabe and Islam Elsadek conducted the fieldwork for this study. Michael L. Berumen provided the funding for the research.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author, L. K. T., upon request.

ETHICS STATEMENT
Research was conducted under KAUST Institutional Animal Care and Use Committee (IACUC) approval #18-022-OS.

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REFERENCES
Ackerman, R. (1997). The nest environment and the embryonic development of sea turtles. In P. L. Lutz & J. A. Musick (Eds.), The biology of sea turtles (pp. 83–106). Boca Raton, FL: CRC Press.
Al-Merghani, M., Miller, J. D., Pilcher, N. J., & Al-Mansi, A. M. A. (2000). The green and hawksbill turtles in the Kingdom of Saudi Arabia: Synopsis of nesting studies 1986-1997. Fauna of Arabia, 18, 369–384.
Attum, O., Kramer, A., Mahmoud, T., & Fouda, M. (2014). Post-nesting migrations patterns of green turtles (Chelonia mydas) from the Egyptian Red Sea. Zoology in the Middle East, 60, 299–305.
Booth, D. T., & Evans, A. (2011). Warm water and cool nests are best. How global warming might influence hatching green turtle swimming performance. PLoS One, 6, e23162.
Brohan, P., Kennedy, J. J., Harris, I., Tett, S. F. B., & Jones, P. D. (2006). Uncertainty estimates in regional and global observed temperature changes: A new data set from 1850. Journal of Geophysical Research—Atmospheres, 111, 1–21.
Bull, J. J. (1985). Sex ratio and nest temperature in turtles: Comparing field and laboratory data. Ecology, 66, 1115–1122.
Carr, A., Hirth, H., & Ogren, L. (1966). The ecology and migrations of sea turtles, 6: The hawksbill turtle in the Caribbean Sea. American Museum Novitates, 2248, 1–29.
Carvalho, S., Kürtén, B., Krokos, G., Hoteit, I., & Ellis, J. (2018). The Red Sea. In C. Sheppard (Ed.), World seas: An environmental evaluation, Volume II: The Indian Ocean to the Pacific (pp. 49–74). London, England: Academic Press.
CITES (2019). Convention on international trade of endangered species, Geneva, Switzerland: CITES. Retrieved online www.cites.org.
Dalleau, M., Ciccione, S., Mortimer, J. A., Garnier, J., Benhamou, S., & Bourjea, J. (2012). Nesting phenology of marine turtles: Insights from a regional comparative analysis on green turtle (Chelonia mydas). PLoS One, 7, e46920.
DeGregorio, B. A., & Williard, A. S. (2011). Incubation temperatures and metabolic heating of relocated and in situ loggerhead
sea turtle (Caretta caretta) nests at a northern rookery. Chelonian Conservation Biology, 10, 54–61.

Du, W. G., & Ji, X. (2003). The effects of incubation thermal environments on size, locomotor performance and early growth of hatching soft-shelled turtles, Pelodiscus sinensis. Journal of Thermal Biology, 28, 279–286.

Esteban, N., Laloë, J., Kidgen, F. S. P. L., Ubel, S. M., Beckling, L. E., Meesters, E. H., ... Christianen, M. J. A. (2018). Optimism for mitigation of climate warming impacts for sea turtles through nest shading and relocation. Scientific Reports, 8, 1–8.

Frazier, J., & Salas, S. (1984). The status of marine turtles in the Egyptian Red Sea. Biological Conservation, 30, 41–67.

Fuentes, M. M. P. B., Hamann, M., & Limpus, C. J. (2010). Past, current and future thermal profiles of green turtle nesting grounds: Implications from climate change. Journal of Experimental Marine Biology and Ecology, 383, 56–64.

Geis, A., Wibbels, T., Phillips, B., Hillis-starr, Z., Meylan, A., Meylan, P., ... van Dam, R. (2003). Predicted sex ratio of juvenile hawksbill sea turtles inhabiting Buck Island Reef National Monument, U.S. Virgin Islands. Journal of Herpetology, 37, 400–404.

Gillooly, J. F., Brown, J., & West, G. B. (2001). Effects of size and temperature on metabolic rate. Science, 293, 2248–2251.

Girondot, M. (1999). Statistical description of temperature-dependent sex determination using maximum likelihood. Evolutionary Ecology, 1, 479–486.

Godfrey, M. H., Amato, A. F. D., Marcovaldi, M. Á., & Mrosovsky, N. (1999). Pivotal temperature and predicted sex ratios for hatching hawksbill turtles from Brazil. Canadian Journal of Zoology, 77, 1465–1473.

Godfrey, M. H., & Mrosovsky, N. (2006). Pivotal temperature for green sea turtles, Chelonia mydas, nesting in Suriname. Journal of Herpetology, 16, 55–61.

Godley, B., Broderick, A., Glen, F., & Hays, G. (2002). Temperature-dependent sex determination of Ascension Island green turtles. Marine Ecology Progress Series, 226, 115–124.

Godley, B. J., Broderick, A. C., Downie, J. R., Glen, F., Houghton, J. D., Kirkwood, I., ... Hays, G. C. (2001). Thermal conditions in nests of loggerhead turtles: Further evidence suggesting female skewed sex ratios of hatching production in the Mediterranean. Journal of Experimental Marine Biology and Ecology, 263, 45–63.

Hanafy, M. H., & Sallam, A. (2003). Status of marine turtles nesting on the Egyptian beaches of the Red Sea (p. 45). Jeddah, Saudi Arabia: National Report to PERSGA.

Hanafy, M. H. (2012). Nesting of marine turtles on the Egyptian beaches of the Red Sea. Egyptian Journal of Aquatic Biology and Fisheries, 16, 59–71.

Harris, A. H. (1993). Wisconsinan pre-Pleniglacial biotic change in southeastern New Mexico. Quaternary Research, 40, 127–133.

Hawkes, L. A., Broderick, A. C., Godfrey, M. H., & Godley, B. J. (2007). Investigating the potential impacts of climate change on a marine turtle population. Global Change Biology, 13, 923–932.

Hawkes, L. A., Broderick, A. C., Godfrey, M. H., & Godley, B. J. (2009). Climate change and marine turtles. Endangered Species Research, 7, 137–154.

Hays, A. G. C., Ashworth, J. S., Barnsley, M. J., Broderick, A. C., Emery, D. R., Henwood, A., ... Henwood, A. (2001). The importance of sand albedo for the thermal conditions on sea turtle nesting beaches. Nordic Society Okkos, 93, 87–94.

Hays, G. C., Broderick, A., Glen, F., & Godley, B. J. (2003). Climate change and sea turtles: A 150 year reconstruction on incubation temperatures at a major marine turtle rookery. Global Change Biology, 9, 62–646.

Hill, J. E., Paladino, F. V., Spotila, J. R., & Tomillo, P. S. (2015). Shading and watering as a tool to mitigate the impacts of climate change in sea turtle nests. PLoS One, 10, 1–14.

Horrocks, J. A., & Scott, N. M. (1991). Nest site location and nest success in the hawksbill turtle Eretmochelys imbricata in Barbados West-Indies. Marine Ecology Progress Series, 69, 1–8.

Houghton, J. D. R., Myers, A. E., Lloyd, C., King, R. S., Isaacs, C., & Hays, G. C. (2007). Protracted rainfall decreases temperature within leatherback turtle (Dermochelys coriacea) clutches in Grenada, West Indies: Ecological implications for a species displaying temperature dependent sex determination. Journal of Experimental Marine Biology and Ecology, 345, 71–77.

Howard, R., Bell, I., & Pike, D. A. (2014). Thermal tolerances of sea turtle embryos: Current understanding and future directions. Endangered Species Research, 26, 75–86.

Humber, F., Godley, B. J., & Broderick, A. C. (2014). So excellent a fishe: A global overview of legal marine turtle fisherie. Diversity and Distributions, 20, 579–590.

IPCC (2014). In R. K. Pachauri & L. A. Meyer (Eds.), Climate change 2014: Synthesis report. Contribution of working groups I, II and III to the Fifth assessment report of the intergovernmental panel on climate change. [eds] Core Writing Team (p. 151). Geneva, Switzerland: IPCC.

IUCN (2019). IUCN red list of threatened species. Retrieved from http://www.redlist.org.

Janzen, F. J. (1994). Climate change and temperature-dependent sex determination in reptiles. Proceedings of the National Academy of Sciences of the United States of America, 91, 7487–7490.

Jensen, M. P., Allen, C. D., Eguchi, T., Bell, I. P., LaCasella, E. L., Hilton, W. A., ... Dutton, P. H. (2018). Environmental warming and feminization of one of the largest sea turtle populations in the world. Current Biology, 28, 154–159.

Jourdan, J., & Fuentes, M. M. P. B. (2015). Effectiveness of strategies at reducing sand temperature to mitigate potential impacts from changes in environmental temperature on sea turtle reproductive output. Mitigation and Adaptation Strategies for Global Change, 20, 121–133.

Kamel, S. J. (2013). Vegetation cover predicts temperature in nests of the hawksbill sea turtle: Implications for beach management and offspring sex ratios. Endangered Species Research, 20, 41–48.

Kaska, Y., Ilgaz, Ç., Özdemir, A., Başkale, E., Türkozan, O., Baran, I., & Stachowitsch, M. (2006). Sex ratio estimations of loggerhead sea turtle hatchlings by histological examination and nest temperatures at Fethiye beach, Turkey. Naturwissenschaften, 93, 338–343.

King, R., Cheng, W. H., Tseng, C. T., Chen, H., & Cheng, I. J. (2013). Estimating the sex ratio of green sea turtles (Chelonia mydas) in Taiwan by the nest temperature and histological methods. Journal of Experimental Marine Biology and Ecology, 445, 140–147.

Laloë, J. O., Cozens, J., Renom, B., Taxonera, A., & Hays, G. C. (2014). Effects of rising temperature on the viability of an
Tezak, B., Sifuentes-Romero, I., Milton, S., & Wyneken, J. (2020). Identifying sex of neonate turtles with temperature-dependent sex determination via small blood samples. *Scientific Reports, 10*, 1–8.

Tilley, D., Ball, S., Ellick, J., Godley, B. J., Weber, N., Weber, S. B., & Broderick, A. C. (2019). No evidence of fine scale thermal adaptation in green turtles. *Journal of Experimental Marine Biology and Ecology, 514–515*, 110–117.

Tuttle, J., & Rostal, D. (2010). Effects of nest relocation on nest temperature and embryonic development of loggerhead sea turtles (*Caretta caretta*). *Chelonian Conservation Biology, 9*, 1–7.

Valenzuela, N., & Lance, V. A. (2004). *Temperature-dependent sex determination in vertebrates*. Washington, DC: Smithsonian Institution.

Williamson, S., Robinson, N. J., Williamson, S. A., Evans, R. G., Robinson, N. J., & Reina, R. D. (2017). Hypoxia as a novel method for preventing movement-induced mortality during translocation of turtle eggs. *Biological Conservation, 216*, 86–92. https://doi.org/10.1016/j.biocon.2017.10.009

Witzell, W. N., & Banner, A. C. (1980). The hawksbill turtle in Western Samoa. *Bulletin of Marine Science, 30*, 571–579.

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

Yalçın Özdílek, Ş., Sönmez, B., & Kaska, Y. (2016). Sex ratio estimations of *Chelonia mydas* hatchlings at Samandağ Beach, Turkey. *Turkish Journal of Zoology, 40*, 552–560.

Zbinden, J. A., Margaritoulis, D., & Arlettaz, R. (2006). Metabolic heating in Mediterranean loggerhead sea turtle clutches. *Journal of Experimental Marine Biology and Ecology, 334*, 151–157.

Zeebe, R. E., Ridgwell, A., & Zachos, J. C. (2016). Anthropogenic carbon release rate unprecedented during the past 66 million years. *Nature Geoscience, 9*, 325–329.

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