Nest temperatures of the Piai and Sayang Islands green turtle (*Chelonia mydas*) rookeries, Raja Ampat Papua, Indonesia: Implications for hatchling sex ratios

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Abstract. *Tapilatu RF, Ballamu F. 2015. Nest temperatures of the Piai and Sayang Islands green turtle (*Chelonia mydas*) rookeries, Raja Ampat Papua, Indonesia: Implications for hatchling sex ratios. Biodiversitas 16: 102-xxx.* Sex determination and hatching success in sea turtles is temperature dependent. Warmer sand temperatures may skew sea turtle population sex ratios towards predominantly females and high sand temperatures may also decrease hatching success. Therefore, understanding nest temperatures is important for conservation programs, including the evaluation of the potential impact of global climate change. Nest temperatures were monitored during the 2013 nesting season of the green sea turtle, *Chelonia mydas*, at Piai and Sayang Islands, Raja Ampat, Papua Barat, Indonesia. Nest temperatures increased from 29°C early in the incubation to 34-36°C in the middle, before decreasing again. Monitored nest temperatures were similar across all beaches. Nest temperatures increased 2-4°C during the middle third of incubation due to metabolic heating. Hatchling sex ratio inferred from nest temperature profiles indicated a strong female bias. This finding is consistent with the relatively warm thermal profiles of the majority of the nesting beaches. This also included some extremely warm nest temperatures that were associated with lower hatching success. Information from this study provides a foundation for developing conservation strategies for enhancing hatchling production with optimal sex ratios at the most important nesting beaches for the western Pacific green sea turtle. This study is the first comprehensive assessment of sex ratios for green sea turtles in Raja Ampat and represents the initiation of a long-term database that can be used at a local level to develop strategies that could potentially offset the impact of long-term climate change on the western Pacific green sea turtle.

Keywords: *Chelonia mydas*, hatching success, Piai, Sayang, sex determination.

INTRODUCTION

Sea turtle populations, globally, are experiencing dramatic population decline. This is due, primarily, to nesting habitat destruction and disturbance, the incidence of commercial fishing by-catch, hunting, shell marketing and raiding of nests by villagers in subsistence economies. Reviews of the status of sea turtle populations in Southeast Asia by Limpus (1994; 1997) determined that all marine turtle populations in the Indo Pacific region, outside Australia, are severely depleted through overharvesting and excessive incidental mortality. Limpus (1997) further estimated that the rate of turtle harvest exceeds the replacement capacity of existing populations in the entire Pacific region. Raja Ampat is a unique site, located on the northwestern side of Papua Barat province - Indonesia. The archipelago contains a full range of marine and coastal habitats that are important for the breeding, foraging and migration of several species of sea turtles. Similar to other areas in the Indo Pacific region, the commercial harvest is identified as one of the major threats to turtle populations in Raja Ampat. Exploitation of sea turtles for both subsistence and commercial purposes is a long-standing practice in Raja Ampat. Sea turtles have long been a source

of protein for local villagers. Further, the expansion of the Balinese turtle fishery towards eastern Indonesia in the mid 1970s caused the depletion of populations in Green turtle rookeries in Sulawesi, Maluku and Irian Jaya (Polunin and Nuitja 1982). Hunting for subsistence and poaching for commercial benefit are most likely to occur during the nesting season abundance.

Like all sea turtles, the green turtle possesses temperature-dependent sex determination (TSD). Incubation temperature determines the sex of hatchling sea turtles during the middle third of embryonic development, with low temperatures producing males, and high temperatures producing females (Yntema and Mrosovsky 1982). Because the environmental conditions at the nest site are a major determinant of nest temperature, it may be expected that females choose their nest site carefully. However, even within a rookery there must be some variation in nest temperature in order to maintain phenotypic diversity. As such, it is of interest to evaluate naturally occurring sex ratios in green turtle populations. In this study, nest temperature profiles were reported from green turtle rookeries on Piai and Sayang Islands of Raja Ampat. Nest temperature profiles were also used to predict the sex ratio of turtles emerging from nests. Documenting

variation in nest temperatures within a rookery is the first step toward a detailed understanding of how incubation temperature influences sea turtle populations. The results provide insight on spatial and temporal dynamics of nest temperatures and predicted hatchlings sex ratios produced on the primary nesting beaches for the western Pacific green turtles at Raja Ampat. These data provide an initial step in establishing a long-term database of hatchling sex ratios for assessing the ecological and conservation implications of TSD in this population, including the potential impact of global climate change. This type of information can prove valuable when attempting to understand the reproductive ecology of sea turtles and when developing conservation strategies for enhancing the recovery of threatened and endangered populations (Coyne et al. 2007; Wibbels 2007).

MATERIALS AND METHODS

This study took place on Piai and Sayang Islands (Figure 1) of Raja Ampat, West Papua, Indonesia during the 2013 green turtle (*Chelonia mydas*) nesting season.

Sayang island is the largest island with approximately 9km of beaches, which are fragmented by karsts. A large number of nests were found on the western beach. Piai island, which is much smaller than Pulau Sayang, is also an important rookery for Green turtles. Sandy beaches, with an approximate length of 3km, are situated on the northern and southern parts of the island. Nest temperatures below the surface were monitored every hour at two sites on Piai and Sayang islands at two different beach zones, open area and under vegetation with temperature data loggers from January to April 2013. In general, the topography of the beaches includes an intertidal zone, then a gently sloping zone, followed by a vegetative zone. Most of the nesting typically occurs well above the high tide line on the gently sloping zone of the beach. Due to logistical difficulties related to placing and retrieving data loggers, they were used primarily during the main nesting season (i.e. the austral summer, January to April). A type of temperature data loggers (HOBO Pendants, Onset Computer Corporation, Pocassete, MA) was used to record sand and nest temperatures. The data logger accurately record temperatures to approximately $\pm$ 0.3-0.4°C.
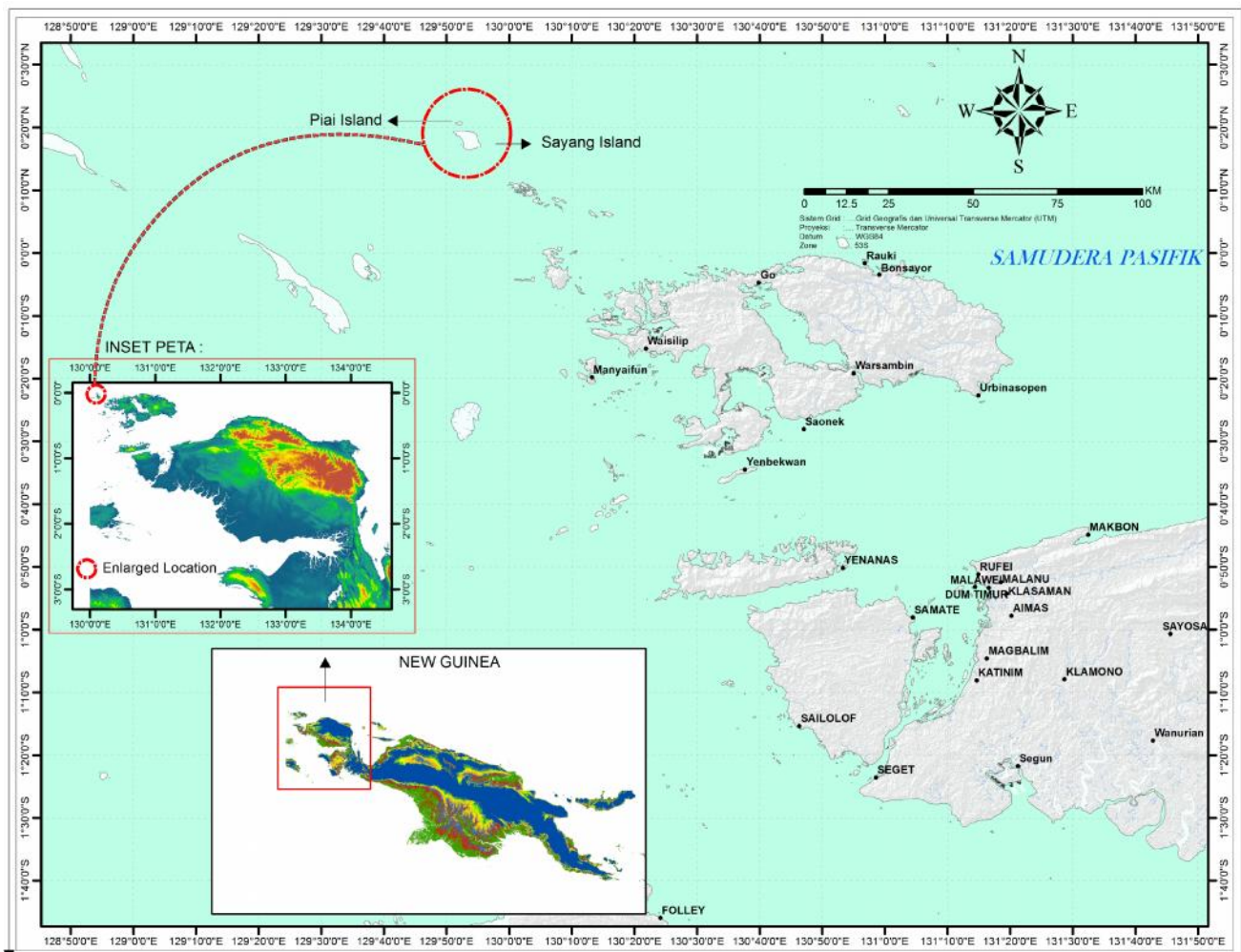

Figure 1. Map of study area in the Piai and Sayang Islands, Raja Ampat, West Papua, Indonesia
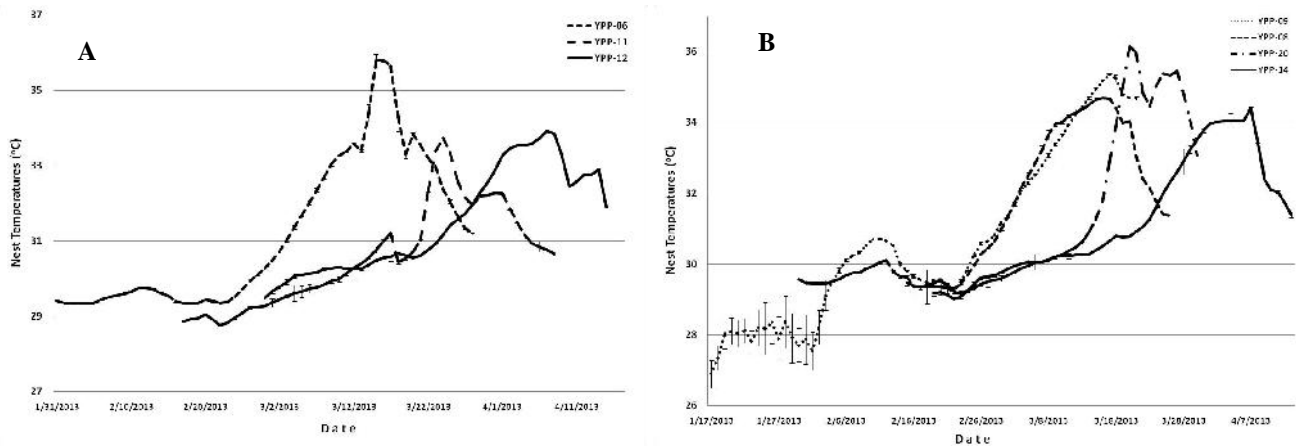
Figure 2. Nest temperature traces. A. Nests constructed in the open beach section. B. Nests constructed close and or under vegetation zones.
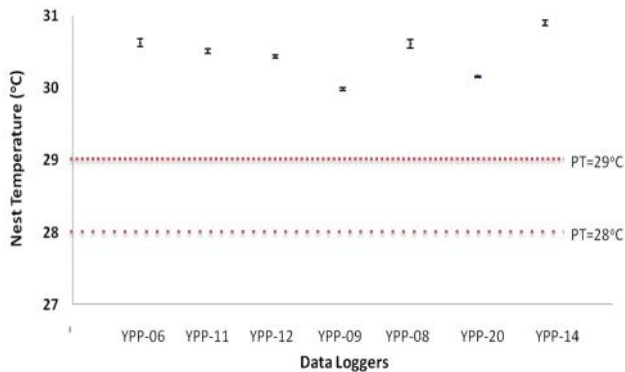

Figure 3. Nests temperatures (Mean±SE) during middle third of development in nests that were monitored with dataloggers at open and vegetation zones at Piai and Sayang Islands.

Nesting females were located during the night and the nests were excavated after nesting had finished. Eggs were removed and counted and a temperature data logger was positioned in the centre of the egg mass during egg replacement. The nest was reburied and the eggs incubated naturally without further interference. Temperature data loggers were used to record incubation temperatures during incubation period of in situ nests. Three nests and four nests were opportunistically selected from open beach zones and under/close to vegetation zones respectively for data logger placement. Nests were excavated after hatching when hatchlings hatched from eggs and dug upwards to retrieve the data logger. The data loggers were programmed in the laboratory to record temperature every hour. The hour measurements were averaged over 24h to obtain a single daily temperature.

Nest temperature traces from the time of oviposition to the time of emergence were used to model the thermal reaction norm of embryonic development using the R package, embryogrowth (5.2, http://cran.r-project.org) (Girondot and Kaska 2014). Hatchling sex ratios were predicted from incubation temperature, knowing that incubation at a constant temperature of $26^{o}C$ produces 100% males, incubation at a constant temperature of $29^{o}C$ produces 100% females, and assuming a linear transition from all males to all females within this temperature range (Booth and Astill 2001; Miller and Limpus 1981). Further, to evaluate the effect of nest temperature on hatchling sex ratio accurately, the mean nest temperature during the middle third of incubation was calculated for each nest. The middle third of development for each nest was estimated from the model of embryonic growth to determine the TSP duration. Hatchling sex ratio was estimated by the mean temperature, weighted by embryonic growth, during the middle third of development for each nest. The mean nest temperature during the middle third in each nest was compared to available pivotal temperature for western Pacific green turtle population. Previous studies indicate that mean nest temperature during the middle third of incubation represents an accurate method for predicting sex ratio in nest that do not experience large daily fluctuations in temperature (Georges et al. 2004; Georges et al. 1994). No direct validation of the sex predictions at this reporting period was made because this would have involved sacrificing the hatchlings for dissection to determine their sex.

RESULTS AND DISCUSSION

Results

The profiles of nest temperatures could be divided into three distinct phases: early, heating, and cooling (Figure 2). During the January-April period, nest temperatures at nests laid both open and vegetation zones are likely to track the sand temperature at 60 cm for the early portion of incubation and then increased to be $2\text{-}4^{o}C$ above sand temperature at middle portion (Figure 2A-B). The climb in nest temperatures was relatively smooth with temperature fluctuations within any given week being less than $0.9^{o}C$.

Fluctuations in nest temperatures over a period of a few days probably due to rainfall events. Nest temperature increased slightly so that at the time of middle incubation period, nest temperatures were 2-4°C distinctively warmer than sand temperature. An increase in nest temperature above surrounding sand temperature during middle portion of incubation is commonly reported in sea turtle nests (Bustard 1972; Booth and Astill 2001; Broderick et al. 2001; Godley et al. 2002), and is caused by the metabolic heat produced by developing embryos which increases greatly during the final stages of incubation.

The patterns of nest temperature profiles early nesting season 2013 were consistent across two monitoring zones. Nest temperatures for nests laid at open beach zones are relatively similar with zones close to vegetation with the exception of Nest with YPP-09 datalogger. During the early phase, nest temperatures of this nest tracked the beach temperatures at 27°C while majority of nests initiated at 29°C. Furthermore, mean nest temperatures recorded in the current study were typically above the pivotal temperature (PT, Figure 3) that had been reported for green turtles in Costa Rica, 28°C (Morreale et al. 1982), in Mediteranean, 29°C (Kaska et al. 1998), and tend to cluster at 29°C (Mrosovsky 1988) in all sea turtles.

An extreme female-biased sex ratio was predicted by the mean temperature during the middle third of development regardless of whether temperatures were weighted by embryonic growth or time. However, the mean temperature of nests during the TSP weighted by embryonic growth was higher ($\overline{X}$ = 32.14°C±0.69) than when weighted by time ($\overline{X}$ = 31.71°C±0.59). In addition, a female-biased sex ratio was also predicted for all but one nest when TSP length in this study was compred to TSP length where hatchling sex ratios were known (Miller and Limpus 1981). It is possible that a few intersex individuals may have been produced in these nests (Miller and Limpus 1981); however, the overall sex ratio would remain strongly female biased.

Discussion

It has been suggested that numerous factors affect nest temperatures (Binckley et al. 1998). These vary from large scale annual and seasonal differences in climate (patterns of precipitation and air temperature) during nesting seasons to mesoscale factors such as nest placement on a beach (sun versus shade, distance to high tide) or sand color (black versus white) down to factors at the individual nest such as depth, egg position (bottom versus top) and metabolic heat generated by developing embryos (Godfrey et al. 1997; Binckley et al. 1998; Mickelson and Downie 2010).

Sexual differentiation in sea turtles is strongly influenced by ambient incubation temperature or TSD (Standora and Spotila 1985; Mrosovsky 1994). Specifically, the sustained temperature to which the embryo is exposed during the middle trimester of incubation determines the eventual gonadal differentiation and sex of the hatchling (Wibbels 2003). The pivotal temperature may vary with species and locale. The pivotal

temperatures for green turtles have been estimated to cluster at 28°C in Costa Rica (Morreale et al. 1982) and at 29°C in Mediterranean (Kaska et al. 1998). The simple method predicted that all successfully monitored nests during the January-April 2013 period should produce female hatchlings,even in a nest (YPP-20) that initiated incubation temperature at 27°C. The overall prediction from monitored nests was an extreme female bias. In addition, all nests monitored with dataloggers, mean temperatures were above the pivotal temperature (Figure 3) suggesting female biased sex ratios. Thus, collectively these results support the hypothesis that female-biased hatchling sex ratios may predominate on the green turtles nesting beaches of Piai and Sayang Islands. This female biased hatching ratio is similar with reports of green turtle nests on Heron Island (Limpus et al. 1983; Booth and Astill 2001), but the bias found in this current study is more extreme than reported in Heron Island. This difference might be attributed to a warmer than average nesting season in 2002-2003on Heron island. There is a trend for a female hatchling bias from sea turtle rookeries worldwide (Spotila et al. 1987; Mrosovsky 1994; Broderick et al. 2001; Godley et al. 2001, 2002). The evolutionary reason(s) for the apparent strong female hatchling bias in most sea turtle rookeries remain unexplained (Mrosovsky1994).

In an evolutionary context, a female visiting a particular rookery has the potential to influence the sex ratio of her offspring by varying her nest timing, nesting beach and nest depth. Given the importance of nest temperature in determining hatchling attributes, a prediction might be that once ashore females use cues to choose nest sites of optimal thermal characteristics. Studies investigating the role of thermal cues in nest site choice have not been done for green turtles from Piai and Sayang, but green turtles nesting at Tortugero, Costa Rica do not actively select nest sites based on temperature (Bjorndal and Bolten 1992).

Global climate change (IPCC 2013) could have a significant impact on reptiles with TSD (Janzen 1994; Mitchell and Janzen 2010) including sea turtles (Hawkes et al. 2007; Chaloupka et al. 2008; Poloczanska et al. 2009; Fuentes et al. 2009, 2010; Hays et al. 2010; Witt et al. 2010; Patino-Martinez et al. 2012). It has been suggested that increases in sand temperature associated with climate change will affect both leatherback sex ratios (Binckley et al. 1998; Hulin et al. 2009; Patino-Martinez et al. 2012) and hatchling fitness (Mickelson and Downie 2010). These changes are predicted to increase the proportion of female leatherback hatchlings and reduce hatching success and fitness primarily at sites already experiencing biases toward female-producing temperatures (Binckley et al. 1998; Mickelson and Downie 2010; Patino-Martinez et al. 2012). On Piai and Sayang islands, nest temperatures may indicate the production of a range of sex ratios but with an overall female bias. This could potentially pose a problem if future temperatures increase due to global climate change. Considering that green sea turtles on these islands are already producing a female biased hatchling sex ratio and that some nest temperatures reach as high as 35°C, the projected increases (IPCC 2013) could result in extreme female biases and potentially impact hatching success.

This initial study suggested that the potential of hatchling sex ratio produced on Piai and Sayang is female-biased and the finding adds to the general paradigm emerging for sea turtles that female biases are prevalent (Mrosovsky 1994; Wibbels 2003). There are several implications for the conservation of the western Pacific sea turtle population at Bird's Head since this population has significantly declined due to over-harvesting (Hitipeuw 2003). It is plausible that possessing TSD and producing female-biased sex ratios from Piai and Sayang islands could be advantageous to the recovery of the Raja Ampat green sea turtle population. It has been proposed that biased sex ratios could significantly impact the recovery of sea turtle populations in both positive and negative ways (Vogt 1994; Mrosovsky and Godfrey 1995; Wibbels 2003; Coyne et al. 2007; Wibbels 2007). Coyne (2007) provides a model showing that female-biased sex ratios could potentially increase recovery rates, since increasing the number of females will increase the egg (and therefore juvenile) production in future years. As an example, the recovery rate of the Kemp's ridley turtle may have been accelerated by the artificial skewing of sex ratios (Wibbels 2007). However, these projections are based upon the assumption that lack of males does not become a limiting factor as the proportion of females increases.

The concept of manipulating sex ratios has drawn some controversies (Vogt 1994; Mrosovsky and Godfrey 1995). In particular the impact of biased sex ratios on the reproductive ecology of sea turtles is not well understood and it is plausible that extreme biases could have a negative impact (Mrosovsky and Godfrey 1995). Extreme biases could potentially affect reproduction in regards to fertility, probability of nesting, and multiple paternity (Wood and Wood 1980; Harry and Briscoe 1988; Chan and Liew 1991). It is plausible that an extreme female bias could lead to the Allee effect due to reduction in number of males. For example, it has been suggested that low hatch rates of leatherback in Malaysia could have related to insufficient number of males to fertilize clutches (Chan and Liew 1991). However, it has been suggested that the Allee effect may not impede the recovery of relatively small population of sea turtles (Hays 2004).

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