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Factors affecting green turtle (*Chelonia mydas*) hatching success, and their temporal variation over 7 study years

UNIVERSIDADE DO ALGARVE

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Mestrado em Biologia Marinha

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Abstract

Sea turtles are considered one of the most important key species in marine ecosystems, leading top-down regulations in food-webs, transferring nutrients and functioning as sentinel-species for monitoring effects of climate change. However, heavy exploitation over years due to the trade and consumption of turtle meat and their eggs, and the challenges faced by climate change, have resulted in a concerning global population decline of sea turtles. On Playa Norte, Costa Rica, in the surrounding area of Tortuguero, which gives nesting habitat to the largest green turtle (*Chelonia mydas*) population of the Atlantic Ocean, the main threats for turtle eggs and hatching success are poaching, predation by dogs, erosion and flooding, overheating and microorganisms. This study examined if the relative impact of each cause changes within a nesting season, and if occurrences have a constant trend over a study period of 7 years. A local study of the seasonality of each impact could endorse the development of effective conservation actions and an adjusted management plan that can be applied strategically to each cause expected at a certain time of the year. Hatching success averaged 38.65% for the 7 study years and was not significantly different among years, nor between climatic periods within a nesting season. Primary reasons for egg loss were “poaching” (24.72%) and “dog predation” (18.11%), followed by natural predation (3.81%), temperature (3.59%), flooding (3.51%), moisture/wet (3.22%), erosion (2.89%) and maternal effects (1.50%). The impact of causes of egg loss were largely constant over the study period. Factors related to climate and weather generally displayed high fatality but a low number of occurrences. On the other hand, anthropogenic factors accounted for the highest turtle egg mortality aggregating both impact and number of events. In terms of seasonality, not all of the causes displayed significant differences between periods, however all causes reached their highest impact in period 3. This suggests that the focus of conservation actions on Playa Norte should be put on the months between September and December, with particular emphasis on anthropogenic factors rather than environmental stressors. An intensification of beach patrol in this period, and nest reburial into an enclosed hatchery are recommended to increase hatching success and ensure recruitment for this green turtle population.

keywords: causes for egg loss, Costa Rica – Playa Norte, conservation management

Resumo

A importância ecológica das tartarugas marinhas tem sido realçada ao longo do tempo, desempenhando um papel significativo na função e estrutura dos ecossistemas (Pace et al. 2019, Sydeman et al. 2015). As tartarugas marinhas têm um papel importante na transferência de nutrientes e ligação entre locais ricos em alimento e praias de nidificação pobres em nutrientes, através das suas rotas migratórias (Bouchard & Bjorndal 2000), assim como reguladores top-down nas teias tróficas marinhas (Bjorndal & Jackson 2002). Devido à sensibilidade das tartarugas marinhas às alterações ambientais, são também consideradas uma das mais importantes espécies sentinela da mega-fauna para a monitorização das consequências das alterações climáticas nos ecossistemas marinhos (Pace et al. 2019, Aguirre & Lutz 2004, Milton & Lutz 2002).

No entanto, seis das sete espécies de tartarugas marinhas na Terra são categorizadas como vulneráveis a criticamente ameaçadas, com uma tendência populacional global decrescente ou desconhecida (IUCN 2018). As capturas acessórias involuntárias na pesca marinha (Stanford et al. 2020), a caça furtiva de tartarugas nas praias de nidificação (Joseph et al. 2019, Campbell & Lagueux 2005, Fleming 2001), e as consequências da poluição marinha (Aguirre & Lutz 2004) têm contribuído consideravelmente para o declínio das populações.

Além disso, uma série de outros fatores influenciam o desenvolvimento embrionário das crias de tartarugas e podem induzir mortalidade durante a incubação, afetando o sucesso da eclosão e, a longo prazo, o recrutamento de populações de tartarugas (Martín-del-Campo et al. 2021). De facto, o sucesso da eclosão depende das interações de numerosos fatores bióticos e abióticos e varia entre espécies e populações de tartarugas marinhas. A caça furtiva (Stanford et al. 2020, Joseph et al. 2019, Thomas-Walters et al. 2020), os predadores nas praias de nidificação (Ratnaswamy & Warren 1998, Fowler 1979), bem como a temperatura (Pike et al. 2015), a humidade, as marés (Lindborg et al. 2016), as cargas microbianas na areia (Bézy et al. 2015), e os efeitos genéticos/maternos (Perrault et al. 2011), são fatores importantes para o sucesso da eclosão.

Numerosos estudos identificaram efeitos sazonais com correlação negativa de fatores de stress ambiental no sucesso da eclosão, tais como chuvas fortes e precipitação prolongada (Limpus et al. 2020, Rivas et al. 2018), inundações por marés (Carpio Camargo et al. 2020),

ou temperaturas elevadas (Lyons et al. 2022, Bladow & Milton 2019, Booth 2017). No entanto, uma variação sazonal de outros fatores, que incluem a caça furtiva ou a predação, é ainda largamente desconhecida. À luz do conhecimento atual, ainda não foram publicados estudos que consideram não só os fatores de stress ambiental, mas também a sazonalidade das causas biológicas e antropogénicas da perda de ovos, e a sua relação entre si. Além disso, existe uma discordância na literatura, não só a sazonalidade para vários fatores não é clara, mas acontece o mesmo com as tendências anuais de sucesso na eclosão de ovos. De facto, por um lado estudos de longo prazo em Terengganu e Labuan, Malásia (Ghazali & Jamil 2019), ou na ilha de Mnemba, Zanzibar (Dunbar 2011) não encontraram diferenças significativas no sucesso da eclosão ao longo dos anos. Por outro lado, há provas de que o sucesso da eclosão diminuiu significativamente em outras praias de nidificação ao longo dos anos, ou seja, nas Ilhas Marianas do Norte (Summers 2018), na Florida (Lindborg et al. 2016), ou nas Ilhas Galápagos (Zárate et al. 2013). Contudo, os cenários futuros projetam geralmente uma queda global do sucesso da eclosão (Carpio Camargo et al. 2020, Laloë et al. 2017) devido ao aumento da temperatura média global (Pike et al. 2015), e ao aumento da frequência e intensidade de cheias e furacões como consequência das alterações climáticas (Gupta et al. 2019).

Considerando as variações locais a que os ovos de tartaruga marinha estão expostos, é crucial determinar o impacto das ameaças durante toda a época de nidificação e entre anos, pelo menos em algumas praias representativas ou importantes - em termos de conservação da espécie – para a nidificação. Uma avaliação anual e sazonal dos fatores locais de stress para o sucesso da eclosão daria uma compreensão mais ampla das circunstâncias ambientais e antropogénicas numa determinada altura do ano. Isto poderia funcionar como uma referência para medições de preservação e forneceria informação fundamental para estabelecer um plano eficaz de gestão da conservação. A aplicação de ações estratégicas de conservação que foram adaptadas aos fatores locais de perda de ovos, e à sua interação entre si, poderia assegurar a sobrevivência e o recrutamento de populações de tartarugas em risco de eclosão.

O seguinte estudo teve lugar na Playa Norte, localizada na costa das Caraíbas do Norte, da Costa Rica. O local do estudo encontra-se na área circundante de Tortuguero, famoso por ser o habitat de nidificação da maior população de tartarugas verdes (Chelonia mydas) do Oceano Atlântico (Lahanas et al. 1998). As principais causas de perda de ovos de tartarugas marinhas na Playa Norte são a caça furtiva, predação por cães, inundação e erosão, temperatura e cargas microbianas na areia.

O objetivo geral deste estudo é fornecer informações sobre a sazonalidade e as tendências anuais de sucesso da incubação e as principais ameaças para os ovos de tartaruga marinha na Playa Norte, sobre as quais podem ser estabelecidas acções de conservação à medida. Mais em pormenor, os nossos objetivos foram: 1) identificar potenciais diferenças climáticas dentro de uma época de nidificação, e assim definir períodos climáticos distintos, com base na precipitação, temperatura e marés; 2) quantificar o sucesso da eclosão em cada época de nidificação e dentro dos períodos climáticos anteriormente mencionados durante um período de estudo de 7 anos; 3) quantificar a magnitude das causas específicas da perda de ovos (i. e. efeitos maternos, erosão, inundações, humidade, temperatura, predação natural, caça furtiva, predação de cães) em cada um dos anos de estudo, avaliando também a presença de tendências recorrentes; 4) investigar a ocorrência de cada causa de perda de ovos em cada um dos períodos climáticos identificados no ponto 1), avaliando assim a potencial reincidência de causas específicas de perda de ovos em períodos climáticos específicos.

O sucesso da incubação foi em média de 38,65% para os 7 anos de estudo. As principais razões para a perda de ovos foram "caça furtiva" (24,72%) e "predação de cães" (18,11%), seguidas de predação natural (3,81%), temperatura (3,59%), inundações (3,51%), humidade/molhado (3,22%), erosão (2,89%) e efeitos maternos (1,50%). O sucesso da incubação não foi significativamente diferente entre períodos climáticos dentro de uma época de nidificação. As causas da perda de ovos variaram no seu impacto, tendo a maioria das causas tendências constantes ao longo dos anos. A "erosão" e "inundações" causaram o maior número de fatalidades, mas apresentaram o menor número de ocorrências. Por outro lado, "efeitos maternos", "predação natural" e "temperatura" foram das causas mais comuns, com um impacto relativamente baixo. A "predação por via húmida" e a "predação por cães" variavam mais consoante o ano. A "caça furtiva" foi responsável pela maior mortalidade de ovos de tartaruga, somando o impacto e número de eventos. Em termos de sazonalidade, nem todas as causas apresentaram diferenças significativas entre períodos, contudo todas as causas atingiram o seu maior impacto no período 3.

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List of Acronyms

ACTo: Área de Conservación Tortuguero - Tortuguero Conservation Area

COTERC: Canadian Organisation for Tropical Education and Rainforest Conservation

CCL: curved carapace length

CCW: curved carapace width

CCKP: Climate Change Knowledge Portal

ENSO: El Niño Southern Oscillation

GLOSS: Global Sea Level Observing System

IUCN: International Union for Conservation of Nature

MINAE: Ministerio de Ambiente y Energía de Costa Rica - Costa Rican Ministry of Environment and Energy

NCEP: National Centers for Environmental Prediction

NWS: National Weather Service

UHSLC: University of Hawaii Sea Level Center

ERO: eroded; nest suffered from erosion

FLO: flooded; nest found under water due to strong rainfall or floods

HAT: hatching activity; clear hatching activity documented (hatchling tracks, alive hatchlings)

NAT: natural state of the nest

POA: poached; nest has been partially or totally poached

PRE: predated; nest has been partially or totally predated

WET: wet; nest found wet from strong rainfall or floods

I. General introduction

1. Ecology of sea turtles

The ecological importance of sea turtles is known and has been underlined since years (Pace et al. 2019, Sydeman et al. 2015, Bjorndal & Jackson 2002): indeed, this charismatic mega-fauna plays a significant role in ecosystem function and structure, imparting top-down effects on marine foodwebs (Sydeman et al. 2015). A wide range of food is consumed by sea turtles, e.g., the green turtle (*Chelonia mydas*) is one of the few species that grazes on Caribbean seagrasses, the hawksbill turtle (*Eretmochelys imbricata*) is the largest known consumer of sponges, and the leatherback turtle (*Dermochelys coriacea*) feeds primarily on jellyfish. Since these are species that are consumed only on a limited extent by other predators, sea turtles are important top-down regulators of above-mentioned species and are therefore crucial in shaping structure and dynamics of ecosystems (Bjorndal & Jackson 2002).

Further, through their migratory routes, they act as vector for the transfer of nutrients, and as connection between rich foraging grounds and nutrient poor nesting beaches (Bouchard & Bjorndal 2000). Because of the direct responses (e.g. cold stunning, behavioral adaptations to heat, trauma and infection) and indirect responses (e.g. depression of immune system, epizootic tumors of marine turtle fibropapillomatosis, compromised physiology) of sea turtles to environmental changes, they are also considered one of the most important sentinel species of mega-fauna for monitoring consequences of climate change, as well as for assessing the general health of marine ecosystems (Pace et al. 2019, Aguirre & Lutz 2004, Milton & Lutz 2002). Therefore, the conservation of turtle populations is of high interest to evaluate and preserve marine ecosystems, which are amongst the most economically and ecologically valuable systems worldwide (Barbier 2017), and emphasize their management and restoration.

Referring to the International Union for Conservation of Nature’s Red List of Threatened Species (IUCN), however, of the seven marine turtle species on Earth, six are categorized as “vulnerable” to “critically endangered” and exhibit a decreasing or unknown global population trend (IUCN 2018). The primary reason for these declines is the vulnerability of sea turtles to anthropogenic activities in all life stages, from eggs to adults. In fact, the biggest threat is likely the illegal/legal (depending on the countries' policies) harvest of both eggs and turtles from beaches and nesting grounds (Fleming 2001). Additionally, a further increase of the mortality rate results from indirect causes, such as the unintentional bycatch in marine fisheries, habitat degradation of nesting beaches and feeding areas, i.e. deforestation, desertification and conversion of habitat for agriculture and tourism (Stanford et al. 2020). Additional stressors are diseases caused by pollution, bioaccumulation of heavy metals, or the ingestion of biotoxins (Aguirre & Lutz 2004).

On top of that, a number of other factors not directly linked to anthropogenic activities influence the embryonic development of turtle hatchlings and can induce mortality during incubation, affecting hatching success and, on long term, the recruitment for turtle populations (Martín-del-Campo et al. 2021). Indeed, hatching success, defined as the proportion of eggs from which hatchlings emerge in the nest chamber, depends upon the interactions of numerous biotic and abiotic factors and varies among species and populations of sea turtles. Genetics and maternal effects, as well as temperature, moisture, tidal wash-over and predators on nesting beaches are important drivers of hatch success (Ackerman 1997).

The relationships among anthropogenic and natural factors that influence more or less directly the hatching success are pictured in a conceptual model in Figure I-1. This model is based on general, global factors; thus adaptations at local situations in real-life applications should be expected (mainly absence of some specific factors). Each single factor is further discussed in the next section.

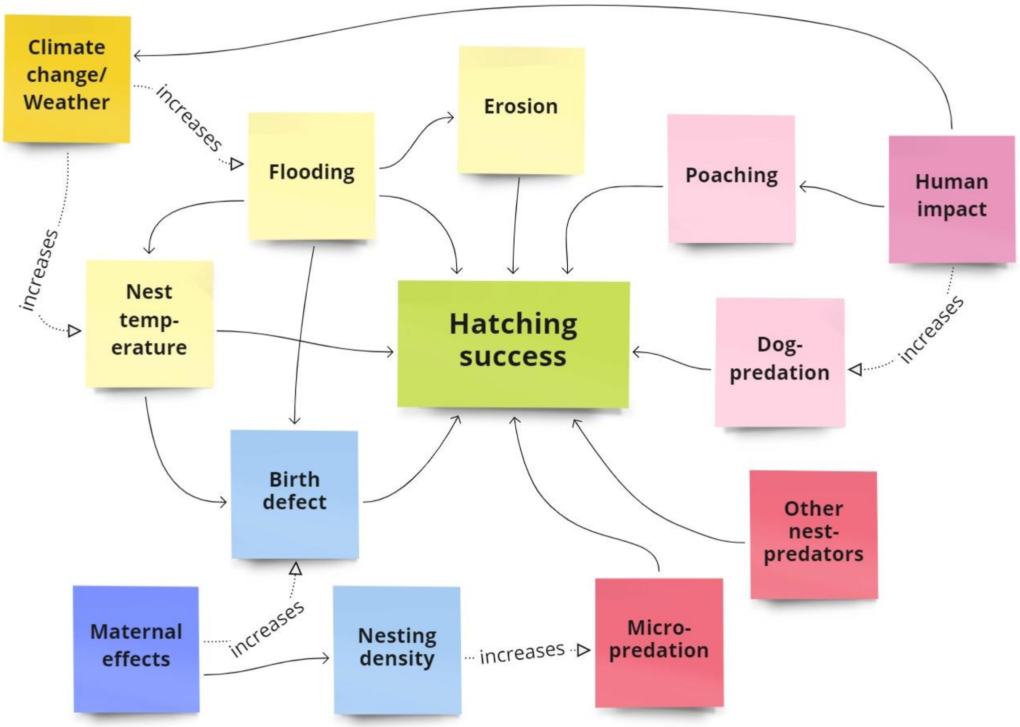


Figure I-1: Factors and influences affecting hatching success. Yellow squares picture factors associated to climate change/meteorological factors, blue squares to maternal effects and genetics, pink squares to anthropogenic influence. Red squares represent natural nest predation occurring due to micro-predation and other nest predators.

2. Causes for egg loss

Sea turtles have been exploited for human consumption for centuries, with both meat and eggs collected as a traditional activity especially among coastal communities all over the world (Mejías-Balsalobre et al. 2021, Veríssimo et al. 2020, Joseph et al. 2019, Koch et al. 2006). In fact, sea turtle meat and eggs are still widely considered a delicacy, so that the hunting of endangered turtles and the collection of their eggs, often illegally, continues in many locations (Stanford et al. 2020, Joseph et al. 2019, Campbell & Lagueux 2005). Turtle poaching activities e.g., reported from Southeast Asia, including dumped carcasses on beaches and seized turtles found on vessels, suggest that there is an ongoing trade of sea turtles from the Coral Triangle to Vietnam and China (Joseph et al. 2019). In Baja California Sur, Mexico, human consumption accounts for 63–91% of the total mortality reported for sea turtles (Koch et al. 2006), and consumer research in Sao Tomè has shown a large-scale demand of turtle meat and eggs in communities, regardless of the local release of policies that criminalise the poaching and trade of sea turtles (Thomas-Walters et al. 2020). Beach patrols and local policies are effective measurements to inhibit the illegal collection of eggs and sea turtles on nesting beaches (Fleming 2001), however, a concerning number of eggs are still taken yearly for human consumption. For example, Santidrián Tomillo et al. (2015) recorded 7% of the nests being poached between 2011–2013 on Playa Cabuyal, Gulf of Papagayo, Northwest Costa Rica, or on Saipan up to one third of nesters were poached between 2006-2016 (Summers et al. 2018).

Nest predators are a major threat for hatchlings and eggs, significantly affecting hatching success all over the world, with carnivore mammals that are considered to be the most important turtle nest predators (Ratnaswamy & Warren 1998). In Turkey, Akayatan beach, for example, at least 75% of turtle nests failed due to predation by foxes and jackals (Brown & MacDonald 1995). In Florida, Jupiter Island, up to 95% of nests were recorded being depredated each year by invasive armadillos and raccoons before predator removal from beaches (Engeman et al. 2005). At Tortuguero in Costa Rica, chief predators traced are dogs, coatis, and black and turkey vultures, destroying 38% of the nests. Dogs, man-introduced predators feeding mostly at night, are considered to be responsible for the greatest nest destruction (Fowler 1979). Generally, nests laid close to the vegetation line are under higher risk of depredation than those on low or mid-beach positions (Brown & MacDonald 1995, Fowler 1979). Additionally, a significant number of turtle nests are predated by ghost crabs (*Ocypode* spp.), considered one of the most important predators on many nesting beaches (Marco et al. 2015). On the Cape Verde Islands for instance, up to 50% of turtle eggs are predated by ghost crabs (Marco et al. 2015), or on Cousine Island, Seychelles, 16,3% of egg losses of hawksbill turtles are attributed to ghost crabs (Hitchins et al. 2004). Besides direct predation of nests by different species, nest predation also presents a threat due to the exposure of nests and therefore making them more vulnerable to unfavorable climatic events (Engeman et al. 2005).

Nest predator removal has been an effective measurement to increase hatching success (Engeman et al 2005). However, since carnivore mammals often also predate on ghost crabs, their reduction can cause a significant increase in ghost crab abundance and therefore an overall increase in turtle nest predation (Marco et al. 2015, Barton & Roth 2008).

This highlights the importance of understanding food web links to assess the relative impact of predators on hatching success.

The rise of global average temperature and associated widespread changes in weather patterns as well as sea level rise are expected to put additional pressure on turtle populations in future (Pike et al. 2015, Fuentes et al. 2010a, Fuentes et al. 2010b). Extreme weather events such as heat waves and large storms are likely to become more frequent or more intense with human-induced climate change (Bindoff et al. 2013). Consequently, the increasing number of storm events and hurricanes will result in more flooding and higher wave run-up. Resulting nest inundations can cause egg mortality from suffocation due to the lack of respiratory oxygen, as well as a result of exposure to higher salinity (Caut et al. 2010). As an additional consequence of nest inundations, major egg mortality is given through complete wash-outs, threats of being uncovered and the exposure of nesting areas more likely to erosion (Fuentes et al. 2010a, Pike & Stiner 2007). Both flooding and erosion can cause an egg mortality of 100% (Witherington et al. 2011). While sea turtles have evolved to actively select nesting sites that are less subject to erosion, probably influenced by the slope of the ground, climate change and shoreline alterations have increased storm intensity, caused sea level rise and changes of beach profiles, making it difficult for females to determine safe nesting locations (Spanier 2010). Despite beaches are dynamic and seasonally changing, as a result of both frequency and intensity of floods rising in recent years, hatching success is decreasing (Carpio Camargo et al. 2020). For example, Lindborg et al. (2016), observed a seasonal change in hatching and emergence success for green turtles as well as Loggerheads, in correlation to the frequency of tropical cyclones in Florida. Green turtle hatching success peaked during the early–middle portion of the nesting season (May and June) and reached its lowest at the end of the nesting season during hurricane season (October-November), with some nests experiencing complete wash-outs and naught hatching success. Therefore, to ensure a higher egg and hatchling survival, nest relocations into safer areas are an important action in terms of species conservation (Wyneken et al. 1988).

The chosen nesting environment such as nest depth and location play a crucial role in the embryonic development, since they determine temperature and humidity inside the nest chamber. The temperature range at which turtle eggs develop is relatively narrow (26°-34°C) (Booth 2017), and even small changes in incubation temperatures can have dramatic effects on hatchling mortality, size and performance within a nest (Bustard & Greenham 1968).

Generally, temperatures in extreme low and high end of the viable incubation range produce poorer hatchlings in quality. Temperatures beyond an upper and lower thermal limit can induce mortality, which can reach 100% (Caut et al. 2010). Moreover, climate change can further amplify the negative effects of temperature. Firstly, as sand temperatures are correlated with air temperatures, due to the global rise of temperatures above a thermal maximum there is an increasing risk of heat mortality. Secondly, the climate change impacts rain patterns, with an intensification of rainfalls and so humidity of nesting beaches. The subsidiary water is likely to cool down incubation temperatures under a critical thermal minimum and therefore increases mortality (Laloë et al. 2017). In a study conducted by Bladow and Milton (2019), results have shown that mortality due to hyperthermia was expressed especially in the embryonic stages 29-30 on Miller scale, meaning that embryos near the end of development appear to be particularly vulnerable to thermal stress. In order to mitigate the impacts of high temperatures, some management strategies have been proposed, such as the use of shade structures and sprinkling water on nesting hatcheries; however, it is necessary to determine the amount of shading and/or water sprinkling necessary for specific natural beach conditions (Martín-del-Campo et al. 2021).

Additionally, a significant factor of hatching mortality are high microbial loads in the sand (Bézy et al. 2015). This applies especially to high density nesting beaches, where overlapped nesting and nest destruction by other turtles causes eggs to break, resulting into high microbial abundance in the sand from the decomposition of broken eggs (Wyneken et al. 1988, Clusella Trullas & Paladino 2007). The decomposition of organic matter through microbes leads to deprivation of oxygen and higher temperatures in the nest, thereby higher embryonic mortality. Fungi seem to play a larger role than bacteria in altering the nest environment and influencing hatching success (Bézy et al. 2015). However, not only the abundance of microbes in the sand are affecting hatching success, but bacterial infections in egg contents are also known to correlate with lower nest success and a higher number of unhatched eggs (Candan & Candan 2020). As organic matter builds up during the nesting season, since the abundance of nests increases, also microbial loads increase, contributing to major egg mortality during high nesting season (Bézy et al. 2015).

Finally, also genetics and maternal effects play a determining role in hatching survival success. Not only they determine egg size and turtle size, but genetic factors such as inbreeding, mutations or chromosomal aberrations can also lead to congenital malformations, abnormalities and eventually to death (Cheng et al. 2008, Perrault et al. 2011). Further, the transfer of diseases and accumulated metals from mother to offspring may contribute to developmental malformations and therefore to low hatching and emergence success (Perrault et al. 2011). A broad study conducted in the Mexican-Caribbean, on green turtle and hawksbill embryos, and in the Pacific-Caribbean, on olive ridley embryos, assessed a 0,2-2% rate of congenital malformations (Martín-del-Campo et al. 2021).

3. Study site

Costa Rica is located in the central or isthmic part of the American Continent, with 50,900 square kilometres of surface. It is confined by Nicaragua in the North and Panama in the Southeast, and by the Caribbean Sea to the East and the Pacific Ocean to the West. The topography of Costa Rica is very varied, with mountains and valleys that cover the small area of the country. Mainly there are two mountain chains that run through the central part of the country longitudinally; the Northern Volcanic Mountain Range and the Talamanca Mountain Range, located to the South. The existence of these two mountain ranges, together with the prevailing winds from the Northeast (trade winds), have framed three climatic regions; the Tropical Humid Atlantic Region, in the North and Atlantic Region, the Central Intermontana Region, with Central Intermontane Valley and South Mountains, and the Tropical Pacific Region, which prevails in the North, South and Central Pacific Regions.

The Caño Palma Biological Station, operated by the Canadian Organization for Tropical Education and Rainforest Conservation (COTERC), is located in the Tropical Humid Atlantic Region on the Northern Caribbean coast of Costa Rica. The climate in this region is denoted as type “Af” in the Köppen-Geiger climate classification. This “wet equatorial climate” is characterized by consistently high temperatures (around 30 °C), high humidity and plentiful precipitation (150–1,000 cm) throughout the year (Arnfield 2020). However, there are two relatively dry periods with less rainy months where rainfalls remain between 100 and 200 mm. The first between February and March and the second between September and October. There are two rainy periods interspersed between the dry ones. The first runs from November to January and is the maximum rainy season. The second runs from May to August and is characterized by a maximum in July that coincides with the summer of the Pacific. The wettest month is December, which is influenced by the effects of cold fronts from the Northern Hemisphere, which occur between November and May, but with a greater possibility of impact between November and March. Rains generally occur at night and in the morning (IMN, 2001).

The data collection operated by Caño Palma Biological Station takes place on Playa Norte, a 5,22 km beach stretch North from Tortuguero National Park (Figure I-2).

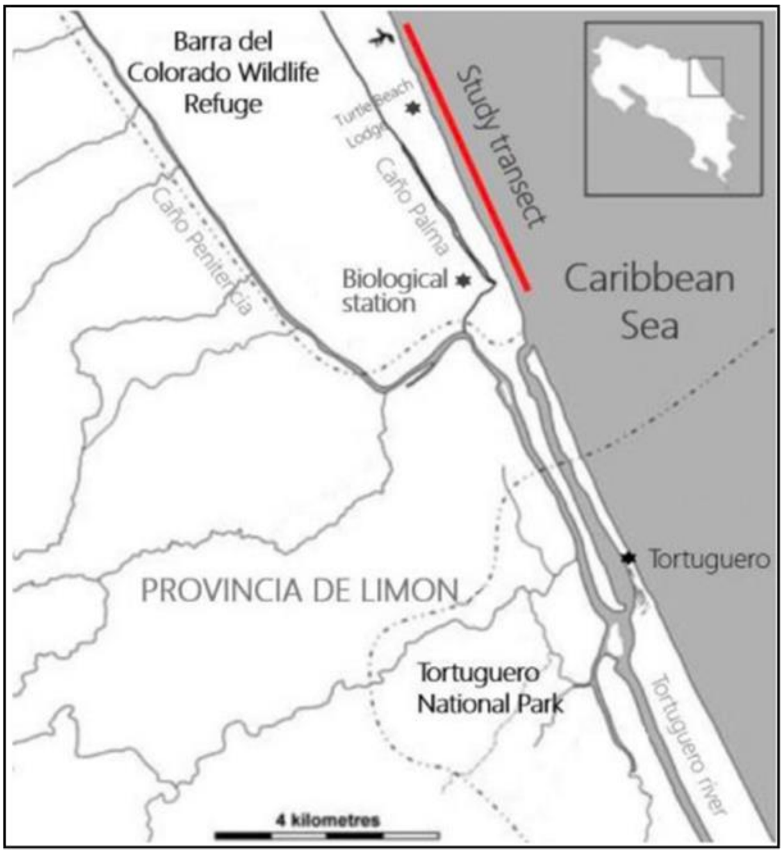


Figure I-2: Localisation of the study site Playa Norte. The beach transect studied is symbolized with a red trait (Allison, 2018).

The area around Tortuguero gives nesting habitat to the largest green turtle population of the Atlantic Ocean (Lahanas et al. 1998), a population which has been signed by heavy exploitation for human consumption since the foundation of the village Tortuguero (Bjorndal et al. 1999). Intensive studies by Archie Carr since 1954 have started the first conservation actions in the area, where the Tortuguero National Park was established in 1975 (Jacobson & Lopez 1994). Since then, actions from local NGO's and the reinforcement of conservation policies have shown positive responses with a local upward green turtle population trend (Bjorndal et al. 1999). In fact, Troëng & Rankin (2005) reported an increase in nesting of 417% in the study period 1971–2003. However, conservation management needs to be continued to increase the population stability of sea turtles, particularly in neighbouring beaches such as Playa Norte which share metapopulations but receive less tourism and conservation attention. In fact, despite years of conservation of sea turtles in the area, the illegal turtle hunting and theft of eggs are still ongoing activities, especially since Playa Norte is located outside the Tortuguero National Park (Mejías-Balsalobre et al. 2021). Additionally, the global challenges stemming from the changing climate still represent a high risk for all turtle populations, as mentioned in the former sub-chapter. With respect to climate change, also the nesting date is moving significantly earlier (Weishampel et al. 2010; Mazaris et al. 2009, Pike et al. 2006). Studies on the Galápagos Islands showed that clutches deposited early in the nesting season show generally a lower hatching success than those in mid-/late-season (Zárate et al. 2013).

It is therefore crucial to quantify and determine local and global threats for eggs and the embryonic development of hatchlings, and assess their magnitude through the nesting season. To the best of my knowledge, such study has not been made yet and would give an important scientific contribution, especially on such an important site for sea turtles as it is the surrounding area of Tortuguero. A local study of the impact of threats throughout the nesting season and on each beach is fundamental to establish an effective conservation management plan and apply strategical conservation actions to reinsure hatching survival success and recruitment for endangered turtle populations. The main threats on Playa Norte are poaching, predation by dogs, erosion and flooding, overheating and microorganisms present in the sand.

References

Ackerman, R. A. (1997). The nest environment and the embryonic development of sea turtles. The Biology of Sea Turtles, Volume I, 83–106.

Aguirre, A. A., & Lutz, P. L. (2004). Marine turtles as sentinels of ecosystem health: is fibropapillomatosis an indicator?. EcoHealth, 1(3), 275-283.

Allison N. 2018. Marine Turtle Monitoring and Tagging Program Green Season Report 2018. Caño Palma Biological Station. Canadian Organisation for Tropical Education and Rainforest Conservation, Playa Norte, Costa Rica.

Arnfield, A. John (2020). *Köppen climate classification*. *Encyclopedia Britannica*. Available online: https://www.britannica.com/science/Koppen-climate-classification (accessed on 26 September 2022)

Barbier, E. B. (2017). Marine ecosystem services. Current Biology, 27(11), R507–R510.

Barton, B. T., & Roth, J. D. (2008). Implications of intraguild predation for sea turtle nest protection. Biological Conservation, 141(8), 2139–2145.

Bézy, V. S., Valverde, R. A., & Plante, C. J. (2015). Olive ridley sea turtle hatching success as a function of the microbial abundance in nest sand at Ostional, Costa Rica. PloS one, 10(2), e0118579.

Bindoff, N. L., Stott, P. A., AchutaRao, K. M., Allen, M. R., Gillett, N., Gutzler, D., Hansingo, K., Hegerl, G., Hu, Y., Jain, S., Mokhov, I. I., Overland, J., Perlwitz, J., Sebbari, R., & Zhang, X. (2013). Chapter 10 - Detection and attribution of climate change: From global to regional. In: Climate Change 2013: The Physical Science Basis. IPCC Working Group I Contribution to AR5. Cambridge: Cambridge University Press.

Bjorndal, K. A., & Jackson, J. B. (2002). 10 Roles of sea turtles in marine ecosystems: reconstructing the past. The biology of sea turtles, 2, 259.

Bjorndal, K. A., Wetherall, J. A., Bolten, A. B., & Mortimer, J. A. (1999). Twenty-six years of green turtle nesting at Tortuguero, Costa Rica: an encouraging trend. Conservation Biology, 13(1), 126-134.

Bladow, R. A., & Milton, S. L. (2019). Embryonic mortality in green (Chelonia mydas) and loggerhead (Caretta caretta) sea turtle nests increases with cumulative exposure to elevated temperatures. Journal of Experimental Marine Biology and Ecology, 518, 151180.

Booth, D. T. (2017). Influence of incubation temperature on sea turtle hatchling quality. Integrative Zoology, 12(5), 352-360.

Bouchard, S. S., & Bjorndal, K. A. (2000). Sea turtles as biological transporters of nutrients and energy from marine to terrestrial ecosystems. Ecology, 81(8), 2305-2313.

Brown, L., & Macdonald, D. W. (1995). Predation on green turtle Chelonia mydas nests by wild canids at Akyatan Beach, Turkey. Biological Conservation, 71(1), 55-60.

Bustard, H. R., & Greenham, P. G. (1968). Physical and chemical factors affecting hatching in the green sea turtle, Chelonia mydas (L.). Ecology,49, 269–276.

Campbell, C. L., & Lagueux, C. J. (2005). Survival probability estimates for large juvenile and adult green turtles (Chelonia mydas) exposed to an artisanal marine turtle fishery in the western Caribbean. Herpetologica, 61(2), 91-103.

Candan, O., & Candan, E. D. (2020). Bacterial diversity of the green turtle (Chelonia mydas) nest environment. Science of The Total Environment, 720, 137717.

Carpio Camargo, A. J., Álvarez Gutiérrez, Y., Jaramillo Véliz, J., & Sánchez Tortosa, F. (2020). Nesting failure of sea turtles in Ecuador-causes of the loss of sea turtle nests: the role of the tide. Journal of Coastal Conservation, 24(5), 1-10.

Caut, S., Guirlet, E., & Girondot, M. (2010). Effect of tidal overwash on the embryonic development of leatherback turtles in French Guiana. Marine Environmental Research, 69(4), 254-261.

Cheng, I. J., Dutton, P. H., Chen, C. L., Chen, H. C., Chen, Y. H., & Shea, J. W. (2008). Comparison of the genetics and nesting ecology of two green turtle rookeries. Journal of Zoology, 276(4), 375-384.

Clusella Trullas, S., & Paladino, F. V. (2007). Micro-environment of olive ridley turtle nests deposited during an aggregated nesting event. Journal of Zoology, 272(4), 367-376.

Engeman, R. M., Martin, R. E., Smith, H. T., Woolard, J., Crady, C. K., Shwiff, S. A., Constantin, B., Stahl, M., & Griner, J. (2005). Dramatic reduction in predation on marine turtle nests through improved predator monitoring and management. Oryx, 39(3), 318-326.

Fleming, E.H. (2001). Swimming against the tide: Recent Surveys of Exploitation, Trade, and Management of Marine Turtles in the Northern Caribbean. Report for TRAFFIC, North America.

Fowler, L. E. (1979). Hatching success and nest predation in the green sea turtle, Chelonia mydas, at Tortuguero, Costa Rica. Ecology, 60(5), 946-955.

Fuentes, M. M. P. B., Hamann, M., & Limpus, C. J. (2010a). Past, current and future thermal profiles of green turtle nesting grounds: Implications from climate change. Journal of Experimental Marine Biology and Ecology, 383(1), 56-64.

Fuentes, M. M. P. B., Limpus, C. J., Hamann, M., & Dawson, J. (2010b). Potential impacts of projected sea-level rise on sea turtle rookeries. Aquatic conservation: marine and freshwater ecosystems, 20(2), 132-139.

Hitchins, P. M., Bourquin, O., & Hitchins, S. (2004). Nesting success of hawksbill turtles (Eretmochelys imbricata) on Cousine Island, Seychelles. Journal of Zoology, 264(4), 383-389.

IMN (Instituto Meteorológico Nacional). 2001. Escenarios de cambio climático para Costa Rica. PrimeraComunicación Nacional para UNFCCC. Instituto Meteorológico Nacional, Ministerio del Ambiente y Energía. SanJosé, Costa Rica. Sp.

Jacobson, S. K., & Lopez, A. F. (1994). Biological impacts of ecotourism: tourists and nesting turtles in Tortuguero National Park, Costa Rica. Wildlife Society Bulletin, 414-419.

Joseph, J., Nishizawa, H., Alin, J. M., Othman, R., Jolis, G., Isnain, I. and Nais, J. (2019). Mass sea turtle slaughter at Pulau Tiga, Malaysia: Genetic studies indicate poaching locations and its potential effects. Global Ecology and Conservation, Vol 17.

Koch, V., Nichols, W. J., Peckham, H., & de la Toba, V. (2006). Estimates of sea turtle mortality from poaching and bycatch in Bahía Magdalena, Baja California Sur, Mexico. Biological Conservation, 128(3), 327–334.

Lahanas, P. N., Bjorndal, K. A., Bolten, A. B., Encalada, S. E., Miyamoto, M. M., Valverde, R. A., & Bowen, B. W. (1998). Genetic composition of a green turtle (Chelonia mydas) feeding ground population: evidence for multiple origins. Marine Biology, 130(3), 345-352.

Laloë, J. O., Cozens, J., Renom, B., Taxonera, A., & Hays, G. C. (2017). Climate change and temperature-linked hatchling mortality at a globally important sea turtle nesting site. Global change biology, 23(11), 4922-4931.

Lindborg, R., Neidhardt, E., Witherington, B., Smith, J. R., & Savage, A. (2016). Factors influencing loggerhead (Caretta caretta) and green turtle (Chelonia mydas) reproductive success on a mixed use beach in Florida. Chelonian Conservation and Biology, 15(2), 238-248.

Marco, A., da Graça, J., García-Cerdá, R., Abella, E., & Freitas, R. (2015). Patterns and intensity of ghost crab predation on the nests of an important endangered loggerhead turtle population. Journal of Experimental Marine Biology and Ecology, 468, 74-82.

Martín-del-Campo, R., Calderón-Campuzano, M. F., Rojas-Lleonart, I., Briseño-Dueñas, R., & García-Gasca, A. (2021). Congenital Malformations in Sea Turtles: Puzzling Interplay between Genes and Environment. Animals, 11(2), 444.

Mazaris, A. D., Kallimanis, A. S., Tzanopoulos, J., Sgardelis, S. P., & Pantis, J. D. (2009). Sea surface temperature variations in core foraging grounds drive nesting trends and phenology of loggerhead turtles in the Mediterranean Sea. Journal of Experimental Marine Biology and Ecology, 379(1-2), 23-27.

Mejías-Balsalobre, C., Restrepo, J., Borges, G., García, R., Rojas-Cañizales, D., Barrios-Garrido, H., & Valverde, R. A. (2021). Local community perceptions of sea turtle egg use in Tortuguero, Costa Rica. Ocean & Coastal Management, 201, 105423.

Milton, S. L., & Lutz, P. L. (2002). Physiological and Genetic Responses to Environmental Stress. The Biology of Sea Turtles, Volume II, 163–197.

Pace, A., Dipineto, L., Fioretti, A., & Hochscheid, S. (2019). Loggerhead sea turtles as sentinels in the western Mediterranean: antibiotic resistance and environment-related modifications of Gram-negative bacteria. Marine Pollution Bulletin, 149, 110575.

Perrault, J., Wyneken, J., Thompson, L. J., Johnson, C., Miller, D. L. (2011). Why are hatching and emergence success low? Mercury and selenium concentrations in nesting leatherback sea turtles (Dermochelys coriacea) and their young in Florida. Mar. Pollut. Bull. 2011, 62, 1671–1682.

Pike, D. A., Antworth, R. L., & Stiner, J. C. (2006). Earlier nesting contributes to shorter nesting seasons for the loggerhead seaturtle, Caretta caretta. Journal of Herpetology, 91-94.

Pike, D. A., Roznik, E. A., & Bell, I. (2015). Nest inundation from sea-level rise threatens sea turtle population viability. Royal Society Open Science, 2(7), 150127.

Pike, D. A., & Stiner, J. C. (2007). Sea turtle species vary in their susceptibility to tropical cyclones. Oecologia, 153(2), 471-478.

Ratnaswamy, M. J., Warren, R. J., (1998). Removing raccoons to protect sea turtle nests: are there implications for ecosystem management? Wildl. Soc. Bull. 26, 846–850.

Santidrián Tomillo, P., Roberts, S. A., Hernández, R., Spotila, J. R., & Paladino, F. V. (2015). Nesting ecology of East Pacific green turtles at Playa Cabuyal, Gulf of Papagayo, Costa Rica. Marine Ecology, 36(3), 506-516..

Spanier, M. J. (2010). Beach erosion and nest site selection by the leatherback sea turtle Dermochelys coriacea (Testudines: Dermochelyidae) and implications for management practices at Playa Gandoca, Costa Rica. Revista de biologia tropical, 58(4), 1237-1246.

Stanford, C. B., Iverson, J. B., Rhodin, A. G., van Dijk, P. P., Mittermeier, R. A., Kuchling, G., Berry, K. H., Bertolero, A., Bjorndal, K. A., Blanck, T. E., Buhlmann, K. A., Burke, R. L., Congdon, J. D., Diagne, T., Edwards, T., Eisemberg, C. C., Ennen, J. R., Forero-Medina, G., ... & Walde, A. D. (2020). Turtles and tortoises are in trouble. Current Biology, 30(12), R721-R735.

Summers, T. M., Martin, S. L., Hapdei, J. R., Ruak, J. K., & Jones, T. T. (2018). Endangered green turtles (Chelonia mydas) of the Northern Mariana Islands: Nesting ecology, poaching, and climate concerns. Frontiers in Marine Science, 4, 428.

Sydeman, W. J., Poloczanska, E., Reed, T. E., & Thompson, S. A. (2015). Climate change and marine vertebrates. Science, 350(6262), 772–777.

Thomas-Walters, L., Vieira, S., Jiménez, V., Monteiro, D., Ferreira, B., Smith, R. J., & Veríssimo, D. (2020). Challenges in the impact evaluation of behaviour change interventions: The case of sea turtle meat and eggs in São Tomé. People and Nature, 2(4), 913-922.

Troëng, S., & Rankin, E. (2005). Long-term conservation efforts contribute to positive green turtle Chelonia mydas nesting trend at Tortuguero, Costa Rica. Biological Conservation, 121(1), 111-116.

Veríssimo, D., Vieira, S., Monteiro, D., Hancock, J., & Nuno, A. (2020). Audience research as a cornerstone of demand management interventions for illegal wildlife products: Demarketing sea turtle meat and eggs. Conservation Science and Practice, 2(3), e164.

Weishampel, J. F., Bagley, D. A., Ehrhart, L. M., & Weishampel, A. C. (2010). Nesting phenologies of two sympatric sea turtle species related to sea surface temperatures. Endangered Species Research, 12(1), 41-47.

Witherington, B., Hirama, S., & Mosier, A. (2011). Sea turtle responses to barriers on their nesting beach. Journal of Experimental Marine Biology and Ecology, 401(1-2), 1–6.

Wyneken, J., Burke, T. J., Salmon, M., & Pedersen, D. K. (1988). Egg failure in natural and relocated sea turtle nests. Journal of Herpetology, 88-96.

Zárate, P., Bjorndal, K. A., Parra, M., Dutton, P. H., Seminoff, J. A., & Bolten, A. B. (2013). Hatching and emergence success in green turtle Chelonia mydas nests in the Galápagos Islands. Aquatic Biology, 19(3), 217-229.

II. Manuscript

Factors affecting green turtle (*Chelonia mydas*) hatching success, and their temporal variation over 7 study years

Plank Karin

Abstract

Sea turtles are considered one of the most important key species in marine ecosystems, leading top-down regulations in food-webs, transferring nutrients and functioning as sentinel-species for monitoring effects of climate change. However, heavy exploitation over years due to the trade and consumption of turtle meat and their eggs, and the challenges faced by climate change, have resulted in a concerning global population decline of sea turtles. On Playa Norte, Costa Rica, in the surrounding area of Tortuguero, which gives nesting habitat to the largest green turtle (*Chelonia mydas*) population of the Atlantic Ocean, the main threats for turtle eggs and hatching success are poaching, predation by dogs, erosion and flooding, overheating and microorganisms. This study examined if the relative impact of each cause changes within a nesting season, and if occurrences have a constant trend over a study period of 7 years. A local study of the seasonality of each impact could endorse the development of effective conservation actions and an adjusted management plan that can be applied strategically to each cause expected at a certain time of the year. Hatching success averaged 38.65% for the 7 study years and was not significantly different among years, nor between climatic periods within a nesting season. Primary reasons for egg loss were "poaching" (24.72%) and "dog predation" (18.11%), followed by natural predation (3.81%), temperature (3.59%), flooding (3.51%), moisture/wet (3.22%), erosion (2.89%) and maternal effects (1.50%). The impact of causes of egg loss were largely constant over the study period. Factors related to climate and weather generally displayed high fatality but a low number of occurrences. On the other hand, anthropogenic factors accounted for the highest turtle egg mortality aggregating both impact and number of events. In terms of seasonality, not all of the causes displayed significant differences between periods, however all causes reached their highest impact in period 3. This suggests that the focus of conservation actions on Playa Norte should be put on the months between September and December, with particular emphasis on anthropogenic factors rather than environmental stressors. An intensification of beach patrol in this period, and nest reburial into an enclosed hatchery are recommended to increase hatching success and ensure recruitment for this green turtle population.

Keywords: causes for egg loss, Costa Rica – Playa Norte, conservation management

Introduction

Green sea turtles (*Chelonia mydas*) have been studied for many years, especially for conservation concern (Summers et al. 2018). Due to the sensitivity of sea turtles to environmental changes (Aguirre & Lutz 2004, Milton & Lutz 2002), they are considered one of the most important sentinel species of mega-fauna for monitoring climate change (Sydeman et al. 2015) and anthropogenic activities (Pace et al. 2019) on marine ecosystems. However, despite international management and local conservation actions, the green turtle is reported as an endangered species in the International Union of Conservation (IUCN) Red List, with a decreasing global population trend. Unintentional bycatch in marine fisheries (Stanford et al. 2020), poaching of turtles on nesting beaches (Joseph et al. 2019, Campbell & Lagueux 2005, Fleming 2001), and consequences of marine pollution (Aguirre & Lutz 2004) have contributed considerably to decline of global populations. Moreover, mortality is extremely high during the egg incubation (Wyneken et al. 1988), induced by the harvest of turtle eggs for human consumption (Summers et al. 2018, Santidrián Tomillo et al. 2015), predation by carnivore mammals (Ratnaswamy & Warren 1998), ghost crabs (*Ocypode* spp.) (Marco et al. 2015, Hitchins et al. 2004), vultures (Fowler 1979), and micropredation (Bézy et al. 2015, Clusella Trullas & Paladino 2007). Additional factors affecting the development of sea turtle embryos are related to environmental stressors (Bladow & Milton 2019), such as flooding and high tide (Caut et al. 2010), erosion (Spanier 2010), and high temperatures (Fuentes et al. 2010a).

These factors greatly vary in space and time, because of the large nesting distribution of sea turtles (Cáceres-Farias et al 2022). Numerous studies have identified seasonal effects with negative correlation of environmental stressors on hatching success, such as heavy rainfalls and prolonged precipitation (Limpus et al. 2020, Rivas et al. 2018), tidal inundation (Carpio Camargo et al. 2020), or elevated temperatures (Lyons et al. 2022, Bladow & Milton 2019, Booth 2017). However, a seasonal variation of other factors, which include poaching or predation, is still widely unknown. To the best of our knowledge, studies that consider not only environmental stressors, but also seasonality in biological and anthropogenic causes of egg loss, and their relationship between each other, have not been published yet. Moreover, not only seasonality for several factors is unclear, but also annual trends in hatching success vary greatly among studies. In fact, on one hand long-term studies in Terengganu and Labuan, Malaysia (Ghazali & Jamil 2019), or Mnemba Island, Zanzibar (Dunbar 2011) have found no significant differences in hatching success over years. On the other hand, hatching success showed a significant decrease on other nesting beaches over years, i.e. on Northern Mariana Islands (Summers 2018), in Florida (Lindborg et al. 2016), or on the Galápagos Islands (Zárate et al. 2013). However, future scenarios generally project a global drop of hatching success (Carpio Camargo et al. 2020, Laloë et al. 2017, Fuentes et al. 2010a) due to the rise of global average temperature (Pike et al. 2015), and increasing frequency and intensity of floods and hurricanes as a consequence of climate change (Gupta et al. 2019).

Considering the local variabilities sea turtle eggs are exposed to, it is crucial to determine impact of threats throughout the nesting season and among years on each nesting beach. Indeed, a thorough understanding of the natural and anthropogenic temporal dynamics governing hatching success are a prominent first step to develop potential tailor-made – in space and time - conservation measures. Thus, the application of strategical conservation actions that have been adapted to local conditioning factors of egg loss, and to their interaction with each other, could reinsure hatching survival and recruitment for endangered turtle populations.

The following study has taken place on Playa Norte, located on the Northern Caribbean coast of Costa Rica. The study site is found in the surrounding area of Tortuguero, known for providing nesting habitat to the largest green turtle (*Chelonia mydas*) population of the Atlantic Ocean (Lahanas et al. 1998). The main causes for egg loss of sea turtles on Playa Norte are poaching, predation by dogs, flooding and erosion, temperature and microbial loads in the sand (Pheasey et al. 2018).

The overall aim of this study was to deliver information about seasonality and year-by-year trends of hatching success and the main threats for sea turtle eggs on Playa Norte, on which specific conservation actions can be established. More in detail, our targets were to 1) given the influence that climatic events may have on the hatching success of the green turtle *Chelonia mydas* (Carpio Camargo et al. 2020, Fuentes et al. 2010a), to identify potential differences in climate within a nesting season, and thus define distinct climatic periods, based on precipitation, temperature and tide; 2) to quantify hatching success in each nesting season and within the previously mentioned climatic periods over a 7-year study period (2014, 2015, 2016, 2017, 2018, 2019, 2021); 3) to quantify the magnitude of specific causes of egg loss (i. e. dog predation, erosion, flooding, maternal effects, moisture, natural predation, poaching, temperature) in each of the study years, while also assessing the presence of recurring trends;

4) to investigate the occurrence of each cause of egg loss in each of the climatic periods identified at point 1), thus assessing the potential reoccurrence of specific causes of egg loss in specific climatic periods.

For the first research question, we expected to identify various distinct climatic periods within a nesting season, with constant differences over the period of the 7-year study. Indeed, the province of Limón in Costa Rica has no identified rainy and dry seasons, but the months from May to August and from November to January are characterized by heavier rainfall than the rest of the year (IMN, 2001). On the contrary, temperature is expected to show a constant trend among the climatic periods and over the years, since the climatic region is characterized by very little annual temperature variation (Arnfield 2020). The same accounts for tide, which in equatorial regions shows only small seasonal changes in the surface tides (Müller et al. 2014). For the second objective, a prediction of annual trends in hatching success could not be made due to the discordance through studies. However, considering natural seasonal trends of hatching success, we expected to find the highest hatching success from August to October. Indeed, clutches deposited early in the nesting season (March-July) generally show a lower hatching success than those in mid-/late-season (July-October) (Zárate et al. 2013). Concerning the third objective, given the paucity of studies on the specific question in the study area, it is difficult to make an explicit prediction. However, some variability of the causes of egg loss among the years may be expected due to climatic variation at different spatial scales (e.g. global as “el Niño”, or more local as flooding of the local river), or due to specific social events and festivities (e.g. independence day, election or sports celebrations when poaching rate is expected to increase in magnitude). In regard to the last goal and the seasonality of threats, we expected causes depending on climate (i.e. erosion, flooding, moisture and temperature) to have a stronger magnitude and occurrence following the results of the first objective. As for “natural predation” and “dog predation”, we assumed to see a specific trend, since nests are usually predated at the end of incubation (Marco et al. 2015, Ali & Ibrahim 2002, Fowler 1979). For the remaining causes a clear prediction can not be done, since they are formally independent from seasonality (Martín-del-Campo et al. 2021, Garcıía et al. 2003).

Materials and methods

1. Study area

Playa Norte is a beach transect of 5.22 km (Datum WGS84 552224.9E 1170322N to Datum WGS84 550043.7E 1175989N) on the North Caribbean coast of Costa Rica (Figure II-1). The area is located north of the village Tortuguero and its Tortuguero National Park, in the province of Limón. Playa Norte is within the Barra del Colorado Wildlife Refuge and subject to the regulation of the Tortuguero Conservation Area (ACTo), and the Costa Rican Ministry of Environment and Energy (MINAE). The established nesting season for green turtles in this area is from June 1st to October 31st (Allison, 2018). However, green turtles have been recorded to nest on Playa Norte as early as March in some nesting seasons.

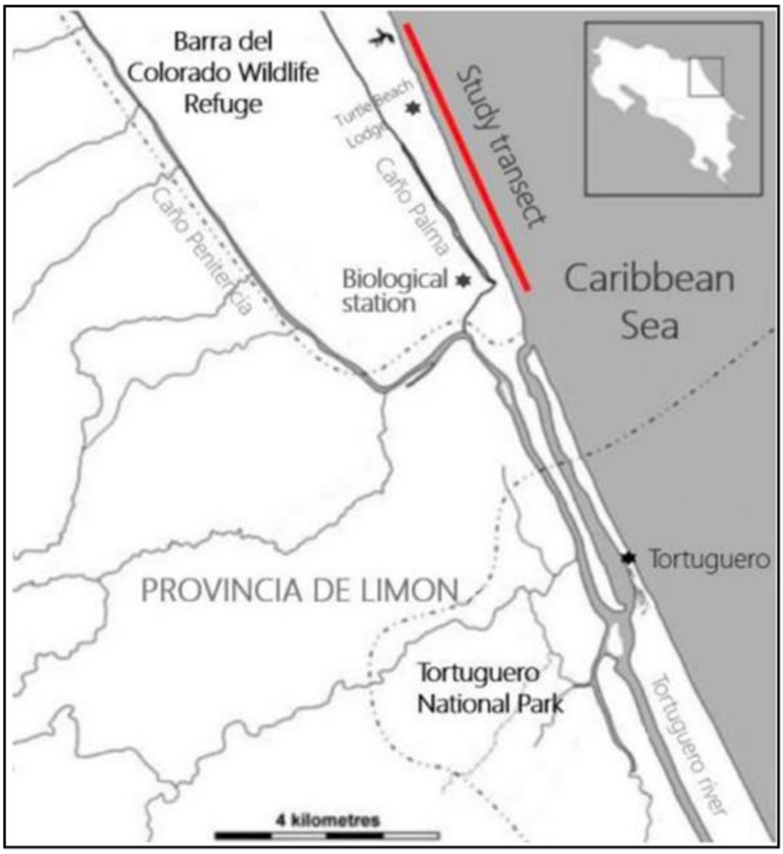


Figure II-1: Localisation of the study site Playa Norte. The beach transect studied is symbolized with a red trait (Allison, 2018).

2. Abiotic data collection

The three climatic variables considered for this study are precipitation (mm), temperature (°C) and tide (cm). Precipitation and temperature were measured on a daily basis by the Caño Palma Biological Station. We attempted to calculate a weekly mean of the two variables for all seven years (from nesting season 2014 to 2021, except for the year 2020). However, the data given by the Research Station were incomplete over long periods in several years. Therefore, monthly means of precipitation and temperature were taken from the Climate Change Knowledge Portal, World Bank Group, for all seven years (Table A 1, A 2, Annex). The data were measured in Limón, the capital city of Limón province, about 37 km from Playa Norte.

Tide data were taken from University of Hawaii Sea Level Center (UHSLC) as part of the Global Sea Level Observing System (GLOSS) (Table A 3, Annex). Research quality data were given from UHSLC station 268 Limón, Costa Rica from 01st January 2014 – 31st December 2018. Further data taken from UHSLC station 268 Limón, Costa Rica were fast delivery data, which, in contrast to research quality data, have not undergone preliminary quality control by Member Nations and are posted within 1-2 months from collection. Fast delivery data covered the period from 01st January 2019 – 18th April 2021. Since there are no further data available from UHSLC station 268 Limón, Costa Rica up to today, research quality data from UHSLC station 739 El Porvenir, Panama (about 450 km distant), were taken to calculate a simple linear regression with research quality data from UHSLC station 268 Limón, Costa Rica. The following formula was calculated for the linear regression between the two datasets:

$$y = 0.8363x - 81.36, \qquad R^2 = 0.6707$$

Thus, missing tide data from 19th April 2021 – 31st December 2021 were estimated through the linear relationship with fast delivery data from UHSLC station 739 El Porvenir, Panama.

3. Biotic data collection

The data collection on Playa Norte took place through Caño Palma Biological Station, operated by COTERC, the Canadian Organisation for Tropical Education and Rainforest Conservation. Data used for this research were collected by volunteers of Caño Palma Biological Station and cover a study period of seven years, from nesting season 2014 until nesting season 2021. Data collection during the nesting season of 2020 was not possible, due to Covid-19 restrictions.

Surveys were conducted in two different shifts, night patrol and morning census. Night patrol was conducted every night since the beginning of March (which usually constitutes the beginning of nesting season) until a no-activity period of two weeks at the end of the nesting season, which usually corresponds to the end of October. Night patrols covered the whole beach transect and were concluded after at least 5 hours of patrol. Laid nests as well as all activities by turtles and humans were recorded. On encounters with turtles, species was determined and, when the animals' behaviour allowed it, turtles were tagged, curved carapace length (CCL) and curved carapace width (CCW) were measured and a body check was performed. Additionally, if the turtle was encountered before or in the process of oviposition, eggs were counted as they were laid and egg-depth assessed. The team supervised the turtles' activity from emergence until re-entering the sea to ensure the turtles' safety from poachers. Laid nests were triangulated, measuring three different distances from marked trees to the nest, to be able to locate egg-chamber further on. Sand was levelled and nest and tracks disguised to cover up the nest and prevent poaching as far as possible. If needed, laid nests in risk areas (e.g. erosion cliff, on high tide line) were relocated into safer locations on the beach. However, the process of nest-relocation was rarely performed, since green turtles on Playa Norte tend to nest close to the vegetation line/in the vegetation rather than on open sand, near the high tide line.

On morning census, the same beach transect was surveyed every morning from the first laid nest in the season, until the last nest hatched at the end of the season, which usually happen at the end of December, or beginning of January. New turtle activities missed by Night patrol were recorded and all present turtle nest states were documented.

The daily evaluation of a nest status throughout the whole incubation period is necessary to indicate reasons that caused egg mortality and why eventual unsuccessful eggs remained undeveloped. The different conditions of the nests were classified as follows:

ERO – eroded; nest suffered from erosion

FLO – flooded; nest found under water due to strong rainfall or floods

HAT – hatching activity; clear hatching activity documented (hatchling tracks, alive hatchlings)

NAT – natural state of the nest

POA – poached; nest has been partially or totally poached

PRE – predated; nest has been partially or totally predated

WET – wet; nest found wet from strong rainfall or floods

In case of a predation by dogs or partial/interrupted poaching activity, scattered empty eggshells were counted and recorded, eventual dead remnants and broken eggs removed from the nest and discarded, and the remaining eggs recovered with sand. Further, excavations were conducted on morning census to determine clutch size (if egg-count was missed at oviposition) and hatching success. An excavation can only be carried out after a nest hatched and hatchlings emerged from egg chamber. Therefore, a nest was excavated at least 2 days after hatching was reported, or 10 days overdue the average incubation period (i.e. 58.5 days for green turtles in Tortuguero, Costa Rica (Pheasey et al. 2018, Tiwari et al. 2006, Fowler 1979)). Egg depth and nest depth were measured and empty eggshells (>50% intact) counted, which determine the number of successful hatched turtle hatchlings. Hatching success was calculated as the number of successful eggs (s) multiplied by 100, divided by the total number of eggs (n):

$$hat.success = \frac{s * 100}{n}$$

Clutch size was determined as the total number of yolked eggs. Yolkless eggs can be clearly distinguished from yolked eggs because of their much smaller size and nonspherical shape (Zárate et al. 2013).

Unhatched eggs were equally counted, opened, and categorized based on the developmental stage of the embryo (Figure II-2). This allowed us to place eggs lost to temperature in the period respectively to the developmental advancement. The classification considered the following stages:

Stage 0: Yolked egg with no embryo

Stage 1: Embryo without any clear developed body parts (embryo occupies <25% of the egg)

Stage 2: Embryo partly developed, head still unproportionally big and great parts of yolk present (embryo occupies 25% - 50% of the egg)

Stage 3: Embryo fully developed with great part of yolk present (Embryo occupies 25% - 50% of the egg)

Stage 4: Embryo fully developed, no yolk (Embryo occupies >75% of the egg)

Figure II-2: From left to right, egg without embryo (stage 0), hatchling stage 1, hatchling stage 2, hatchling stage 3, hatchling stage 4 and hatched turtle found dead.

Further it was determined if eggs were depredated by crabs (identified by a cut in the eggshell and contents appearing “dusty”, sometimes with bones present), by ants (identified by one or several small holes in eggshell, content appears cleaned and “dried out”) or by microorganisms (identified by characteristic odor, bright colors of content, often greenish or pink) (Pheasey et al. 2018). Additionally, dead and alive hatchlings found were reported, and live hatchlings led to the sea.

Once the number of successful eggs and unsuccessful eggs was determined for each nest, we determined the rate of unsuccessful eggs lost to each cause (poaching, dog-predation, natural-predation, flooding, erosion, maternal effects) based on observations of nests and the assessment of the daily state of each nest over the whole incubation period. In the statistical analysis we excluded nests that have been partly predated or eroded and initial clutch size was not determined in oviposition, since an unknown number of eggs were lost.

In case nothing was found in an excavation, whether eggshells nor alive/dead hatchlings, and there is confidence (due to daily nest-check) that the nest has not been washed away or eroded, the “disappearance” of the nest is attributed to poaching which has taken place unnoticed. Unsuccessful eggs in a no-embryo stage suggest being not fertilized and were therefore attributed to “maternal effects”. Clutches that presented eggs containing undeveloped embryos and no apparent reason influenced the status of the nest during incubation, the mortality has been attributed to “temperature”.

4. Statistical analysis

4.1. Seasonality of climatic data

To identify potential differences in the monthly averages of all 7 study years of the three climatic variables precipitation, temperature and tide, we used a set of agglomerative clustering methods: single-linkage agglomerative clustering, complete-linkage agglomerative clustering, average-agglomerative clustering (UPGMA), Ward's Minimum Variance Clustering, and flexible clustering. Cophenetic correlation coefficients were calculated for each dendrogram in order to select the best clustering method, and fusion level values were calculated to form coherent clustering groups.

The different groups resulting from calculation of fusion levels were tested for each single climatic factor (precipitation, temperature, tide). If all the assumptions for a one-way ANOVA were met (no extreme outliers, normality of data, and homogeneity of variances), this analysis was performed. If assumptions were not met, a Welch one-way test was carried out. The post-hoc pairwise comparison was computed with Tukey test and Games-Howell test, respectively. P-Values were adjusted with Tukey.

In order to have more coherent clusters for further analysis, results of the clustering were slightly adjusted. A contingency table helped us identify the frequency distribution of each month within each clustering group, ultimately determining the final climatic periods used for all the following analyses. As for the clustering groups, one-way ANOVA or non-parametric equivalent Welch one-way test were performed to quantify significant differences between the newly formed climatic periods. These resulting periods were also used to separate the nests throughout the nesting season.

4.2. Hatching success

Hatching success was calculated for each nesting season, each period within the nesting season, and over the whole 7 study years. The association to the climatic periods was determined by the laying date of the nest.

First, hatching success as percentage and as raw number of successfully-hatched eggs per each year has been tested through Kruskal-Wallis test – ANOVA assumptions were not met.

Then, to quantify seasonal differences in hatching success, hatching success was plotted per climatic period for every year and tested with a one-way ANOVA or a Welch one-way test if ANOVA assumptions were not met. The post-hoc pairwise comparison was computed with Tukey test and p-values were adjusted with Tukey.

4.3. Annual and seasonal egg losses per cause

To quantify the magnitude of each specific cause of egg loss in each of the study years, annual percentage of egg losses to each cause were plotted and analysed with a Kruskal-Wallis test, since ANOVA assumptions could not be met. Following, a Dunn's Test was performed for a pairwise comparison. Bonferroni correction was applied to adjust p-values.

The calculation of seasonal egg loss to each cause needed additional considerations. In fact, since incubation time for green turtles is about 2 months, some nests covered more than one climatic period. Thus, to define seasonal egg losses, each cause needed to be analysed separately.

In case of loss of eggs to “maternal effects” the date of oviposition determined the assigned period. Genetic alterations leading to egg failure (Martín-del-Campo et al. 2021) and non-fertilisation of eggs (Wyneken et al. 1988) are determined by laying turtle and therefore depend on the moment the clutch is laid.

The period for eggs lost due to predation by ants, ghost crabs and microorganisms, which all were classified as “natural predation”, was determined by the last two weeks of incubation. In fact, ants were observed in previous studies to invade sea turtle nests in late stages of development (McAllister et al. 1965), during pipping or shortly after hatching (Allen et al. 2001). Ghost crabs are reported to predate turtle nests particularly at the end of incubation period, after 40 days of incubation (Marco et al. 2015). Additionally, through predation by ghost crabs, secondary predators might get access to the nest, i.e. ants that use tunnel from ghost crabs as pathway to enter the nest, or fungi find broken eggs and remnants of embryos suitable as medium to spread (Ali & Ibrahim 2002).

For eggs lost to “temperature” we reviewed the embryonic stage of undeveloped eggs and located them in a period respectively to the developmental advancement.

The remaining causes of egg loss (“erosion”, “flooding”, “poaching”, “predation”, “wet”) were causes with a distinctive and decisive impact that was reported on the daily nest check sheet. The date of each of these events was reviewed and associated period identified.

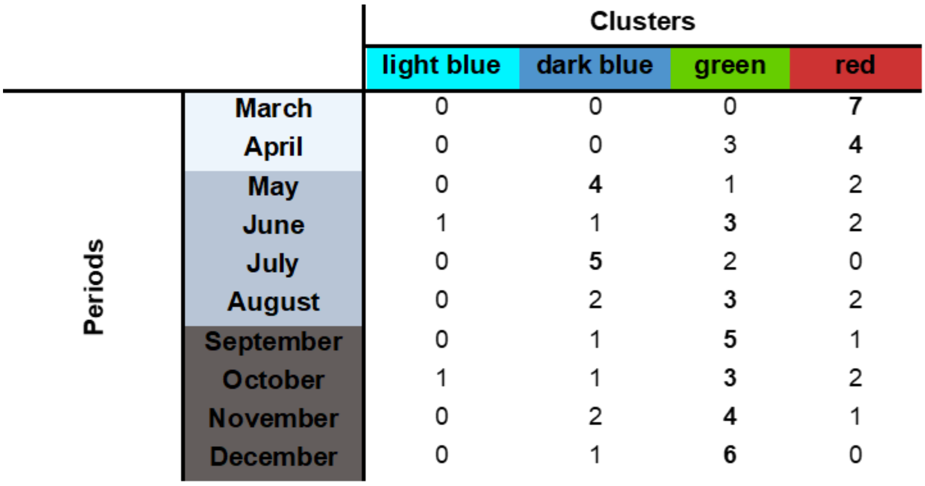
To investigate the occurrence of each cause of egg loss in each of the climatic periods identified, we determined the number of events of each cause aggregated by years and separated by periods. The periods were plotted per total number of events of each cause of egg loss. Significant difference between periods based on threats was examined with a one-way ANOVA. The post-hoc pairwise comparison was computed with a Tukey test and adjustment of p-values made with Tukey.

Results

1. Seasonality of climatic data

Of the clustering methods performed, the average-agglomerative clustering (UPGMA) exhibited the highest cophenetic correlation coefficient = 0.761 (Figure A 1, appendix). Based on fusion level values (Figure A 2, appendix), we cut the UPGMA-dendrogram to separate the data into four groups (Figure A 3, appendix). After compilation of the abundance of each month in a contingency table (Table II-1), 3 climatic periods were identified. The first cluster group (red) showed highest abundance of the months March and April. The second group (dark blue) was constituted mostly of the summer months May-June-July-August. The third group (green) was composed predominantly of the months September-October-November-December. The climatic periods were formed respectively.

Table II-1: Frequency distribution of months in cluster groups (light blue, dark blue, green and red) to determine the three climatic periods March to April, May to August and September to December.



		Clusters			
		light blue	dark blue	green	red
Periods	**March**	0	0	0	**7**
	April	0	0	3	**4**
	May	0	**4**	1	2
	June	1	1	**3**	2
	July	0	**5**	2	0
	August	0	2	**3**	2
	September	0	1	**5**	1
	October	1	1	**3**	2
	November	0	2	**4**	1
	December	0	1	**6**	0

The branch in “light blue” was formed only by two months, 2017-October and 2018-June (Figure II-3). Therefore we did not consider that group as an individual one and aggregated those two months to the respective climatic periods.

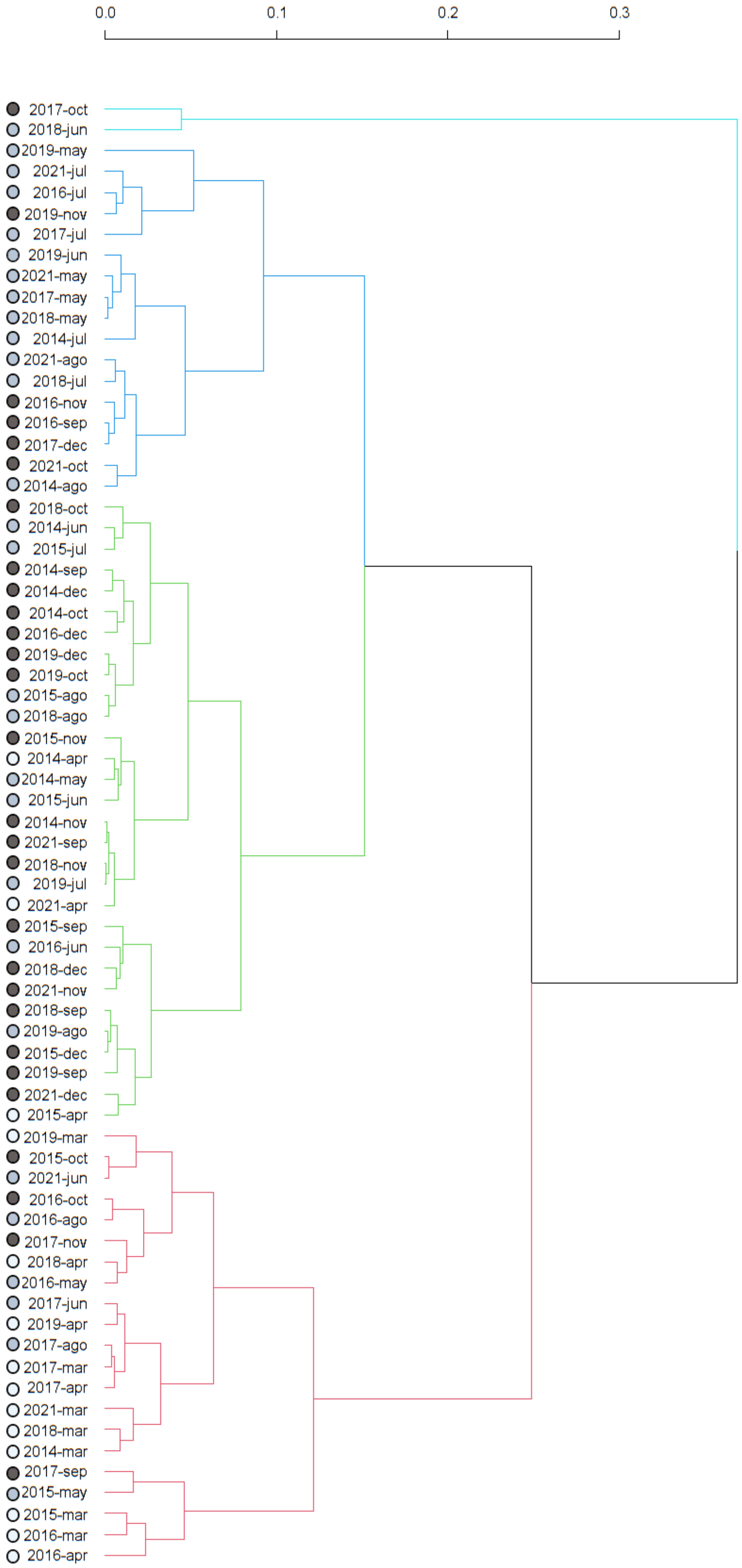


Figure II-3: Cluster groups formed based on fusion level calculation coloured in light blue, dark blue, green and red. Individual months were marked with dots in a grey scale respective to their climatic period.

As we tested the significance between cluster groups, temperature and tide resulted not significantly different with p = 0.052 and p = 0.28, respectively. Precipitation was significantly different (p = 2e- 04) between “dark blue-green”, “dark blue-red" and "green-red” clusters (Figure II-4). The one-way analysis of variance could not detect significant differences from “light blue”, since the data frame contained only two samples.

On the other hand, when we tested the climatic periods, we found significant differences in precipitation (p = 5.66e-06), temperature (p = 9.77e-12) and tide (p = 6.28e-09). In the pairwise comparison, precipitation was significantly different in “period 1-period 3” and “period 1-period 2”. Temperature and tide showed the same trend, as they were both significantly different in “period 1-period 3” and “period 2-period 3” (Figure II-4).

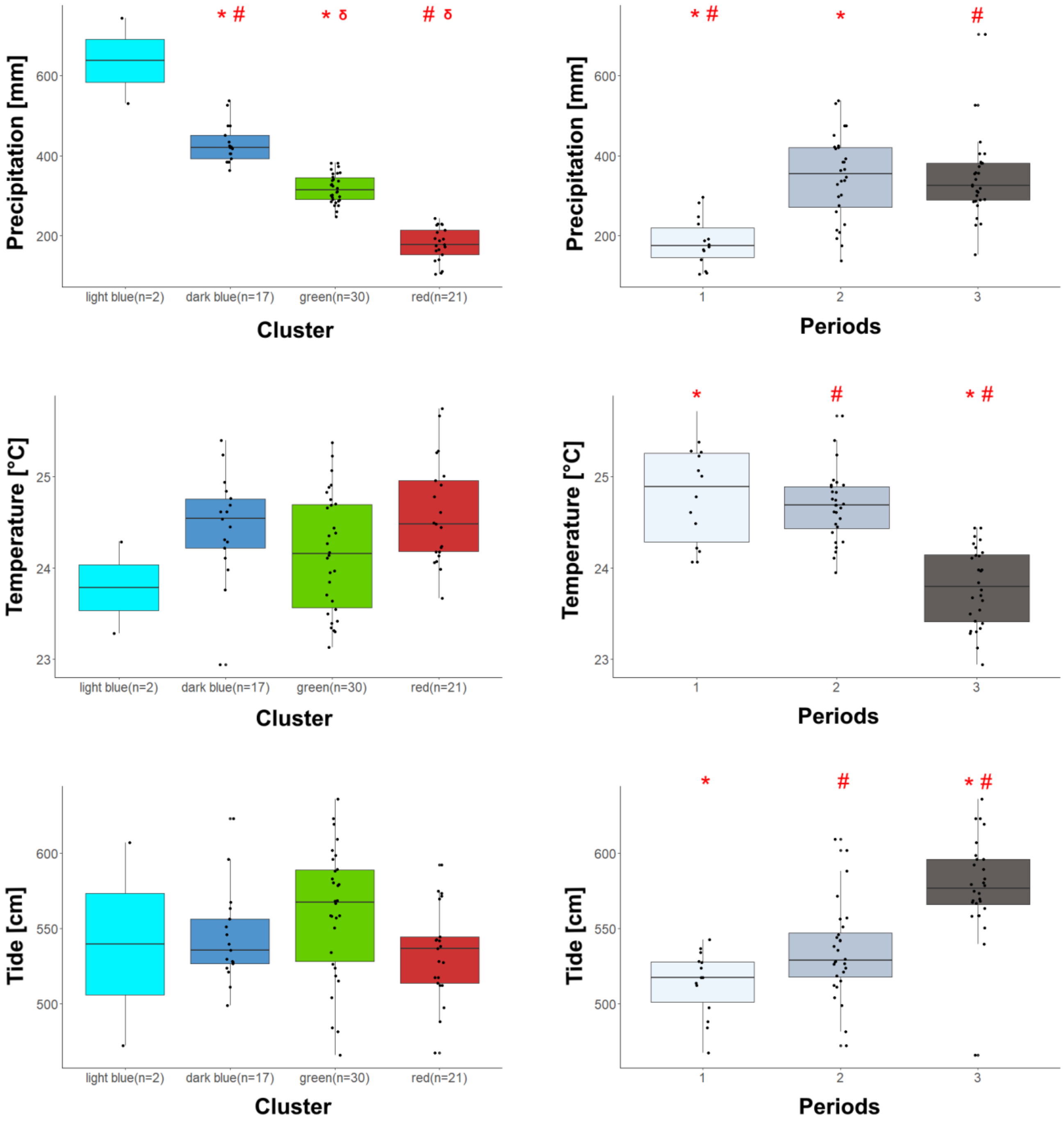


Figure II-4: Cluster groups (light blue, dark blue, green, red) and climatic periods (in grey scale) for precipitation, temperature and tide. Significant differences between single groups are marked with red symbols. The “n=x” in the cluster groups is the number of months forming each cluster group.

2. Hatching success

Of the total 868 green turtle nests laid on Playa Norte in the seven study years, 764 nests were included in our study, which corresponds to 88.02% of all nests. The remaining 104 nests were either partly predated/eroded without an initial egg count, thus an unknown number of eggs were lost, or nests were not excavated.

A total number of 33,512 successful eggs were counted over the period of the 7 study years, which corresponds to an average hatching success of 38.65% ± 4.20% (mean ± standard deviation). "Poaching" and "dog predation" were responsible for an unsuccess of 24.72% ± 5.12% and 18.11% ± 4.57%, respectively. Unsuccess from causes depending on the climate varied between 2.89% ± 1.68% (erosion), 3.51% ± 2.80% (flooding), 3.22% ± 2.19% (wet), 3.59% ± 1.34% (temperature). "Maternal effects" were responsible for 1.50% ± 0.36% of unhatched eggs, and 3.81% ± 1.81% of the eggs were naturally predated. The annual percentages are presented in Figure II-5. Overall, hatching success did not differ significantly among years (Figure A 4, appendix)

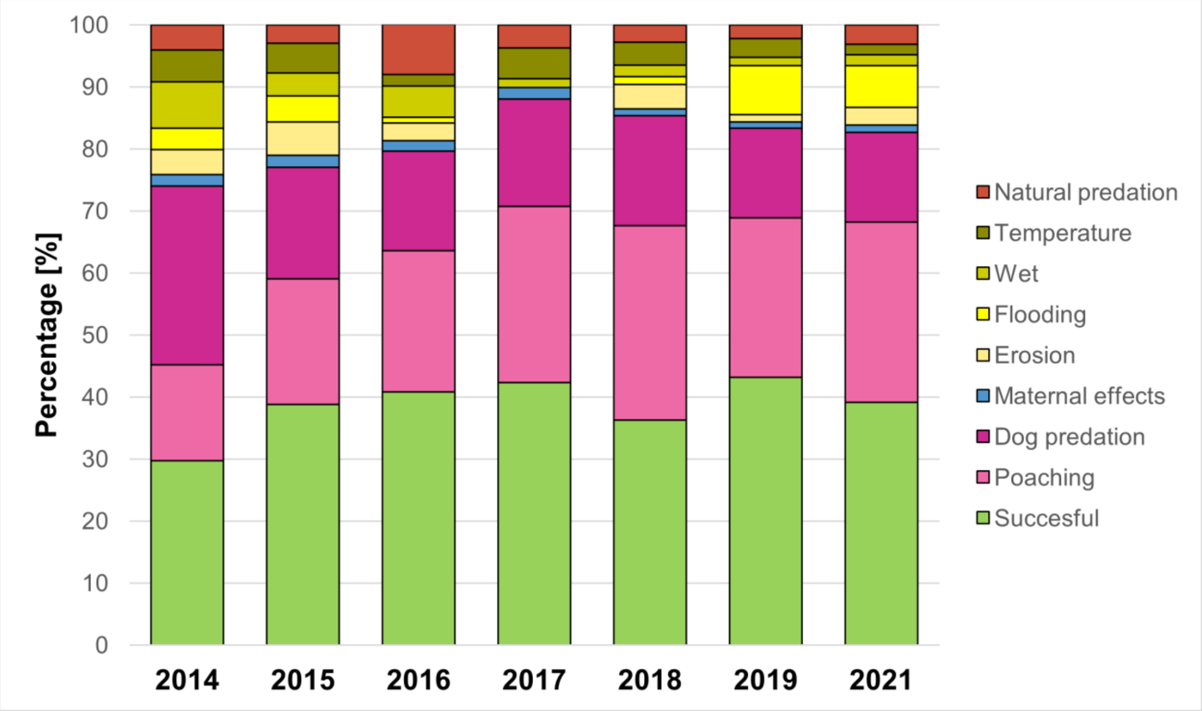


Figure II-5: Annual distribution of successful eggs and eggs lost to poaching, dog predation, maternal effects, erosion, flooding, moisture (wet), temperature and natural predation in percentage.

As we tested the seasonal differences in hatching success in climatic periods in the individual years, the ANOVA general model detected only 2021 to be significant ($p = 0.025$). However, significant differences were not detected in the post hoc pairwise comparison with Tukey-test in 2021 due to the low numerosity of period 1 (Figure II-6). The climatic period 1 of 2014 and 2015 was not accounted in the statistical tests because of the small sample size ($n=2$), and in the other years it was completely absent.

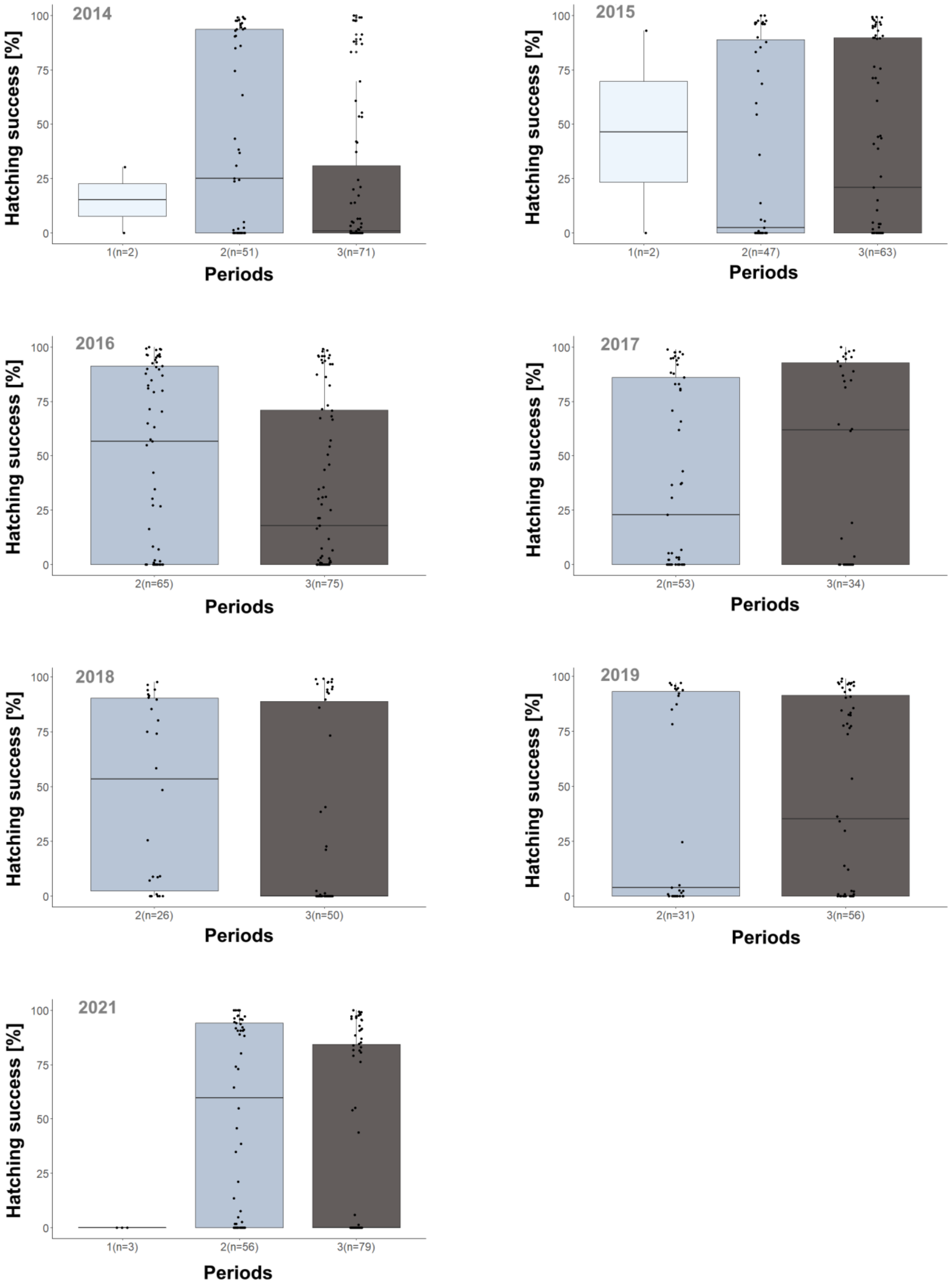


Figure II-6: Differences in hatching success between periods on an annual basis. The "n=x" is the number of nests counted in the respective period.

3. Annual and seasonal egg losses per cause

The Kruskal-Wallis general model in the analysis concerning the annual egg losses resulted significant with a p < 2.2e-16 for all years. Significant differences calculated in a post hoc pairwise comparison with Dunn's Test (Table A 4, Appendix) revealed a set of common results among the 7 study years (Figures II-7, II-8 and II-9). "Dog predation" and "poaching" were found to follow the same trend by being significantly different from "maternal effects", "natural predation" and "temperature" in each year, and from "wet" in 2016 (Figure II-8).

"Erosion" and "flooding" exhibited a very similar trend, in which both causes for egg loss were significantly different from "maternal effects", "natural predation" and "temperature" in the years 2014, 2015 and 2021. Further, significant differences were detected between "erosion" in 2019 (Figure II-9), and among "flooding" in 2016 and 2018 (Figures II-8, II-9), from "maternal effects", "natural predation" and "temperature". In 2018 instead, "erosion" was significantly different exclusively from "maternal effects" (Figure II-9).

Significant differences for "wet" were observed only in the first three study years. In fact, "wet" resulted significantly different from "maternal effects" in 2014, 2015 and 2016 (Figures II-7, II-8 and II-9). Additionally, "wet" was found being significantly different from "natural predation" and "temperature" only in 2014 (Figure II-7).

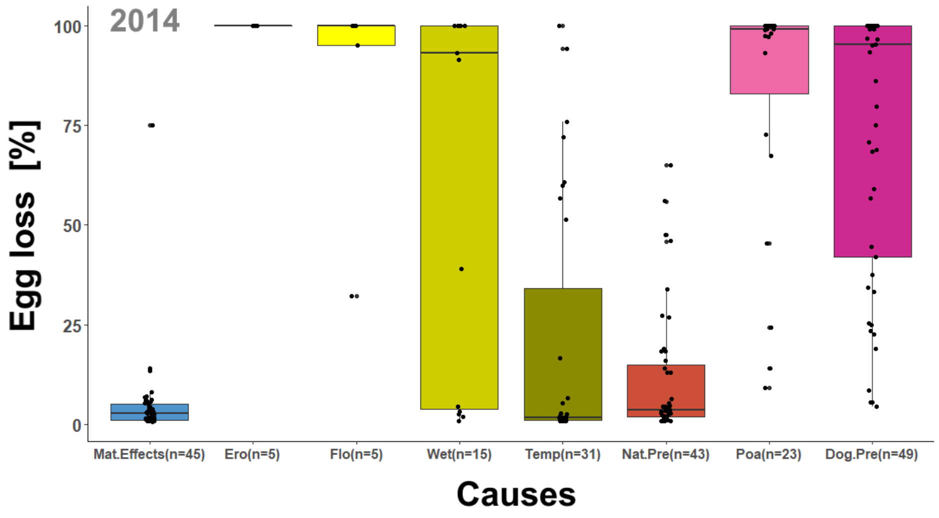


Figure II-7: Illustration of impact of each cause in terms of egg loss for the nesting season 2014. The "n=x" is the number of occurrences of each cause within the nesting season.

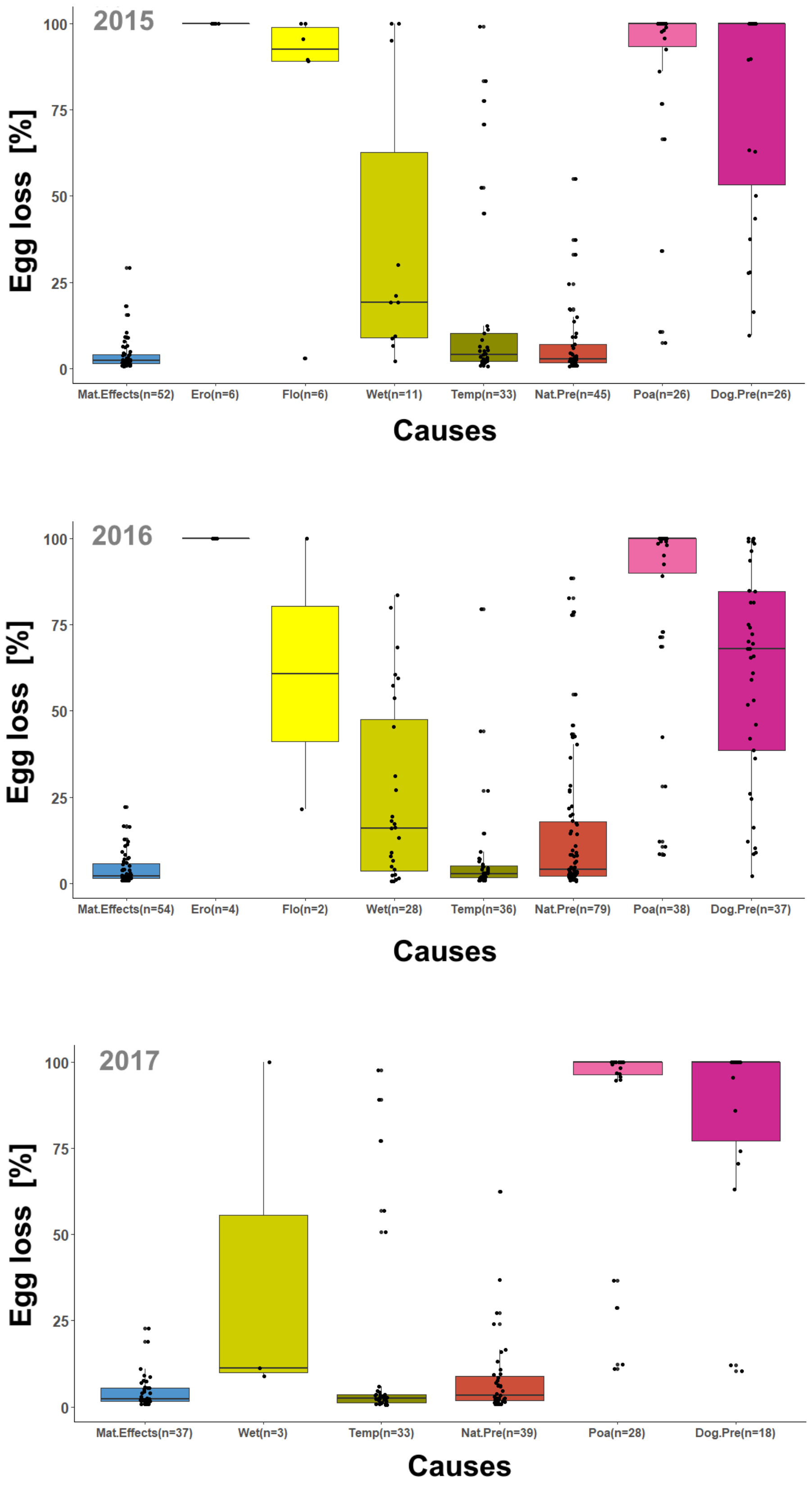


Figure II-8: Illustration of impact of each cause in terms of egg loss for the nesting seasons 2015-2017. The “n=x” is the number of occurrences of each cause within the nesting season.

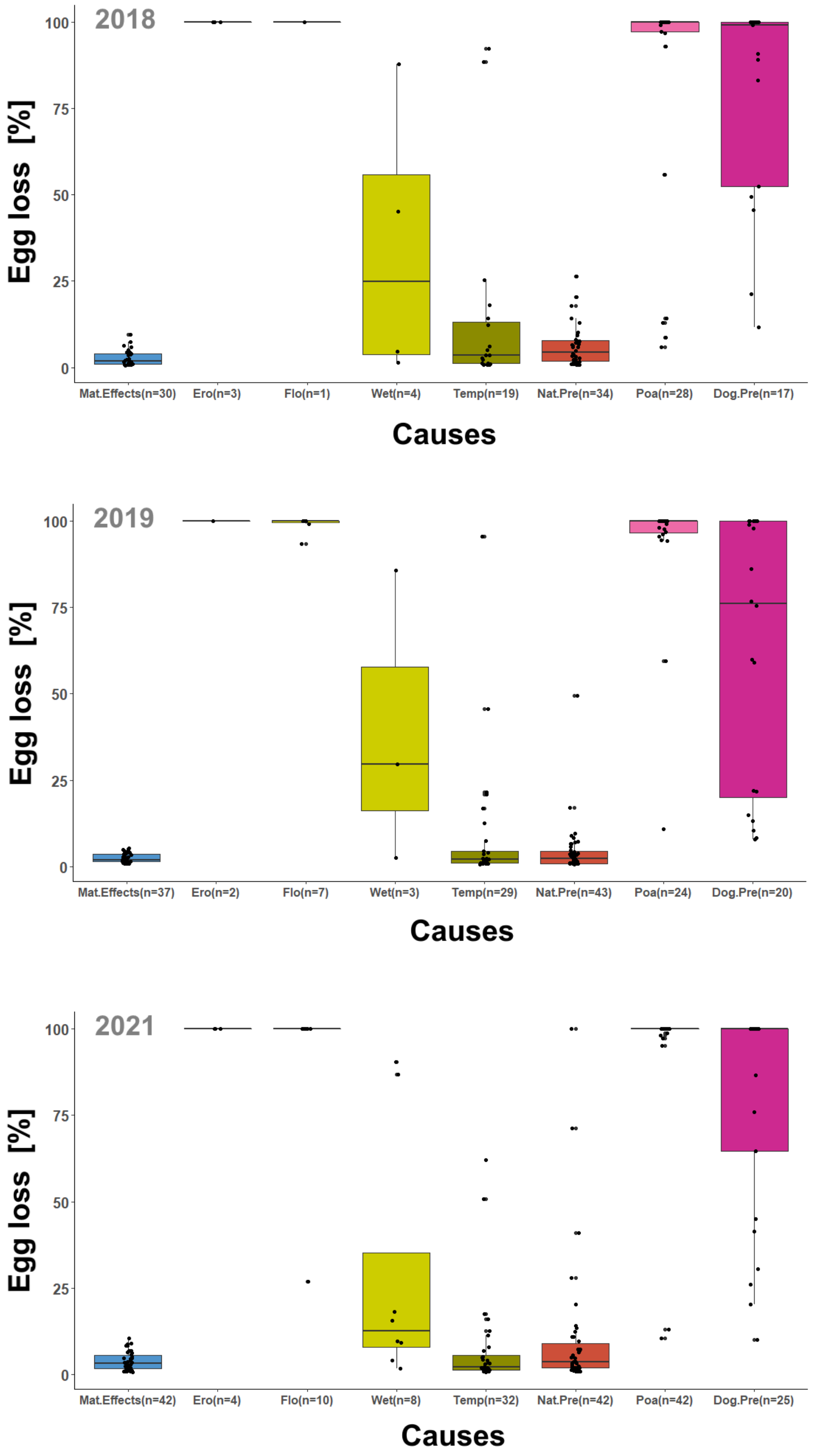


Figure II-9: Illustration of impact of each cause in terms of egg loss for the nesting seasons 2018-2021. The “n=x” is the number of occurrences of each cause within the nesting season.

Similarly, in the seasonal statistical analysis of egg losses per cause, the general model of analysis of variance determined significant differences in all of the causes, except "erosion" ($p = 0.312$). The p-Values calculated are $p = 0.00016$ for "dog predation", $p = 0.013$ for "flooding", $p = 0.00074$ for "maternal effects", $p = 6.31e-05$ for "natural predation", $p = 0.00066$ for "poaching", $p = 0.00013$ for "temperature", and $p = 0.038$ for "wet". The post hoc pairwise comparison found that six of the eight causes of egg loss (with exception of "erosion" and "wet") were significantly different between "period 1-period 3" (Table II-2). "Period 1" differed significantly from "period 2" only in "flooding", "maternal effects" and "poaching". On the other hand, significant differences were detected between "period 2-period 3" in "dog predation", "natural predation" and "temperature". No significant differences were found between the individual climatic periods in "wet", however the p-value in the pairwise comparison of "period 2-period 3" was within a close range of being significantly different ($p = 0.0511$).

Table II-2: Pairwise comparison of climatic periods and listing of causes of egg loss found significantly different in each of them.

	Period 1	**Period 2**
Period 1		
Period 2	Flooding Maternal Effects Poaching	
Period 3	Dog Predation Flooding Maternal Effects Natural Predation Poaching Temperature	Dog Predation Natural Predation Temperature

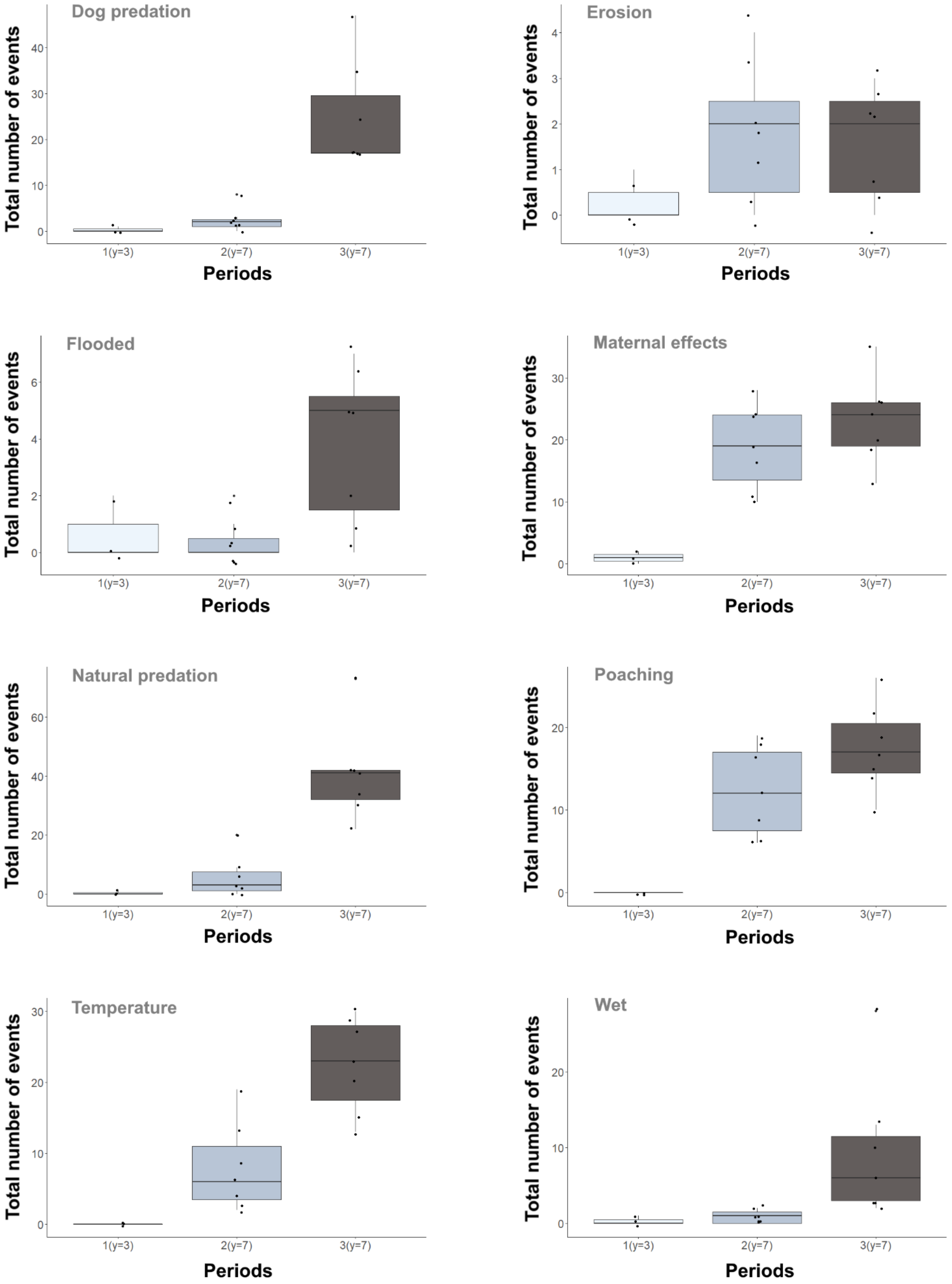


Figure II-10: Total number of occurrences of each egg-loss cause per climatic period. Mean was calculated from number of events in each season per year ("y=x" in periods describes number of years in which nests were faced with threats).

Discussion

Regarding the first research question, our hypotheses were partly met. Differences were found in the cluster groups based only on precipitation. However, as we “compiled” the three climatic periods actually used for the analysis, differences were found in all three climatic variables. Consequently, our analytical approach to define climatic periods was supported, since the adjusted climatic periods were even more significantly different than the groups suggested by the UPGMA-dendrogram. This suggests that all variables display climatic patterns that are more distinct between periods than initially assumed.

Concerning the second objective of the study, the annual hatching success was fairly constant over the 7 study years, similarly to results reported from other nesting beaches around the world (e.g. Ghazali & Jamil 2019, Dunbar 2011). However, a mean hatching success of 38.65% on Playa Norte is relatively low compared to other green turtle populations worldwide (Summers et al. 2018, Brost et al. 2015, Garcıía et al. 2003). Reasons for these relatively low values of hatching success lie in the strong impact of egg losses caused by anthropogenic activities (Figure II-5). Indeed, poaching and domestic dog predation alone are accountable for an egg loss of 42.83% (as average in the 7-year study period) on Playa Norte. However, it is remarkable to observe that hatching success on our study site, without considering these two drivers, would display a hatching success of about 80%, that is a value commonly reported as a “natural” one from many parts of the world (Zárate et al. 2013).

Regarding the hatching success within the climatic periods, we could not assess our hypothesis due to the low numerosity/absence of nests in period 1 (Figure II-6). Therefore, it is not possible to draw a resolute conclusion for the nests lost in period 1 and their relationship with environmental stressors. However, on one hand, our results showed that most of the eggs lost in period 1 depended on not-anthropogenic factors, i.e. “flooding”, “erosion” and “wet” (Figure II-10). On the other hand, precipitation and tide were the lowest in period 1 (Figure II-4). An explanation to this counterintuitive pattern may be found in the resolution of climatic data available for the analysis. Indeed, our data were monthly means, where single, potentially extreme, environmental events were hardly detectable.

Hatching success between period 2 and period 3 did not display any significant differences, despite the variations found in environmental variables. Numerous studies have underlined the threat of climate change for turtle populations worldwide (Clarke et al. 2021, Limpus et al. 2020, Laloë et al 2017, Fuentes et al. 2010b). Interestingly, climate change effects on hatching success were not detected over the study period on Playa Norte, which suggests that: 1) our temporal frame (7-year period), despite relevant in comparison to other similar studies, may still be too short to detect any effect indirectly caused by climatic change; 2) this region suffers less of environmental changes on a global scale – but this needs to be assessed in a longer time scale; 3) the proactive behaviour of sea turtles in selecting appropriate sites for nesting (Spanier 2010). In fact, on the Ascension Island green turtles have been reported to crawl up to 80 m from the high tide line to find an adequate nesting spot (Hays et al. 1995). Also on Playa Norte green turtles were observed to nest far up the beach, distant from the high tide line and under vegetation.

In regard to the third research question, our initial hypotheses were mostly met. Causes of egg loss seem to follow a clear trend throughout the 7 study years (Figures II-7, II-8, II-9), apart for the year 2017. Zero eggs were lost neither to flooding nor erosion in that year. In fact, 2017 has been identified as a particularly dry year. As we examined the individual months of 2017 in the cluster groups (Figure II-3), most of the months were found in the red cluster. Present in this group is also the month of March of all 7 study years, which is characterized by the lowest precipitation throughout the whole year (Table A 1, Annex). In fact, even if just slightly different from other years, the precipitation average among years is still lowest in 2017. Assumably, the mean was evened out by October 2017, which distinguished itself from the other months and years by a substantially high precipitation. This could be explained by the passage of hurricane Nate at the beginning of October 2017, which caused heavy rains and extreme floods (Quesada-Román et al. 2020). However, the reasons for the low precipitation in that year cannot be clearly defined and are supposed to be associated to a more complex local climatical relationship.

It can be easily seen from all the other study years that “erosion” and “flooding” have a low number of occurrences but a very high impact, which is 100% in most cases. In fact, other investigations have identified “erosion” (Brost et al. 2015) and “flooding” (Limpus et al. 2020) as the factors with the greatest effect on egg survivorship by generally reducing success to zero. In comparison, the other two factors related to climatic events and weather, “wet” and “temperature”, displayed usually a much lower average of egg loss with higher standard deviation. On one hand, the case of “wet” could be explained through the time of exposure to the cause and the amount of water retained in the nest, which is much less than in case of flooding. In fact, Pike et al. (2015) have shown that inundation for 1 or 3 h reduced egg viability by less than 10%, whereas inundation for 6 h reduced viability by approximately 30%. On the other hand, temperature dependent hatching success is known to be influenced by sand albedo (Hays et al. 2001), substrate characteristics, such as grain size and organic matter content (Lindborg et al. 2016), and the amount of shade (Fuentes et al. 2010a) that covers the nest. In correspondence to the results of our second research question, temperature seemed to have generally a low impact on egg loss on Playa Norte, despite the high number of events. In fact, as mentioned above, green turtles tended to nest close to/under vegetation on our study site. Hence, the amount of shade on the nests was notably high. As reported from other studies, shading reduces the nest temperature significantly and can protect embryos from lethal incubation temperatures (Bézy et al. 2015, Wood et al. 2014).

With a similar trend, “maternal effects” and “natural predation” were causes with a high number of occurrences, nevertheless their impact was among the lowest. In fact, eggs within a clutch usually exceed 94% fertility (Williamson et al. 2017, Rafferty & Reina 2014, Rafferty et al. 2013, Abella et al. 2006). However, it has to be considered that infertility and embryonic failure are generally underestimated, since unsuccessful eggs are opened only after incubation time was completed, and decomposition or micropredation could mask the true infertility (Wyneken et al. 1988).

Our results concerning “natural predation” were similar to what is reported by Pheasey et al. (2018), who calculated a mortality caused by predation “by ghost crabs, microorganisms and other predators” of 4.086% on Playa Norte. “Natural predation” by microbial abundance within a nest is highest on high density nesting beaches (Bézy et al. 2015), where nests are laid on top of each other and destroyed by other nesting turtles. However, this was very rarely the case on Playa Norte, which could explain the relatively low egg predation by microbial loads. Ant predation was also considerably low on Playa Norte, consistent with the outcomes shown by Mangel et al. (2001), who calculated an ant infestation at 0.5% nests at the close Tortuguero beach. Also ghost crabs seemed to play a minor role as predators on turtle eggs on our study site. As mentioned above, the predation by carnivore mammals on nesting beaches often targets also other predators, such as the ghost crab, and regulates their population (Pheasey et al. 2018, Marco et al. 2015, Barton & Roth 2008). In fact, “dog predation” affected hatching success significantly on our study site and, most likely, ghost crab abundance. Despite already pretty high, the impact of domestic dog predation, however, was likely underestimated, since a number of turtle nests have been excluded from statistical analysis in some cases of partial predation due to the unknown number of lost eggs. Management actions to reduce dog predation on Playa Norte have been put into practice since 2014, by deploying bamboo screens on nests. In fact, the screens have been observed to prevent the complete predation of 48% of the nests, without affecting hatching success nor emergence success (Pheasey et al. 2018). Through the study period, screens usually were deployed 3-2 weeks before hatching date, since predation is highest in the last stages of incubation (Fowler 1979). However, predation was still reported, in many cases only a few days before the screen deployment was scheduled. Nonetheless, it is shown that there is no significant difference between timing of deployment and likelihood of the screen being breached (Pheasey et al. 2018). Therefore, an intensification of conservation measurements could include to move the screen deployment timing forward to limit dog predation.

As it is shown in our results, “poaching” was amongst the highest causes of egg loss in terms of events but also impact. In fact, poaching usually implies the collection and loss of an entire clutch, mainly for the illegal egg trade in the local area (Mejías-Balsalobre et al. 2021). Additionally, partial poaching is an ongoing activity observed on Playa Norte, particularly by the few households that live directly on the beach and collect some of the eggs for their own consumption. In the last study year, 2021, the highest number of poaching events has been observed. We assume this is attributed to Covid-19 restrictions, which might have encouraged sea turtle egg poaching. Indeed, other studies suppose similar hypothesis, i.e. Quesada-Rodríguez et al. (2021) revealed a higher poaching rate from 2019, possibly due to fewer censuses per night because of Covid-19 restrictions. In fact, enforcing beach protection is an effective conservation action, which can practically halt poaching to 100% (Garcıía et al. 2003). Propositions to reduce poaching on Playa Norte could include an increase in the number of patrolling teams and separating the beach transect between them for a higher frequency of passing supervision. Further, the construction of an enclosed hatchery could be taken in consideration. The practice of nest reburial into safer areas has been observed to have some negative effects, i.e. altered sex ratios (Tomillo et al. 2021) and reduced hatching success relative to natural nests (Mortimer 1999). However, the pressure of poaching and domestic dog predation outweighs the egg losses that would be caused by environmental stressors in relocated nests, for which enclosed hatcheries would be an effective conservation action to improve hatching success (Quesada-Rodríguez et al. 2021, Garcıía et al. 2003, Wyneken et al. 1988). Nonetheless, the reburial of nests into an enclosed hatchery would need a more detailed study of natural incubation conditions to ensure natural sex ratios and hatchling survival, i.e. physical characteristics of sand type (Bézy et al. 2015), nest depth (Spanier 2010) and amount of shade (Tomillo et al. 2021).

Concerning the last aim, hypotheses were only partly met. Despite the significant differences of precipitation between climatic period 1 and both period 2 and period 3 (Figure II-4), "wet" did not change significantly throughout the nesting season (Figure II-10). In fact, Costa Rica is categorised as tropical humid region, characterized by daily rainfall (Arnfield 2020). However, as for the cause of egg loss "flooding", it seems that the stressor goes along with tide, since both variables were significantly higher in period 3. This suggests that conservation actions regarding the flooding of nests need to concentrate particularly in the months from September to December, caring more about tide rather than precipitation.

Literature characterizes Costa Rica with consistently high temperatures (around 30 °C) (Arnfield 2020). Over the study period of 7 years however, we found temperature decreasing throughout the nesting season. Remarkable is that, despite the decreasing temperatures, the number of events of temperature impacting sea turtle eggs is significantly higher in period 3 from both periods 1 and 2. If we consider the mean incubation period of 58.5 days for green turtles in Tortuguero, Costa Rica (Tiwari et al. 2006, Fowler 1979), it is coherent that most of the clutches hatch in period 3. In fact, October and November are peak months for hatchling emergence on Playa Norte (Pheasey et al. 2018). This suggests that turtle embryos in late stages of development are more vulnerable to elevated temperatures than early stages. Indeed, Bladow & Milton (2019) observed a significantly higher mortality to elevated temperatures in the Miller-stages 29 and 30, which correspond to the last stages before hatching.

"Natural predation" and "dog predation" were, as expected, and coherently to prior studies, significantly higher in period 3. In fact, predation is positively correlated with hatching and emergence events throughout the nesting season (Marco et al. 2015, Ali & Ibrahim 2002, Fowler 1979) and therefore increased significantly in the months of October and November, close to the end of incubation.

Despite significant and non-significant differences between periods in causes of egg loss, it is remarkable to observe that each stressor followed the same trend with a higher number of events in period 3, even though hatching success did not differ significantly between periods 2 and 3. However, the elevated number of occurrences in period 3 of all causes of egg loss could be explained through the happening of both oviposition and hatching during those months. While turtles were still laying nests almost on a daily basis, particularly through September and October, hatching and emergence reached their seasonal peak in October and November. Therefore, factors that affected the initial period of incubation overlapped with factors that caused egg loss at the end of incubation. This resulted in a general high pressure on hatchling survival by both anthropogenic and environmental stressors. Consequently, it is crucial to concentrate conservation actions in the months comprised between September and December, especially, by aiming to counteract human-induced egg mortality. Indeed, environmental and natural stressors can be mostly neglected on Playa Norte due to their low impact – in terms of total annual egg loss. The enforcement of beach patrols in that period would increase the impact of sea-turtle conservation efforts. A seasonal management of voluntary workers with a lower number of patrols through the early nesting season and contextually an intensification of patrols during the late nesting season would increase the protection of nests with no economic effort. Additionally, we suggest a construction of an enclosed hatchery for nest relocations into safer areas to ensure a higher hatchling survival. In fact, this type management could reduce both anthropogenic and natural factors of egg loss (Garcıía et al. 2003).

Conclusion

The findings of this study allow us to get a general profile of annual trends and the differences found in stressors between climatic periods within a nesting season on Playa Norte, through which some specific guidelines for conservation actions can be determined. Resources and effort in beach protection should concentrate between September to December, when nests are still laid and hatching reaches its seasonal peak. In particular, it is crucial to relieve the pressure coming from anthropogenic factors, since hatching unsuccess deriving from poaching and domestic dog predation overweighs the unsuccess caused by climatic factors by a considerable margin. Suggested conservation actions include an intensification of beach patrols during late nesting season, and a relocation of nests into enclosed hatcheries. Nonetheless, a follow-up of this study is needed to investigate effectiveness of suggested conservation actions in terms of hatching success, but also to understand the effects of additional interventions such as nest relocations – on sex determination, hatchling quality, and the entire beach ecosystem.

References

Abella, E., Marco, A., & López-Jurado, L. F. (2006). Why are egg fertilization rates not correlated with hatching success in sea turtles. In Book of abstracts. Twenty-sixth annual symposium on sea turtle biology and conservation. International Sea Turtle Society, Athens, Greece (p. 43).

Aguirre, A. A., & Lutz, P. L. (2004). Marine turtles as sentinels of ecosystem health: is fibropapillomatosis an indicator?. EcoHealth, 1(3), 275-283.

Ali, A., & Ibrahim, K. (2002). Crab Predation On Green Turtle Eggs Incubated On a Natural Beach And In Turtle Hatcheries. Proceedings of the 3rd Workshop on SEASTAR2000 2002: 95-100.

Allen, C. R., Forys, E. A., Rice, K. G., & Wojcik, D. P. (2001). Effects of Fire Ants (Hymenoptera: Formicidae) on Hatching Turtles and Prevalence of Fire Ants on Sea Turtle Nesting Beaches in Florida. The Florida Entomologist, 84(2), 250–253.

Allison N. 2018. Marine Turtle Monitoring and Tagging Program Green Season Report 2018. Caño Palma Biological Station. Canadian Organisation for Tropical Education and Rainforest Conservation, Playa Norte, Costa Rica.

Arnfield, A. John (2020). *Köppen climate classification*. *Encyclopedia Britannica*. Available online: https://www.britannica.com/science/Koppen-climate-classification (accessed on 26 September 2022)

Barton, B. T., & Roth, J. D. (2008). Implications of intraguild predation for sea turtle nest protection. Biological Conservation, 141(8), 2139–2145.

Bézy, V. S., Valverde, R. A., & Plante, C. J. (2015). Olive ridley sea turtle hatching success as a function of the microbial abundance in nest sand at Ostional, Costa Rica. PloS one, 10(2), e0118579.

Bladow, R. A., & Milton, S. L. (2019). Embryonic mortality in green (Chelonia mydas) and loggerhead (Caretta caretta) sea turtle nests increases with cumulative exposure to elevated temperatures. Journal of Experimental Marine Biology and Ecology, 518, 151180.

Booth, D. T. (2017). Influence of incubation temperature on sea turtle hatchling quality. Integrative Zoology, 12(5), 352-360.

Brost, B., Witherington, B., Meylan, A., Leone, E., Ehrhart, L., & Bagley, D. (2015). Sea turtle hatchling production from Florida (USA) beaches, 2002-2012, with recommendations for analyzing hatching success. Endangered Species Research, 27(1), 53-68.

Cáceres-Farias, L., Reséndiz, E., Espinoza, J., Fernández-Sanz, H., & Alfaro-Núñez, A. (2022). Threats and Vulnerabilities for the Globally Distributed Olive Ridley (Lepidochelys olivacea) Sea Turtle: A Historical and Current Status Evaluation. Animals, 12(14), 1837.

Campbell, C. L., & Lagueux, C. J. (2005). Survival probability estimates for large juvenile and adult green turtles (Chelonia mydas) exposed to an artisanal marine turtle fishery in the western Caribbean. Herpetologica, 61(2), 91-103.

Carpio Camargo, A. J., Álvarez Gutiérrez, Y., Jaramillo Véliz, J., & Sánchez Tortosa, F. (2020). Nesting failure of sea turtles in Ecuador-causes of the loss of sea turtle nests: the role of the tide. Journal of Coastal Conservation, 24(5), 1-10.

Caut, S., Guirlet, E., & Girondot, M. (2010). Effect of tidal overwash on the embryonic development of leatherback turtles in French Guiana. Marine Environmental Research, 69(4), 254-261.

Clarke, L. J., Elliot, R. L., Abella‐Perez, E., Jenkins, S. R., Marco, A., Martins, S., & Hawkes, L. A. (2021). Low‐cost tools mitigate climate change during reproduction in an endangered marine ectotherm. Journal of Applied Ecology, 58(7), 1466-1476.

Clusella Trullas, S., & Paladino, F. V. (2007). Micro-environment of olive ridley turtle nests deposited during an aggregated nesting event. Journal of Zoology, 272(4), 367-376.

Dunbar, T. (2011). Monitoring nesting trends and hatchling success of the green turtle (Chelonia mydas) population on Mnemba Island, Zanzibar.

Fleming, E.H. (2001). Swimming against the tide: Recent Surveys of Exploitation, Trade, and Management of Marine Turtles in the Northern Caribbean. Report for TRAFFIC, North America.

Fowler, L. E. (1979). Hatching success and nest predation in the green sea turtle, Chelonia mydas, at Tortuguero, Costa Rica. Ecology, 60(5), 946-955.

Fuentes, M. M. P. B., Hamann, M., & Limpus, C. J. (2010a). Past, current and future thermal profiles of green turtle nesting grounds: Implications from climate change. Journal of Experimental Marine Biology and Ecology, 383(1), 56-64.

Fuentes, M. M. P. B., Limpus, C. J., Hamann, M., & Dawson, J. (2010b). Potential impacts of projected sea-level rise on sea turtle rookeries. Aquatic conservation: marine and freshwater ecosystems, 20(2), 132-139.

Garcıía, A., Ceballos, G., & Adaya, R. (2003). Intensive beach management as an improved sea turtle conservation strategy in Mexico. Biological Conservation, 111(2), 253-261.

Ghazali, A. F., & Jamil, N. R. (2019). Population and Trend Analysis for Green Turtle (Chelonia mydas) and Hawksbill Turtle (Eretmochelys imbricata) in Marine Park Centre Redang, Terengganu and Marine Park Centre Rusukan Besar, Labuan, Malaysia. Pertanika Journal of Science & Technology, 27(3).

Gupta, S., Jain, I., Johari, P., & Lal, M. (2019). Impact of climate change on tropical cyclones frequency and intensity on Indian coasts. In Proceedings of International Conference on Remote Sensing for Disaster Management (pp. 359-365). Springer, Cham.

Hays, G. C., Ashworth, J. S., Barnsley, M. J., Broderick, A. C., Emery, D. R., Godley, B. J., Henwood, A., & Jones, E. L. (2001). The importance of sand albedo for the thermal conditions on sea turtle nesting beaches. Oikos, 93(1), 87-94.

Hays, G. C., Mackay, A., Adams, C. R., Mortimer, J. A., Speakman, J. R., & Boerema, M. (1995). Nest site selection by sea turtles. Journal of the Marine Biological Association of the United Kingdom, 75(3), 667-674.

Hitchins, P. M., Bourquin, O., & Hitchins, S. (2004). Nesting success of hawksbill turtles (Eretmochelys imbricata) on Cousine Island, Seychelles. Journal of Zoology, 264(4), 383-389.

IMN (Instituto Meteorológico Nacional). 2001. Escenarios de cambio climático para Costa Rica. PrimeraComunicación Nacional para UNFCCC. Instituto Meteorológico Nacional, Ministerio del Ambiente y Energía. SanJosé, Costa Rica. Sp.

Joseph, J., Nishizawa, H., Alin, J. M., Othman, R., Jolis, G., Isnain, I. and Nais, J. (2019). Mass sea turtle slaughter at Pulau Tiga, Malaysia: Genetic studies indicate poaching locations and its potential effects. Global Ecology and Conservation, Vol 17.

Lahanas, P. N., Bjorndal, K. A., Bolten, A. B., Encalada, S. E., Miyamoto, M. M., Valverde, R. A., & Bowen, B. W. (1998). Genetic composition of a green turtle (Chelonia mydas) feeding ground population: evidence for multiple origins. Marine Biology, 130(3), 345-352.

Laloë, J. O., Cozens, J., Renom, B., Taxonera, A., & Hays, G. C. (2017). Climate change and temperature-linked hatchling mortality at a globally important sea turtle nesting site. Global change biology, 23(11), 4922-4931.

Limpus, C. J., Miller, J. D., & Pfaller, J. B. (2020). Flooding-induced mortality of loggerhead sea turtle eggs. Wildlife Research, 48(2), 142-151.

Lindborg, R., Neidhardt, E., Witherington, B., Smith, J. R., & Savage, A. (2016). Factors influencing loggerhead (Caretta caretta) and green turtle (Chelonia mydas) reproductive success on a mixed use beach in Florida. Chelonian Conservation and Biology, 15(2), 238-248.

Lyons, M. P., von Holle, B., & Weishampel, J. F. (2022). Why do sea turtle nests fail? Modeling clutch loss across the southeastern United States. Ecosphere, 13(3), e3988.

Marco, A., da Graça, J., García-Cerdá, R., Abella, E., & Freitas, R. (2015). Patterns and intensity of ghost crab predation on the nests of an important endangered loggerhead turtle population. Journal of Experimental Marine Biology and Ecology, 468, 74-82.

Mangel, J., Troëng, S., Segura, L., Stockmann, M., Ortega, A., Reyes, C., Hudgson, Z., Opazo, A., Fernández, L., Hernández, R., Hussy, D., Ramírez, M., De la Parra, S., Martínez, M., Hajjar, R., & Rankin, E. (2001). Report on the 2000 green turtle program at Tortuguero, Costa Rica. Unpubl report. Carib. Conserv Corp & Ministry Environ Energy CR.

Martín-del-Campo, R., Calderón-Campuzano, M. F., Rojas-Lleonart, I., Briseño-Dueñas, R., & García-Gasca, A. (2021). Congenital Malformations in Sea Turtles: Puzzling Interplay between Genes and Environment. Animals, 11(2), 444.

McAllister, H. J., Bass, A. J., Van Schoor, H. J., (1965). The marine turtles of Tongaland, Natal. Lammergeyer. 3: 10–40.

Mejías-Balsalobre, C., Restrepo, J., Borges, G., García, R., Rojas-Cañizales, D., Barrios-Garrido, H., & Valverde, R. A. (2021). Local community perceptions of sea turtle egg use in Tortuguero, Costa Rica. Ocean & Coastal Management, 201, 105423.

Milton, S. L., & Lutz, P. L. (2002). Physiological and Genetic Responses to Environmental Stress. The Biology of Sea Turtles, Volume II, 163–197.

Mortimer, J. A. (1999). Reducing threats to eggs and hatchlings: hatcheries. 175-178. Research and management techniques for the conservation of sea turtles. IUCN/SSC Marine Turtle Specialist Group Publ, 4.

Müller, M., Cherniawsky, J. Y., Foreman, M. G., & von Storch, J. S. (2014). Seasonal variation of the M 2 tide. Ocean Dynamics, 64(2), 159-177.

Pace, A., Dipineto, L., Fioretti, A., & Hochscheid, S. (2019). Loggerhead sea turtles as sentinels in the western Mediterranean: antibiotic resistance and environment-related modifications of Gram-negative bacteria. Marine Pollution Bulletin, 149, 110575.

Pheasey, H., McCargar, M., Glinsky, A., & Humphreys, N. (2018). Effectiveness of concealed nest protection screens against domestic predators for green (Chelonia mydas) and hawksbill (Eretmochelys imbricata) sea turtles. Chelonian Conservation and Biology, 17(2), 263-270.

Pike, D. A., Roznik, E. A., & Bell, I. (2015). Nest inundation from sea-level rise threatens sea turtle population viability. Royal Society Open Science, 2(7), 150127.

Quesada-Rodríguez, C., Orientale, C., Diaz-Orozco, J., & Sellés-Ríos, B. (2021). Impact of 2020 COVID-19 lockdown on environmental education and leatherback sea turtle (Dermochelys coriacea) nesting monitoring in Pacuare Reserve, Costa Rica. Biological Conservation, 255, 108981.

Quesada-Román, A., Ballesteros-Cánovas, J. A., Granados-Bolaños, S., Birkel, C., & Stoffel, M. (2020). Dendrogeomorphic reconstruction of floods in a dynamic tropical river. Geomorphology, 359, 107133.

Rafferty, A. R., Evans, R. G., Scheelings, T. F., & Reina, R. D. (2013). Limited oxygen availability in utero may constrain the evolution of live birth in reptiles. The American Naturalist, 181(2), 245-253.

Rafferty, A. R., & Reina, R. D. (2014). The influence of temperature on embryonic developmental arrest in marine and freshwater turtles. Journal of Experimental Marine Biology and Ecology, 450, 91-97.

Ratnaswamy, M. J., Warren, R. J., (1998). Removing raccoons to protect sea turtle nests: are there implications for ecosystem management? Wildl. Soc. Bull. 26, 846–850.

Rivas, M. L., Spínola, M., Arrieta, H., & Faife - Cabrera, M. (2018). Effect of extreme climatic events resulting in prolonged precipitation on the reproductive output of sea turtles. Animal Conservation, 21(5), 387-395.

Santidrián Tomillo, P., Roberts, S. A., Hernández, R., Spotila, J. R., & Paladino, F. V. (2015). Nesting ecology of East Pacific green turtles at Playa Cabuyal, Gulf of Papagayo, Costa Rica. Marine Ecology, 36(3), 506-516..

Spanier, M. J. (2010). Beach erosion and nest site selection by the leatherback sea turtle Dermochelys coriacea (Testudines: Dermochelyidae) and implications for management practices at Playa Gandoca, Costa Rica. Revista de biologia tropical, 58(4), 1237-1246.

Stanford, C. B., Iverson, J. B., Rhodin, A. G., van Dijk, P. P., Mittermeier, R. A., Kuchling, G., Berry, K. H., Bertolero, A., Bjorndal, K. A., Blanck, T. E., Buhlmann, K. A., Burke, R. L., Congdon, J. D., Diagne, T., Edwards, T., Eisemberg, C. C., Ennen, J. R., Forero-Medina, G., ... & Walde, A. D. (2020). Turtles and tortoises are in trouble. Current Biology, 30(12), R721-R735.

Summers, T. M., Martin, S. L., Hapdei, J. R., Ruak, J. K., & Jones, T. T. (2018). Endangered green turtles (Chelonia mydas) of the Northern Mariana Islands: Nesting ecology, poaching, and climate concerns. Frontiers in Marine Science, 4, 428.

Sydeman, W. J., Poloczanska, E., Reed, T. E., & Thompson, S. A. (2015). Climate change and marine vertebrates. Science, 350(6262), 772–777.

Tiwari, M., Bjorndal, K. A., Bolten, A. B., & Bolker, B. M. (2006). Evaluation of density-dependent processes and green turtle Chelonia mydas hatchling production at Tortuguero, Costa Rica. Marine Ecology Progress Series, 326, 283-293.

Tomillo, P. S., Wallace, B. P., Paladino, F. V., Spotila, J. R., & Genovart, M. (2021). Short-term gain, long-term loss: How a widely-used conservation tool could further threaten sea turtles. Biological Conservation, 261, 109260.

Williamson, S. A., Evans, R. G., & Reina, R. D. (2017). When is embryonic arrest broken in turtle eggs?. Physiological and Biochemical Zoology, 90(5), 523-532.

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.

Wyneken, J., Burke, T. J., Salmon, M., & Pedersen, D. K. (1988). Egg failure in natural and relocated sea turtle nests. Journal of Herpetology, 88-96.

Zárate, P., Bjorndal, K. A., Parra, M., Dutton, P. H., Seminoff, J. A., & Bolten, A. B. (2013). Hatching and emergence success in green turtle Chelonia mydas nests in the Galápagos Islands. Aquatic Biology, 19(3), 217-229.

Appendix

Table A 1: Precipitation mean (mm) of each month for all 7 study years; calculated annual mean in the last column and calculated monthly mean over the 7 years in the last row. Data has been taken from Climate Change Knowledge Portal, World Bank Group.

	March	April	May	June	July	August	September	October	November	December	Annual mean
2014	139.87	282.68	297.89	346.01	417.73	363.50	358.19	356.08	318.41	382.17	291.57
2015	110.97	246.91	136.75	275.26	337.12	366.43	299.95	242.65	341.44	286.64	269.47
2016	106.53	103.61	209.07	259.92	451.74	214.48	405.51	229.18	405.78	291.22	255.11
2017	177.67	172.11	422.21	192.59	474.49	174.50	153.26	744.62	225.98	384.29	247.89
2018	162.88	191.08	392.58	530.55	383.78	338.02	309.39	372.99	311.00	285.63	338.31
2019	229.33	187.62	538.03	424.79	327.82	301.40	289.87	355.49	526.93	381.09	311.99
2021	164.86	295.95	420.36	228.51	474.84	383.93	323.81	433.95	326.44	275.98	310.03
Monthly mean	156.02	211.42	345.27	322.52	409.65	306.04	305.71	390.71	350.85	326.72	

Table A 2: Temperature mean (°C) of each month for all 7 study years; calculated annual mean in the last column and calculated monthly mean over the 7 years in the last row. Data has been taken from Climate Change Knowledge Portal, World Bank Group.

	March	April	May	June	July	August	September	October	November	December	Annual mean
2014	24.78	25.38	24.91	24.66	25.40	24.62	23.97	23.84	23.30	23.13	24.32
2015	24.22	25.23	24.96	24.39	24.75	24.83	24.44	24.44	24.17	24.35	24.43
2016	25.27	25.28	25.67	24.70	24.94	24.91	24.31	23.98	22.94	23.34	24.42
2017	24.06	25.75	25.24	24.48	24.84	24.18	24.13	23.28	23.67	23.76	24.16
2018	24.18	24.61	24.28	24.29	24.45	23.95	24.11	23.64	23.42	23.31	23.82
2019	24.07	25.01	24.54	24.69	24.89	24.69	24.27	23.54	23.98	23.70	24.19
2021	24.49	25.07	24.61	24.23	24.76	24.11	24.14	24.22	23.50	23.39	24.18
Monthly mean	24.44	25.19	24.89	24.49	24.86	24.47	24.20	23.85	23.57	23.57	

Table A 3: Tide mean (cm) of each month for all 7 study years; calculated annual mean in the last column and calculated monthly mean over the 7 years in the last row. Research quality data has been given by UHSLC station 268 Limón, Costa Rica from 01st January 2014 to 31st December 2018. Fast delivery data from UHSLC station 268 Limón, Costa Rica covered the period from 01st January 2019 to 18th April 2021. Data from 19th April 2021 to 31st December 2021 was estimated through the linear regression with fast delivery data from UHSLC station 739 El Porvenir, Panama.

	March	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**	**Annual mean**
2014	467.45	484.37	515.19	526.20	545.81	523.74	558.50	566.74	568.63	598.48	528.77
2015	488.42	523.93	542.13	481.67	518.68	601.97	568.13	574.71	589.30	580.26	541.92
2016	497.65	528.13	571.39	504.30	511.16	538.32	567.40	573.23	563.27	466.06	529.75
2017	527.39	513.67	535.68	544.30	556.39	512.45	569.63	607.13	592.03	539.84	543.77
2018	536.61	512.20	499.06	472.27	527.71	557.13	622.80	558.71	558.07	550.26	531.92
2019	517.61	542.60	551.26	528.17	588.23	609.23	595.87	578.55	596.00	619.16	556.70
2021	517.42	533.97	529.65	542.00	526.68	521.29	579.17	622.87	635.90	582.77	552.07
Monthly mean	507.51	519.84	534.91	514.13	539.24	552.02	580.21	583.13	586.17	562.41	

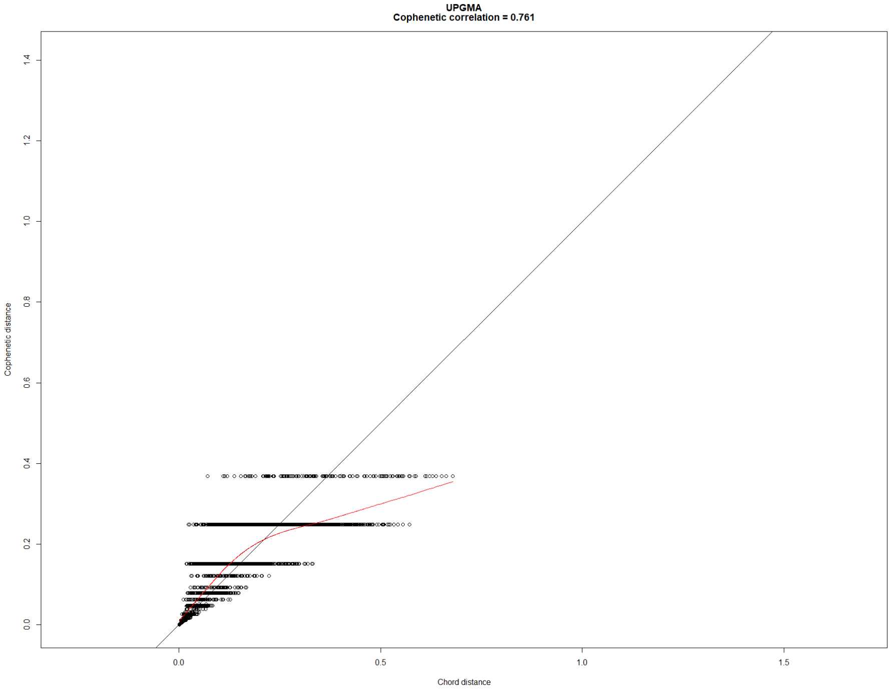


Figure A 1: Cophenetic correlation of UPGMA-dendrogram and cophenetic correlation coefficient = 0.761.

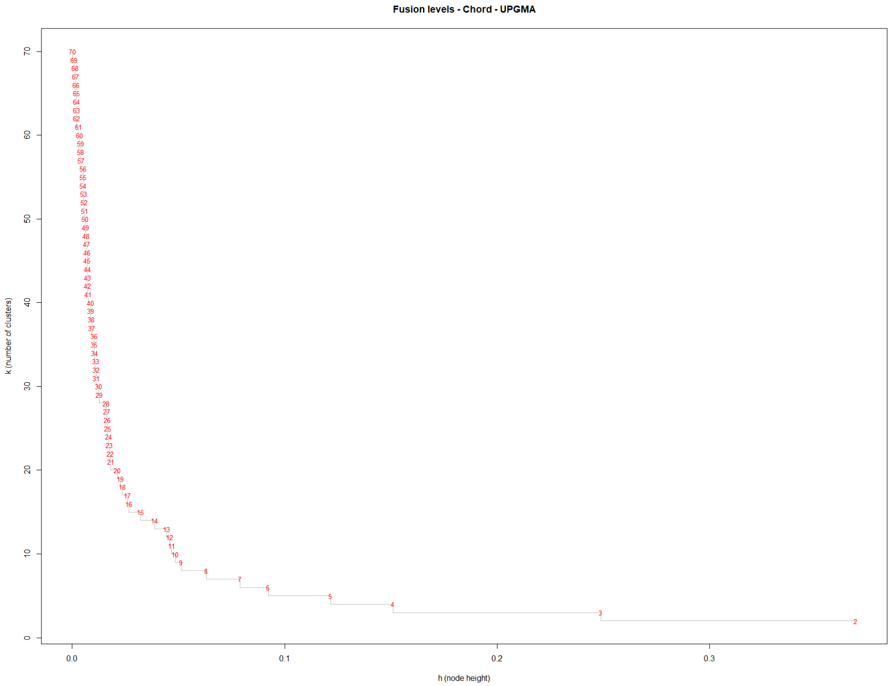


Figure A 2: Fusion levels calculated for the UPGMA-dendrogram.

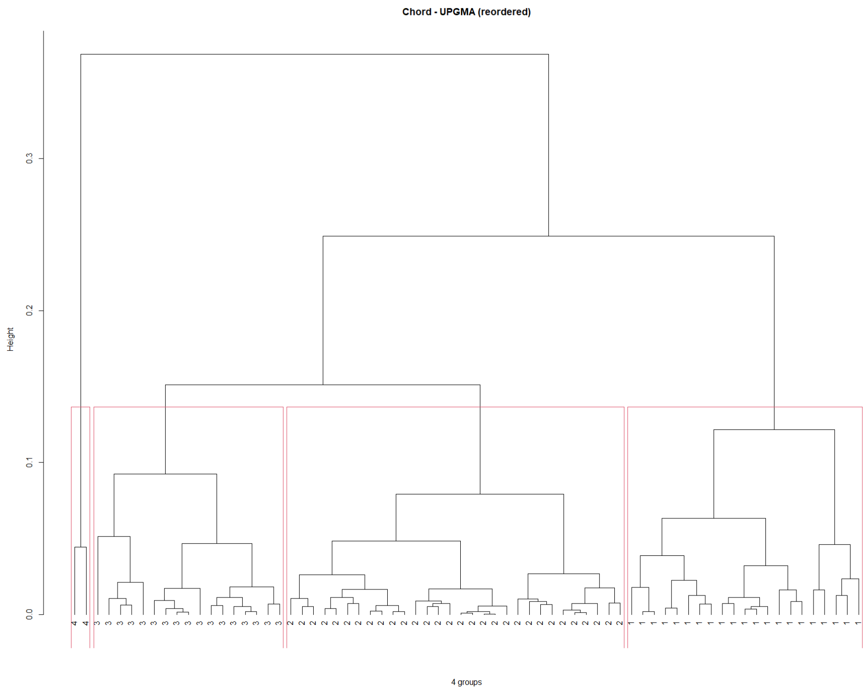


Figure A 3: Branches of UPGMA-dendrogram were cut to form 4 groups (in red squares)

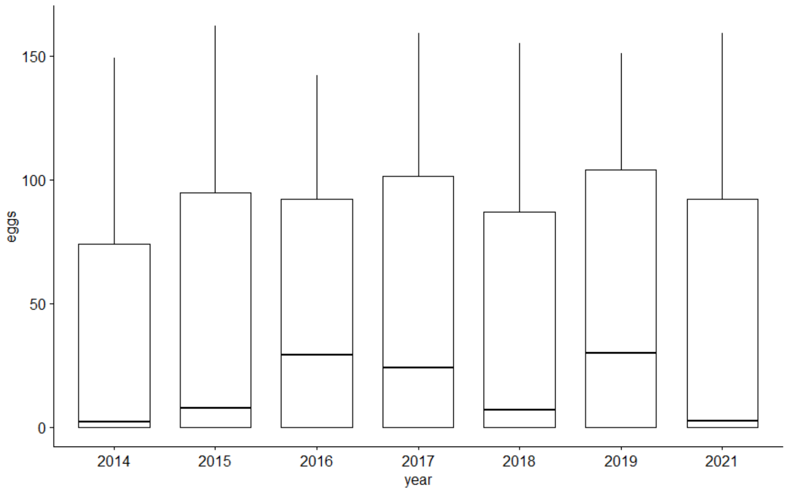


Figure A 4: Raw number of successful eggs per year.

Table A 4: Significant differences between causes of egg loss in each year.

	Dog Predation	Erosion	Flooding	Maternal Effects	Natural Predation	Poaching	Temperature
Dog Predation							
Erosion							
Flooding							
Maternal Effects	2014 2015 2016 2017 2018 2019 2021	2014 2015 2016 2018 2021	2014 2015 2019 2021				
Natural Predation	2014 2015 2016 2017 2018 2019 2021	2014 2015 2016 2021	2014 2015 2019 2021				
Poaching				2014 2015 2016 2017 2018 2019 2021	2014 2015 2016 2017 2018 2019 2021		
Temperature	2014 2015 2016 2017 2018 2019 2021	2014 2015 2016 2021	2014 2019 2021			2014 2015 2016 2017 2018 2019 2021	
Wet	2016			2014 2015 2016	2014	2016	2014