HATCHING FAILURE IN *EX-SITU* NESTS OF HAWKSBILL TURTLE (*Eretmochelys imbricata*) IN MELAKA, MALAYSIA

SEE KHAI WEI

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by

SEE KHAI WEI

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KEGAGALAN PENETASAN DALAM SARANG *EX-SITU* BAGI PENYU KARAH (*Eretmochelys imbricata*) DI MELAKA, MALAYSIA

ABSTRAK

Aktiviti bertelur bagi penyu karah (*Eretmochelys imbricata*, Linnaeus 1766) di Melaka, Malaysia menghadapi banyak tekanan daripada aktiviti manusia di sepanjang pesisiran pantai. Tapak penetasan penyu oleh Jabatan Perikanan di Padang Kemunting, Melaka merupakan tempat untuk pemuliharaan *ex-situ* serta pengurusan yang penting kerana terdapat pelbagai ancaman di pantai di mana penyu bertelur seperti pemungutan telur tanpa lesen, pembanjiran sarang serta pemangsa semula jadi. Pemuliharaan *ex-situ* berkemungkinan menyebabkan peratus penetasan yang rendah dalam sarang penyu. Kajian ini menyelidiki faktor-faktor yang menyebabkan kegagalan penetasan dalam sarang *ex-situ* bagi penyu karah dengan menggunakan beberapa pendekatan selama tiga musim dari 2018 hingga 2020, dari 13 pantai di Melaka. Untuk menganalisis peratus penetasan dan kematian telur, 241 sarang telah digali untuk menentukan jumlah telur yang berjaya menetas dan tahap perkembangan bagi telur yang tidak menetas. Purata peratus penetasan yang diperolehi ialah 58.2 ± 28.2% (n = 241). Di antara telur yang tidak menetas (n = 9,872), 70.8% tidak berkembang, 11.2% merupakan tahap embrio awal, 1.1% merupakan tahap embrio pertengahan, 6.3% merupakan tahap embrio akhir, 5.3% menghadapi pemangsa semula jadi dan 5.3% lagi telah reput. Dalam lapan pembolehubah yang dianalisis, hanya dua pembolehubah didapati mempengaruhi peratus penetasan atau kematian telur dalam kajian ini, iaitu tempoh pengeraman telur dan jumlah telur yang dieram dalam satu sarang. Sebahagian besar telur yang tidak menunjukkan tanda-tanda perkembangan telah menimbulkan persoalan bahawa telur tersebut tidak disenyawakan. Kesuburan telur telah ditentukan dengan menggunakan kaedah untuk menemui tompok putih pada telur yang dilakukan pada awal pengeraman telur dalam 50 sarang. Purata kesuburan telur ialah 85.9 ± 16.5% (n = 50), tetapi peratus penetasannya lebih rendah pada 57.6 ± 24.3% (n = 50). Hasil kajian ini menunjukkan bahawa kesuburan telur tidak menjamin peratus penetasan dan ketidaksuburan bukan penyebab kepada kegagalan penetasan. Kehadiran kulat yang dinamakan kompleks spesies *Fusarium solani* (FSSC) dalam sampel penyu, pasir sarang dan telur juga diperiksa sebagai salah satu penyebab kepada kegagalan penetasan. Sampel telah dikumpul daripada tujuh individu penyu karah betina dan sarangnya untuk pemencilan FSSC, yang dikenal pasti berdasarkan ciri-ciri morfologi. Walaupun FSSC dipencilkan daripada 96.9% (n = 32) daripada telur yang tidak menetas dari tujuh sarang yang dikaji, sarang-sarang tersebut turut didapati mempunyai peratus penetasan yang tinggi, dengan purata 85.8 ± 10.5% (n = 7). Kehadiran FSSC dalam sarang tidak menjejaskan peratus penetasannya. Terdapat dua kemungkinan bagi peranan FSSC dalam telur penyu, iaitu patogen primer dan saprofit. Patogen primer menyebabkan penyakit dan kematian dalam telur penyu yang masih hidup, manakala saprofit mendiami telur yang telah mati. Kajian ini menunjukkan kepentingan untuk mengekalkan amalan yang ketat dalam pengurusan tapak penetasan untuk mencapai peratus penetasan yang tinggi dalam pemuliharaan *ex-situ*. Selain peratus penetasan dan kematian telur, penyelidikan dan pemantauan aspek-aspek seperti parameter persekitaran sarang, kecergasan anak penyu dan nisbah jantina anak penyu dalam tapak penetasan juga perlu diutamakan.

HATCHING FAILURE IN *EX-SITU* NESTS OF HAWKSBILL TURTLE (*Eretmochelys imbricata*) IN MELAKA, MALAYSIA

ABSTRACT

Hawksbill turtle (*Eretmochelys imbricata*, Linnaeus 1766) nesting in Melaka, Malaysia faces enormous pressures from various human activities along its coastline. The hatchery of the Department of Fisheries at Padang Kemunting, Melaka provides an important *ex-situ* conservation and management tool as threats such as egg poaching, inundation, and natural predation are prevalent at nesting beaches. *Ex-situ* conservation has been implicated in causing low hatching success to sea turtle nests. This study aims to identify the factors that contribute to hatching failure in *ex-situ* nests of hawksbill turtle using several approaches during three nesting seasons from 2018 to 2020, from 13 nesting beaches in Melaka. To analyse hatching success and egg mortality, post-emergence nest excavation was conducted on 241 clutches to determine the number of successfully hatched eggs and the stages of the unhatched eggs. The mean hatching success was 58.2 ± 28.2% (n = 241). Among the unhatched eggs (n = 9,872), 70.8% were undeveloped, 11.2% were in early embryonic stage, 1.1% were in mid embryonic stage, 6.3% were in late embryonic stage, 5.3% were depredated, and another 5.3% were decayed. Analysing eight potential determinants, only two variables were found to affect hatching success or egg mortality in this study, which were the incubation period and clutch size of the nests. The large number of undeveloped eggs led to the concern that the eggs were unfertilised. Egg fertility was determined using the white spot method conducted at the beginning of egg incubation on 50 clutches. The mean egg fertility was 85.9 ± 16.5% (n = 50), but the mean hatching success was significantly lower at 57.6 ± 24.3% (n = 50). In this study, the

results showed that egg fertility did not reflect hatching success and infertility was unlikely the cause of hatching failure. Presence of the fungi called *Fusarium solani* species complex (FSSC) in nesting sea turtles, nest sand, and eggs was also assessed as a cause of hatching failure. Samples were collected from seven nesting sea turtles and their corresponding nests for the isolation of FSSC, identified based on its morphological characteristics. Although FSSC was isolated from 96.9% (n = 32) of the unhatched eggs sampled from the seven study nests, the nests from which it was sampled were found to have high hatching success as well, with a mean percentage of 85.8 ± 10.5% (n = 7). FSSC was present in the nest but did not compromise the hatching success of the entire egg clutch. There are two possible roles of FSSC in sea turtle eggs, namely primary pathogen and saprophyte. A primary pathogen causes diseases and mortalities in live sea turtle eggs, while a saprophyte colonises eggs that have died. For *ex-situ* conservation, it is important to maintain best hatchery practices to attain high hatching success. Apart from hatching success and egg mortality, it is also essential to prioritise the research and monitoring of aspects like nest environment parameters, hatchling fitness, and hatchling sex ratio in the hatchery.

CHAPTER 1

INTRODUCTION

1.1 Background

Human activities causing the decline of sea turtle populations is a global problem. Modification of habitat, overutilisation, fishery bycatch, pollution and debris are some of the factors affecting sea turtles in all of their life stages (Seminoff et al., 2015). The world's seven extant sea turtle species include the hawksbill turtle (*Eretmochelys imbricata*), green turtle (*Chelonia mydas*), leatherback turtle (*Dermochelys coriacea*), olive ridley turtle (*Lepidochelys olivacea*), Kemp's ridley turtle (*Lepidochelys kempii*), loggerhead turtle (*Caretta caretta*), and flatback turtle (*Natator depressus*) (Meylan & Meylan, 1999), with the first four of these species found in Malaysia (Chan, 2006), and the study species is hawksbill turtle. Globally, the hawksbill turtle faces an extremely high risk of extinction in the wild and it is listed as Critically Endangered (CR) in International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2021). Hawksbill turtles are distributed in the tropical and subtropical regions of the Atlantic, Pacific and Indian Oceans, with their habitats including coastal reefs, bays, lagoons, and estuaries (Mortimer & Donnelly, 2008; Witzell, 1983). They nest in at least 70 countries but have low densities in most of the countries. There is concern about continuing losses of hawksbill populations in Southeast Asia (Mortimer & Donnelly, 2008). Hawksbill turtle nesting is uncommon in Malaysia, with only two remaining sites with high nesting frequency, which are on the northern beaches of Melaka and Sabah Turtle Islands, with remnant populations in

Terengganu (e.g., Redang Island), Johor (e.g., Tengah Island), and Pahang (e.g., Tioman Island) (Chan, 2006; Mortimer et al., 1993).

Sea turtle eggs are vulnerable to multiple natural threats such as inundation by sea water (Pike et al., 2015), nest destruction by beach erosion (García et al., 2003), and predation risk by animals like ghost crabs, monitor lizards, and insect larvae (Ali et al., 2004; Donlan et al., 2004; Maulany et al., 2012b). Apart from the natural threats, a serious threat to sea turtle eggs in Malaysia is poaching activity. Due to the demand for sea turtle eggs for consumption, eggs are poached and sold as a source of income for some villagers living near sea turtle nesting beaches (Mohd Jani et al., 2020; WWF-Malaysia, 2009). As a result, relocating eggs to a sea turtle hatchery is an important strategy for sea turtle conservation (*ex-situ* conservation) in Malaysia, which is also used worldwide. However, opposing views exist between researchers on the impact of hatcheries as a conservation strategy due to the contrasting outcomes of *ex-situ* nest hatching success among different nesting sites around the world (García et al., 2003; Sari & Kaska, 2017). Some studies found that *ex-situ* nests had lower hatching success than *in-situ* nests (Baptistotte et al., 2003; Candan, 2018), while others showed higher hatching success in *ex-situ* nests compared to *in-situ* nests (Ilgaz et al., 2011; Tuttle & Rostal, 2010). Despite this controversy, studies on hatching success and egg mortality have been lacking for the hatcheries in Malaysia. Studies on green turtles in Malaysia (Salleh & Sah, 2014; Abd Mutalib et al. 2015) assessed the effects of nest depth, hatchery shading and nesting season on hatching success in hatcheries in Kerachut, Penang and Setiu, Terengganu, but did not study the potential causes of egg mortality at different developmental stages. No other research has been done on the other factors that could influence the hatching success of sea turtle eggs incubated in the hatcheries in Malaysia.

In Melaka, the conservation of hawksbill turtles is managed jointly by the Department of Fisheries Melaka (DoF Melaka) and World Wide Fund for Nature Malaysia (WWF-Malaysia). Padang Kemunting hatchery, located on one of the primary nesting beaches in Melaka, has been established since 1990 (Mortimer et al., 1993). The hatchery acts as an *ex-situ* site for the incubation and hatching of sea turtle eggs. Hawksbill turtles nest year-round in Melaka, with peak nesting season from May to August (Salleh et al., 2018). Hawksbill turtles' total nest numbers in a year have been ranging from 383 to 807 nests in the recent five years from 2016 to 2020, with an average of 583 nests per year (DoF Melaka, unpublished data). Eggs from the *in-situ* nests of nesting sea turtles are collected and relocated to the hatchery site for incubation. The establishment of the hatchery is crucial because eggs can be secured from poaching activity and the incubation of turtle eggs can be monitored. Due to the high level of poaching, the focus of conservation efforts has been on securing more eggs for protection and incubation in the hatchery. While it is critical to protect sea turtle eggs from poaching, egg mortality and the factors that have resulted in hatching failure have never been investigated in Melaka. Even though hatching success data is being collected, the data has not been analysed as to what had caused low hatching success. Consequently, the determinants of hatching success are not known at this study site.

Hatching success is the percentage of hatchlings hatched from the eggs relative to the total number of eggs incubated in each clutch (Miller, 1999). For this research work, the term hatching success is used when referring to the hatching percentage of sea turtle clutches, as well as when analysing the determinants of hatching success. This is also a term commonly used in sea turtle studies. On the other hand, the term hatching failure is used to emphasise the condition whereby the eggs failed to hatch. Both of these terms are used interchangeably in this study.

1.2 Problem statement

Despite the hawksbill turtle's status as a Critically Endangered sea turtle species, in addition to Melaka as an important hawksbill turtle nesting rookery in Malaysia, there is a paucity of published research on hawksbill turtles in Malaysia. Studies on Malaysia's hawksbills include nesting ecology (Chan, 2010; Mortimer et al., 1993; Salleh et al., 2018), hatchlings' offshore migration (Chung et al., 2009), multiple paternity in clutches (Joseph & Shaw, 2011), and genetic differences of turtles between nesting rookeries (Nishizawa et al., 2016). Apart from the study by Chan (2013) that briefly reported the hatching success of hawksbills in Terengganu, no other study has looked into hatching success and the factors that could affect egg mortality. In Melaka, hawksbill turtle eggs are relocated to the hatchery to protect them from poaching threats, but they face another threat in the hatchery, which is low hatching success, according to DoF Melaka (S. Mastura, personal communication, April 3, 2018). Due to the scarcity of information, it is not possible to indicate the factors that may have contributed to the hatching failure of sea turtle eggs in Melaka. This issue could limit the number of hatchlings that could make it to the sea and recruit into the adult population in the future.

Since the causes of low hatching success has not been established by previous works at this study site, this present study will explore different variables that could affect hatching success. Inspection of the developmental stages of dead embryos can reveal potential causes of egg mortality (Miller et al., 2017). Eckert & Eckert (1990) suggested that if egg mortality is observed in very early embryonic stages, it may be caused by rough handling during relocation, while for deaths observed in late embryonic stages, the causal factors may be the microenvironment of the hatchery site or the shape of the new egg chamber. Since the determinants for hatching success can vary as stated above, different variables must be included in the analysis of hatching success and egg mortality in order to identify or to rule out the causes of hatching failure.

Before this study was conducted, it was observed in the hatchery that the unhatched eggs primarily failed at the undeveloped stage. The hatchery manager, WWF-Malaysia, suggested that undeveloped eggs at this study site could be unfertilised due to the possible infertility of sea turtles (N. Bariyah, personal communication, April 3, 2018). Infertility of sea turtles could happen if there is a lack of males in the adult population (Jensen et al., 2018). However, several studies at different nesting sites indicated that sea turtle egg fertility is generally higher than 93% (Abella et al., 2017; Miller et al., 2003; Rafferty & Reina, 2014; Rings et al., 2015). It is a problem in Melaka where both adult sex ratio and egg fertility are not determined at this site. Consequently, it is essential to determine the egg fertility of hawksbill turtles in Melaka before concluding that hatching failure is due to infertility and the lack of males.

Previous research from other nesting sites has further implicated that sea turtle egg mortality is caused by the fungal infection of the *Fusarium solani* species complex (FSSC) (Sarmiento-Ramírez et al., 2010, 2014). FSSC has been isolated from sea turtle eggs in nesting sites ranging from Central and South America to Africa and Australia (Sarmiento-Ramírez et al., 2014). A preliminary analysis conducted for this study also discovered that *Fusarium* sp. was indeed present in the eggshells of unhatched hawksbill turtle eggs, but could not be found in the hatchery nest sand. It is therefore important to sample extensively from the nesting beaches to the hatchery site to determine the source of FSSC. Data on the developmental stages of unhatched eggs and hatching success of nests associated with FSSC must also be collected to assess if FSSC is truly a threat to sea turtle eggs at this study site.

The information available for sea turtle's hatching failure in Melaka is very limited. It is not known if the relocation of eggs and the use of a hatchery to incubate the eggs have implicated negative outcomes on the eggs or whether the mother turtles have contributed to hatching failure. To address these gaps, the present study will look at the determinants of hatching success and egg mortality, egg fertility, and FSSC at nesting and hatchery sites in order to identify the causes of hatching failure.

1.3 Significance of research

Sea turtle conservation and management efforts should not be limited to securing eggs from the threats of natural causes and poaching. The multi-faceted approach used in this study is significant in identifying the threats to low hatching success, which included uncovering the determinants of hatching success and egg mortality, determining egg fertility, and recognising the impact of FSSC. This study aimed at filling in the gaps for a better understanding of hatching failure, as well as to provide recommendations and future directions for improving hawksbill turtle conservation in Padang Kemunting hatchery, Melaka. This study can also provide the local context of hatching failure in Melaka's hatchery, with implications for other hatcheries in Malaysia and around the world. In fact, determining the factors crucial for sustained hatchling production was recognised by an international group of 35 sea turtle scientists as one of the research priorities for management and conservation of sea turtles around the world (Hamann et al., 2010). Improving our knowledge of the factors that influence hatching success and thus hatchling production will help determine the measures required to ensure the long-term survival of sea turtle populations (Rees et al., 2016).

1.4 Aim and objectives

Given the significance of hatchling production in a Critically Endangered sea turtle species, this study aimed to identify the factors that contribute to hatching failure in *ex-situ* nests of hawksbill turtles in Melaka. Therefore, the main objectives of this study were:

- To analyse hatching success, egg mortality, and the determinants in *ex-situ* nests of hawksbill turtles in Melaka.
- To determine the egg fertility for hawksbill turtles in Melaka.
- To assess the presence of *Fusarium solani* species complex (FSSC) in nesting sea turtles, nest sand, and eggs of hawksbill turtles in Melaka.

1.5 Research questions and hypotheses

This study aimed to address research questions regarding the hatching failure of hawksbill turtles and the hypotheses of this study were as follows:

- Do nest incubation variables, sea turtle nesting variables, and spatiotemporal variables affect hatching success and egg mortality of the *ex-situ* nests of hawksbill turtles in Melaka?

  H_0: There is no relationship between nest incubation variables, sea turtle nesting variables, spatiotemporal variables and hatching success and egg mortality.

  H_a: There are relationships between nest incubation variables, sea turtle nesting variables, spatiotemporal variables and hatching success and egg mortality.

- Is there any difference in determining egg fertility using two different methods (i.e., white spot method and post-emergence nest excavation method) and does egg fertility affect hatching success?

  H_0: There is no significant difference between white spot fertility, apparent fertility, and hatching success.

  H_a: There are significant differences between white spot fertility, apparent fertility, and hatching success.

- What is the source of *Fusarium solani* species complex (FSSC) in the eggs of hawksbill turtles in Melaka?

  As this topic did not involve any hypothesis testing, the general hypothesis was that *Fusarium solani* species complex (FSSC) is expected to be isolated from nesting sea turtles, nest sand, and eggs of hawksbill turtles in Melaka.

1.6 Structure of thesis

Chapter 1 gives an introduction to the study including the background of the study. Problem statement and significance of research are also discussed, leading to the main objectives and research questions of the study. Limitations encountered during the execution of this study are stated as well.

Chapter 2 presents the literature on the general life cycle of sea turtles, egg development, threats to sea turtles, and conservation efforts. The chapter closes with a review on the protection and management of sea turtles in Malaysia.

Chapter 3 examines the hatching success and egg mortality of *ex-situ* nests of hawksbill turtles. The effects of several variables on the hatching success and egg mortality at different developmental stages are analysed.

Chapter 4 assesses egg fertility of hawksbill turtles in Melaka. Egg fertility was determined using white spot method and nest excavation method, and both methods were then compared between each other. The relationship between egg fertility and hatching success is also discussed.

Chapter 5 addresses the presence of *Fusarium solani* species complex (FSSC) in nesting sea turtles, including their nests and eggs, discussing the sources and roles of FSSC in unhatched eggs.

Chapter 6 represents the overall discussion from all findings to establish explanations to the research questions. Recommendations for hatchery practices and management, and opportunities for further research in this field are described.

Chapter 7 concludes the study along with future directions for the conservation of hawksbill turtles in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 General life cycle of sea turtles

Sea turtle is a long-lived, wide-ranging, and migratory marine species (Meylan & Meylan, 1999). Many of the behaviours and life cycle patterns of sea turtles are similar across species, and thus, the approaches for studying and managing sea turtles are relatively similar as well (Meylan & Meylan, 1999). There are, however, some variations between different species, for example, leatherbacks prefer a diet with gelatinous organisms (e.g., jellyfish) while the green turtles live on a herbivorous diet (e.g., seagrass) (Bjorndal, 1997). Nevertheless, the life cycle of sea turtles is described in general in the following, with a focus on the study species, the hawksbill turtle.

Beginning with the early life stage of sea turtles, newly hatched sea turtles typically emerge from the nests in groups at night. Hatchlings from the same clutch work together to dig upwards through the nest chamber to escape to the surface sand (Witzell, 1983). This synchronous activity of the hatchlings reduces the time taken to emerge and saves each individual's energy reserve (Rusli et al., 2016). Hatchlings then find their way to the sea using visual cues, moving towards the bright ocean horizon (Lohmann & Lohmann, 1996). Upon reaching the sea, hatchlings engage in swimming frenzies so that they can swiftly escape from the predator-rich neritic zone and into the relatively safer oceanic zone (Bolten, 2003; Witzell, 1983). Movements of the post-hatchlings in the oceanic zone are mostly passive because they drift with the ocean currents (Bolten, 2003; Meylan & Meylan, 1999), though they may employ magnetic cues for orientation (Lohmann & Lohmann, 1996). Post-hatchlings mainly feed on small and non-motile food items like hydroids, copepods, pleuston, macroalgae, and seagrasses (Witherington, 2002). Sea turtles remain in the oceanic zone and enter the early juvenile development which may last for several years.

Once juvenile hawksbill turtles grow to a carapace length of around 20 to 35 cm, they undergo ontogenetic habitat shifts and move from the oceanic zone to the neritic foraging grounds to maximize their growth rates (Bolten, 2003; Meylan & Meylan, 1999). They settle in the neritic zone and continue to grow and mature to adulthood. Neritic juveniles primarily feed on invertebrates including sponges and tunicates, and may opportunistically consume macroalgae (Carrión-Cortez et al., 2013). Hawksbills' age to maturity can range from 15 to 22 years (Diez & van Dam, 2002; Snover et al., 2012). As with other sea turtle species, hawksbill turtles undertake long distance migrations from the foraging grounds to breeding grounds when they reach sexual maturity (Bolten, 2003; Meylan & Meylan, 1999). Both males and females exhibit natal homing where they perform reproductive migrations to the waters near their natal beach (Velez-Zuazo et al., 2008). Successful reproduction in sea turtles necessitates a unique set of environmental factors and an ideal reproductive habitat is uncommon (Lohmann et al., 1999). Determining whether a new location is favourable for reproduction is probably challenging for a sea turtle. The fact that an adult animal exists proves that the natal beach possesses the necessary qualities for successful reproduction. This could be why natural selection favoured sea turtles returning to their natal beach to reproduce (Lohmann et al., 2013).

During copulation, a male turtle mounts onto the female's carapace using his claws and tail. A female turtle may mate with several males in a nesting season, showing a polyandrous mating behaviour. Only females will return to the terrestrial habitats to lay eggs (Witzell, 1983). Sea turtles in general also act as nutrients and energy transporters between marine and terrestrial ecosystems. By laying eggs on the beach, nutrients and energy are transferred from their foraging grounds in the oceans to nesting beaches. Nest remains after hatchling emergence (e.g., eggshells, unhatched eggs, and dead hatchlings) become nutrients to the inhabitants of beach ecosystems including plants, detritivores, and decomposers (Bouchard & Bjorndal, 2000). After mating in the breeding grounds, the adult turtles return to their foraging grounds. They spend the most of their lives in the foraging grounds except during reproduction (Meylan & Meylan, 1999). Hawksbill turtles have important ecological roles in the marine and coastal food webs (Mortimer & Donnelly, 2008). They help maintain healthy reef ecosystems by feeding on several benthic invertebrates like sponges and corallimorpharians, which are the aggressive competitors to the corals for space on reefs (León & Bjorndal, 2002).

2.2 Nesting

A few weeks after mating in the coastal waters near nesting beach, female turtles will emerge to nest at the beach generally at night (Sea Turtles of India, 2011; Witzell, 1983). Females deposit their eggs, then return to the sea without providing parental care for the eggs or hatchlings (Ali et al., 2004). The nesting behaviour of sea turtles is called natal homing in which hatchlings migrate long distances away from their natal beaches, and later go back to the same geographic area where they were born to lay eggs as adults (Lohmann et al., 2013). Hawksbill turtles have been observed laying one to five clutches in a nesting season, with interclutch intervals of two to three weeks, and they remigrate to nesting beaches every two to four years (Ditmer &

Stapleton, 2012; Witzell, 1983). Some of these variations in sea turtle nesting were analysed in the present study and will be presented in subsequent chapters.

A female turtle displays several stages during the nesting process. Firstly, the turtle will emerge and move up to the beach from the sea. She then begins to select a site suitable for nesting above the high tide line (Ali et al., 2004; Sea Turtles of India, 2011). Hawksbill turtles have shown preferences for nesting in the woody vegetation zone on the beach (Salleh et al., 2018). After selecting a site, the turtle will start to position herself in a body pit by clearing the surface sand using her front and rear flippers. She will then start to construct an egg chamber in the shape of a flask by digging in and clearing more sand with her rear flippers (Ali et al., 2004; Sea Turtles of India, 2011; Witzell, 1983). Oviposition begins and two to three eggs are dropped from the cloaca at one time until all eggs are deposited. There are great variations in the number of eggs laid in each clutch for different hawksbill turtle populations, the mean could range from around 70 to 180 eggs per clutch (Witzell, 1983). After oviposition is completed, the turtle starts to cover and fill in the egg chamber with sand by using her rear flippers. Nest sand is compressed and compacted with the tail or the body. The turtle then covers the body pit and camouflages the nest site by throwing sand all around using both front and rear flippers. Lastly, the turtle traverses down the beach and returns to the sea (Ali et al., 2004; Sea Turtles of India, 2011; Witzell, 1983).

At this point onwards, the eggs are very vulnerable because they are left unattended and face the most risks, such as natural predation or an even greater threat, i.e., egg poaching. In this study, oviposited eggs were all translocated to the nearest hatchery of the Department of Fisheries (DoF) to save them from being poached.

2.3 Threats to sea turtles

2.3.1 Natural threats

Natural threats to sea turtle nests on the beaches include sea water wash over or tidal inundation of the nests. Tidal inundation can have a significant impact on hatching success, and the worst case scenario is the destruction of the entire egg clutch (Başkale & Kaska 2005; Pike et al., 2015; Tuttle & Rostal, 2010). The development of eggs is harmed by incubation in sand with extremely high level of moisture content (McGehee, 1990; Tuttle & Rostal, 2010). When the eggs are soaked in an inundated nest, gas exchange is hampered, affecting oxygen diffusion from the surroundings to the eggs (McGehee, 1990; Ware & Fuentes 2018). Besides, high tides during tropical storms can also cause beach erosion which can result in nest loss (Ahles & Milton, 2016; García et al., 2003). Beach erosion has destroyed around 40 to 60% of sea turtle nests in places like St. Croix, US Virgin Islands (Eckert & Eckert 1990), and Playa Cuixmala, Mexico (García et al., 2003). Sea level rise would make nesting beaches even more vulnerable by increasing the frequency of erosion and inundation, which would result in the destruction of more nests (Pike et al., 2015; Reece et al., 2013).

Sea turtles also face the risks from natural predation. Sea turtle eggs and hatchlings suffer higher mortalities from natural predators compared to the adults. Sea turtle eggs have been found to be predated by a variety of animals including foxes, monitor lizards, ghost crabs, and insect larvae (Ali et al., 2004; Başkale & Kaska, 2005; Donlan et al., 2004; Maulany et al., 2012b). Although the rates of nest loss varied, predation can sometimes result in 100% of nest losses (Başkale & Kaska, 2005; Maulany et al., 2012b). Fire ants have also been observed to cause mortalities or inflicting serious injuries on hatchlings just after they had emerged from the eggs

(Parris et al., 2002). For emerging hatchlings on their frenzied crawl from the beach to the sea, they are commonly predated by ghost crabs and birds (Tomillo et al., 2010). After reaching the ocean, hatchlings can be vulnerable to predators due to their small size. They can be eaten by a wide variety of fishes and birds. The risk of predators to sea turtles decreases significantly as they grow due to the changes in body size (Heithaus, 2013). However, adult turtles have also been preyed upon by animals like crocodiles, killer whales, and sharks (Fertl & Fulling, 2007; Heithaus, 2013; Whiting & Whiting, 2011).

2.3.2 Anthropogenic threats

Hawksbill populations worldwide suffered substantial decreases in the 19th and 20th centuries as a result of the extensive and long term exploitation of turtle parts and eggs for food and tortoiseshell trade (Mortimer & Donnelly, 2008). Adult and juvenile hawksbills are hunted for their beautiful carapace to be made into tortoiseshell products. Between 1950 and 1992, Japan as the world's leading importer of tortoiseshell, imported shells equivalent of 1.3 millions of large turtles from throughout the world (Mortimer & Donnelly, 2008). Sea turtles, regardless of species, are also poached for their parts for consumption, such as their meat, blood, and fat, or to make turtle soup and turtle oil (IOSEA, 2014). Besides, egg exploitation is still rampant in many countries, including Malaysia, Indonesia, Mozambique, Maldives, and Bangladesh (IOSEA, 2014). Sea turtle eggs are mostly consumed as a delicacy, as a source of protein, for medicinal purposes, or as an aphrodisiac (IOSEA, 2014; WWF-Malaysia, 2009). The exploitation of sea turtles is mainly driven by socioeconomic factors (e.g., source of income and demand for exotic goods), cultural factors (e.g., traditional beliefs), and political factors (e.g., lack of legislation and enforcement) (IOSEA, 2014). In general, sea turtle's slow development into maturity along with ongoing harvests of sea turtle eggs causing no new recruitments, made it difficult for their populations to recover (Seminoff et al., 2015).

Apart from the exploitation and direct take of sea turtles, their ecosystems are under threat as well in response to the fast growing human population, particularly in the coastal areas with high human densities that often are also major tourist destinations (Antworth et al., 2006). Coastal development, pollution, and fisheries bycatch are some of other anthropogenic factors threatening sea turtle populations. The impacts are extensive, and have been documented as some of the causes of global sea turtle population decline (Witherington & Martin, 2000).

Coastal development causes habitat alteration and increases human presence on sea turtle nesting beaches (Antworth et al., 2006; Kaska et al., 2010; Roe et al., 2013). Changes to the environmental features of the beaches could discourage sea turtles from nesting. For instance, altered chemical and physical properties of sand, beach slope and elevation on developed beach can serve as cues to determine nest site quality by nesting sea turtles (Rizkalla & Savage, 2011; Roe et al., 2013). Sea turtle's nesting activities are higher at undeveloped beaches compared to developed beaches (Kaska et al., 2010; Rizkalla & Savage, 2011; Roe et al., 2013). Turtles were also found to prefer laying their eggs on the undisturbed parts along the beach, where there are little or no human activities (Kaska et al., 2010). In addition, they were found to more likely abandon their nesting attempts at the developed beach rather than at the undeveloped beach (Roe et al., 2013). Another disturbance to turtles caused by humans along coastal areas is light pollution. Artificial light on nesting beaches hinder turtles from emerging from the sea to the beach for nesting (Witherington & Martin, 2000). Hatchlings emerging from the nests also suffer from misorientation because they are attracted to the stronger artificial light instead of the natural light from the sea. When the hatchlings cannot find the sea, they are subjected to death due to dehydration, fatigue, and predation (Harewood & Horrocks, 2008; Witherington & Martin, 2000).

Sea turtles are very vulnerable to the impacts of plastic pollution due to the prevalence of plastic debris in their habitats (Nelms et al., 2016). Sea turtles have been known to ingest marine debris, which can damage and block their digestive system and subsequently causing death (Bugoni et al., 2001; Nelms et al., 2016). Debris pieces instead of food may also take up space in the gut, resulting in dietary dilution and malnutrition in sea turtles, slowing their growth rate (McCauley & Bjorndal, 1999). Plastic ingestion may occur when sea turtles mistake plastics for their natural prey or when plastics are mixed in their usual diet (Nelms et al., 2016). In addition to that, plastic debris accumulated on nesting beaches can impact nesting turtles and emerging hatchlings by obstructing their way to the sea (Ivar do Sul et al., 2011; Nelms et al., 2016; Tomillo et al., 2010).

Fisheries bycatch is recognised as one of the greatest hazards to sea turtle populations (Donlan et al., 2010; Wallace et al., 2010). The use of fishery resources by coastal communities who rely on them to make a living has led to human-turtle conflicts (Yeo et al., 2007). Sea turtles are often accidentally caught as non-target species in fishing gears which cause their mortalities (Gilman et al., 2009). As sea turtles swim in the ocean moving between nesting beaches and foraging grounds, they might encounter fishing operations and become entangled in different types of fishing nets, such as trawl nets, gill nets, and longlines (Gilman et al., 2009; Wallace et al., 2010; Yeo et al., 2007). Sea turtles caught as bycatch might get drowned or seriously injured (Gilman et al., 2009). Despite the fact that certain fishing gears, such as ray nets, can entangle sea turtles and have been banned from use in Malaysia, fishers have been found to continue using them as there were no other substitutes for them and enforcement has been lacking (Yeo et al., 2007). When discarded, these fishing nets (known as ghost nets) continue to become a hazard to sea turtles at sea. Sea turtles can become entangled by them, causing deep cuts to their bodies, most of the time leading to mortalities (Nelms et al., 2016). Entanglement can prevent a sea turtle from escaping threats like predators or boat strikes, as well as restricting a sea turtle's movement to forage, which can lead to death from starvation (Gregory, 2009; Nelms et al., 2016). Propeller and collision injuries in sea turtles have also been observed as a result of boat strikes (Seminoff et al., 2015). Other than these, in areas with high boat traffic near nesting beaches is likely to cause nesting turtles to abandon their nesting attempts as well (Seminoff et al., 2015).

2.4 Sea turtle nest protection

2.4.1 *In-situ* conservation

In-situ conservation of sea turtle nests refers to protecting nests in their original location where the female turtles lay the eggs without disturbing or manipulating the nests during incubation (Boulon, 1999; Shanker et al., 2003). For *in-situ* conservation, the involvement of surveillance personnel such as government officials, staff of conservation programmes, local communities, and volunteers, is important to monitor and to undertake patrolling activities on the nesting beaches (Baptistotte et al., 2003; Boulon, 1999; Shanker et al., 2003). Continuous and regular monitoring and patrolling activities can minimise or prevent a number of threats which include predation and the poaching of eggs. Predators like small mammals, as well as poachers, are less likely to appear when humans are present (Boulon, 1999). Another measure which is crucial for the *in-situ* incubation of sea turtle eggs is to install mesh or screen over the nests to hinder natural predators. Nests are covered with screens buried in the sand to keep predators from digging into the nests (Antworth et al., 2006; Boulon, 1999; Kornaraki et al., 2006). Screening has been shown to be the most effective way of reducing nest predation and thus increasing hatching success (Antworth et al., 2006; Boulon, 1999). In the cases where egg poaching is a problem, sea turtle nests are disguised by removing physical signs of nesting such as turtle tracks and smoothing out the sand to blend in with the surrounding beach area, reducing the possibility of a poacher to find sea turtle nests in the area (Boulon, 1999; Revuelta et al., 2013; Shanker et al., 2003).

2.4.2 *Ex-situ* conservation

Ex-situ conservation of sea turtle nests refers to the relocation of eggs to a protected hatchery site after the oviposition of nesting sea turtles (Abella et al., 2007; Mortimer, 1999; Shanker et al., 2003). It is best not to relocate nests and they should be left *in-situ* whenever possible (Mortimer, 1999; Phillott & Shanker, 2018). For nests to be relocated to a hatchery, there must be continuous threats to eggs in their natural habitat which could cause egg mortality, for example inundation, depredation, poaching, and erosion, whereby *in-situ* protection is not possible (Mortimer, 1999; Phillott & Shanker, 2018). Hatcheries should be constructed as close to nesting beaches as possible to reduce relocation time and physical impact on sea turtle eggs.

Besides, to avoid underground flooding as well as tidal inundation of the eggs, hatcheries should also be placed above the high tide line (Mortimer, 1999).

For egg relocation to the hatchery, it is the most desirable to collect, transport, and rebury eggs in the hatchery within two hours of oviposition (Ali et al., 2004; Mortimer, 1999; Shanker et al., 2003). For eggs relocated more than two hours after oviposition, it is recommended that eggs are handled with caution to avoid breaking the fragile embryonic membranes and dislodging the embryos (Ali et al., 2004; Mortimer, 1999). Eggs can be collected straight from the cloaca of the turtle during oviposition, or dug out from the egg chamber after the turtle returns to the sea (Mortimer, 1999; Shanker et al., 2003). Eggs should be transported while retaining the original orientation of the eggs, avoid physical shocks to eggs, and are carried in clean and rigid containers (Abella et al., 2007; Ali et al., 2004; Mortimer, 1999; Shanker et al., 2003).

In the hatchery, each egg clutch should be reburied in a microhabitat that is as similar to its *in-situ* nest as possible (Mortimer, 1999; Shanker et al., 2003). The *ex-situ* nest should be built in the form of a flask, whereby the egg chamber has a flask shaped rounded bottom with a narrow neck (Ali et al., 2004; Mortimer, 1999; Shanker et al., 2003). The depth of the *in-situ* nest should be measured and replicated in the *ex-situ* nest (Mortimer, 1999; Shanker et al., 2003). If the eggs are relocated within two hours of oviposition, they can be placed a few at a time into the *ex-situ* nest, and if relocated more than two hours of oviposition, they should be placed one at a time carefully. The eggs should never be poured into the nests (Mortimer, 1999). *Ex-situ* nests should be spaced at least one metre apart to avoid their impacts on each other (e.g., heat and respiratory gas supply) and so that hatchery personnel can move around without stepping on the nests (Mortimer, 1999; Phillott & Shanker, 2018). Each nest should have a marker and be numbered, with all the associated data documented (e.g., nesting date, original nest location, and clutch size) (Mortimer, 1999; Shanker et al., 2003). Nests are enclosed with cylindrical netlon mesh buried about 10 cm under the sand to deter crabs from burrowing into the nests. To keep hatchlings in the nest enclosure when they emerge, the netlon mesh should have a small mesh size of less than 1 cm to avoid hatchlings from extending their heads and flippers through the openings and become injured (Ali et al., 2004; Mortimer, 1999). Hatcheries should be kept free of any leftover food material to avoid attracting red ants that are predators of eggs and hatchlings (Ali et al., 2004).

Hatchlings must be released on the same night as they emerge. They will have more energy to swim away from the shore and away from predators if they are released sooner (Ali et al., 2004). Hatchlings should be allowed to crawl over the beach and into the sea unassisted, so that they will be able imprint on their natal beach (Ali et al., 2004; Mortimer, 1999; Sea Turtles of India, 2011; Shanker et al., 2003). To reduce hatchling mortality to predation, they should be released in groups at various locations on the beach during each release to avoid creating a predator feeding station (i.e., predators may learn that hatchlings are released at a specific point and may wait for them). When immediate release is not possible, hatchlings should be kept in a styrofoam box or other container and placed in a cool and dark environment. They should not be kept in water as they will go on a swimming frenzy and deplete their energy reserves (Ali et al., 2004; Mortimer, 1999; Shanker et al., 2003). Another management technique is headstarting, which involves keeping hatchlings in captivity for a length of time, usually between 9 and 12 months, until they reach a size that could minimise natural mortality factors, in order to increase recruitment to declining sea turtle populations (Bell et al., 2005). This technique is controversial because of the potential altering of sea turtle's behaviour and there is a lack of research on its risks and effectiveness (Bennett et al., 2017). Thus, it should be carefully considered before employing this technique.

Hatcheries should be operated in accordance with best practice guidelines, with personnel who are highly trained and adequate resources to safeguard and monitor nests throughout the nesting season (Mortimer, 1999; Phillott & Shanker, 2018). It is recommended that hatchery managers evaluate the necessity for nests to be relocated from their original location on a regular basis, since poaching activities and predator density may decrease with time, eliminating the need for nests to be protected in the hatcheries (Phillott & Shanker, 2018).

2.5 Egg development

Hatching success of sea turtle nests depends on successful egg and embryonic development. The early stages of egg development are exposed to the risk of high mortality. Sea turtles' embryonic development comprises 31 stages in chronological sequence and are described based on morphological structures by Miller (1985) and Miller et al. (2017) (Figure 2.1). The 31 stages of development were classified into early, mid and late embryonic stages by Rafferty et al. (2011) and Rings et al. (2015), whereby the early embryonic stage corresponds to stage 1 to 16, the mid embryonic stage to consist of stage 17 to 23, and stage 24 to 31 are the late embryonic stages. The egg and embryonic developmental stages are described in the following in reference to Figure 2.1.

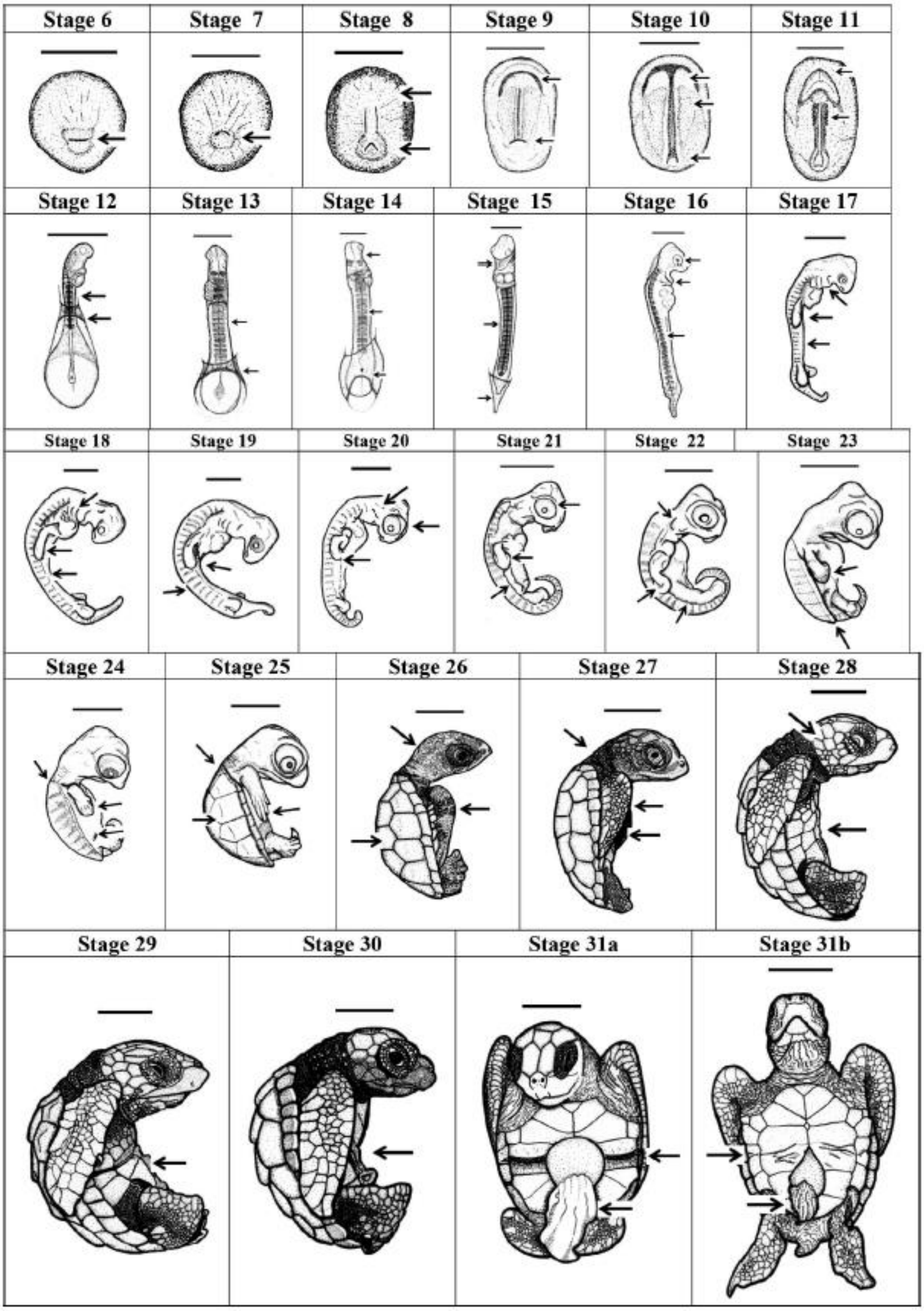

Figure 2.1 Sea turtle embryos' 31 stages of development (stage 6 to 31) from oviposition to hatching. Figure was adopted from Miller et al. (2017), (c) Chelonian Research Foundation, used with permission.