



Faculty of Resource Science and Technology

**Green Sea Turtle, *Chelonia mydas* (Linnaeus 1758) Conservation in
Lundu, Sarawak: Historical Perspective, Framework for Headstart and
Community-based Effort**

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Green Sea Turtle, *Chelonia mydas* (Linnaeus 1758) Conservation in
Lundu, Sarawak: Historical Perspective, Framework for Headstart and
Community-based Effort

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2018

DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been dully acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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9th August 2018

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ABSTRACT

Green turtle exists in Sarawak waters and its small scale conservation effort had already started since 1950s. The success of the effort is still debatable, as the data collected was not made readily available, while public participation in the conservation effort is still at infancy stage. The current study involved analysing nesting data of green turtles of Sarawak Turtle Islands, headstarting to obtain survival and growth of turtles in captivity while predicting the size at maturity for Sarawak's green turtle, disease profiling of turtle carcass from headstart program and conducting community based awareness program. Nesting, egg collection, incubation and hatching success data for 36 years has shown that Sarawak's green sea turtle population has stabilized (approximately 3000 nesters) after the drop in nesting trend in the 1950s. The number of eggs collected and incubated since 1990 until 2016 was 90 to 100 %, which helped to improve the number of nesters as more hatchlings were released into the sea. Estimation shows that Sarawak has an average of 3,088 mature nesters from 2010 until 2016 that corresponds to a calculated remigration interval of 3 to 4 years with interesting frequency of 3 times per season for mature nesters. Eighteen months of headstart program has been successful with survival rate of 22.5 % and 82 % for Batch 1 and 2, respectively. The growth rate of the turtles was 9.86 cm/year (Batch 1) and 15.92 cm/year (Batch 2). Batch 2 was found to be heavier and longer compared to Batch 1 with mean of 2.65 ± 0.64 g for weight and mean of 2.16 ± 0.02 cm for straight carapace length (SCL) compared to Batch 1's mean of 2.51 ± 0.63 g for weight and mean of 2.08 ± 0.19 cm for SCL. Based on both growth data from the headstart program and secondary data, a von Bertalanffy growth function was obtained for green turtles of Sarawak, where a first time female nester in 1982 with SCL of 101.03 cm was estimated to be between 12.5 to 13 years old. Commonly observed physical appearances of

dead hatchlings were eye infection, bite mark, red or blue marks, bloated plastron and emaciation. The types of diseases found were classified as infectious and non-infectious. The commonly found bacteria that are associated with infectious diseases are *Proteus* sp., *Pseudomonas* sp., *Klebsiella pneumonia*, *Morganella morganii* and *Citrobacter freundii* while 4 taxa of fungi namely *Cladosporium* spp., *Penicillium* sp., *Aspergillus* spp. and *Gibberella* sp. were found in the carcasses. Batch 1 showed a higher disease and mortality count when compared to Batch 2 as the handling and management technique was better for the latter. A Standard Operating Procedure comprising of human-turtle interaction manual, husbandry practice plus behavioural and disease management tactics to be used for small scale headstart program was produced. The awareness programs resulted in a positive community-based effort involving local communities of Lundu, the public, as well as private and government agencies. Development of an educational toolkit on green sea turtle at the end of the project is hoped to be of help in the conservation of sea turtle in the future. The survey conducted during the program has reflected a positive vibe as 96.5 % of the total participants were willing to attend another sea turtle awareness program.

Keywords: Green sea turtle, headstart, disease, awareness program, education toolkit.

Konservasi Penyu Agar, Chelonia mydas (Linnaeus 1758) di Lundu, Sarawak: Dari Perspektif Sejarah, Rangka Kerja untuk Program 'Headstart' dan Usaha Melibatkan Komuniti

ABSTRAK

Penyu agar wujud di perairan Sarawak dan usaha konservasi berskala kecil penyu agar telah bermula sejak tahun 1950an lagi. Namun begitu, kejayaan program konservasi penyu agar di Sarawak sering dipersoalkan kerana data yang dikumpul tidak tersedia untuk tatapan umum manakala usaha konservasi bersama orang tempatan adalah minimum. Kajian ini meliputi analisis data berkaitan penyu hijau yang bertelur di Pulau Penyu Sarawak, projek 'headstart' untuk mendapatkan data kelangsungan hidup dan tumbesaran untuk penyu yang ditenak lantas menggunakan data itu untuk mengenalpasti saiz penyu agar yang matang, pengenalpastian penyakit dan agen pembawanya dari bangkai penyu yang dikumpul dari program 'headstart' dan mengendalikan program konservasi penyu berteraskan komuniti. Data berkaitan bilangan penyu bertelur, pengumpulan dan pengeraman telur penyu serta kadar penetasan selama 36 tahun telah menunjukkan populasi penyu agar di Sarawak dalam status stabil (lebih kurang 3000 sarang) setelah penurunan mendadak populasi sekitar 1950an. Jumlah telur yang dikutip dan dieramkan semenjak 1990 hingga 2016 adalah sekitar 90 hingga 100 peratus dan kemungkinan besar telah membantu menambahkan bilangan penyu bertelur kerana lebih banyak anak penyu telah dilepaskan ke laut. Dianggarkan Sarawak kini mempunyai purata 3,088 penyu matang yang bertelur dari tahun 2010 hingga 2016 dengan julat migrasi 3 ke 4 tahun dan frekuensi kekerapan bertelur sebanyak 3 kali semusim. Program 'headstart' penyu agar selama 18 bulan telah berjaya dilaksanakan dengan kadar kelangsungan hidup sebanyak 22.5 dan 82 % bagi kumpulan 1 dan 2 masing-masing. Kadar tumbesaran

penyu sekitar 9.86 cm/tahun (Kumpulan 1) dan 15.92 cm/tahun (Kumpulan 2). Berdasarkan data terkumpul 'headstart' dan data sekunder, fungsi tumbesaran von Bertalanffy bagi penyu agar Sarawak mendapati bahawa seekor penyu yang pertama kali bertelur pada tahun 1982, dengan 101.03 cm SCL dianggarkan berumur sekitar 12.5 hingga 13 tahun. Pemeriksaan fizikal bangkai anak-anak penyu telah menemukan kesan jangkitan mata, kesan gigitan, kesan lebam warna merah atau biru tua pada badan, 'plastron' membengkak dan sindrom 'emaciation'. Penyakit berjangkit dan bukan berjangkit yang terdapat pada bangkai penyu merekodkan kehadiran bakteria *Proteus* sp., *Pseudomonas* sp., *Klebsiella pneumonia*, *Morganella morganii* dan *Citrobacter freundii*. Di samping itu, empat taksa fungi juga dijumpai iaitu *Cladosporium* spp., *Penicillium* sp., *Aspergillus* spp. dan *Gibberella* sp..Kumpulan 2 menunjukkan kadar kematian dan jumlah penyakit yang lebih sedikit berbanding Kumpulan 1 disebabkan oleh teknik pengurusan dan pengendalian yang lebih baik. Prosedur Operasi Standard yang mengandungi manual interaksi manusia-penyu, teknik penternakan dan juga taktik pengurusan perangai dan penyakit untuk digunakan oleh program 'headstart' berskala kecil telah dihasilkan. Program kesedaran berkenaan konservasi penyu agar Sarawak berteraskan komuniti telah memberi impak positif dengan penglibatan komuniti setempat sekitar Lundu, orang awam, pihak swasta dan pelbagai agensi kerajaan. Penghasilan kit pembelajaran berkenaan penyu agar pada akhir projek ini diharap dapat membantu program konservasi penyu yang berterusan pada masa hadapan. Survei yang dibuat selepas program kesedaran memberikan keputusan yang positif kerana sebanyak 96.5 % peserta mahu mengikuti program begini lagi.

Kata kunci: Penyu agar, program 'headstart', penyakit, program kesedaran, kit pembelajaran.

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic Acid
bp	Base pair
min	Minute
sec	Seconds
rRNA	Ribosomal Ribonucleic Acid
ppm	Parts per million
CTAB	Cetyltrimethyl ammonium bromide
NaCl	Sodium Chloride
MgCl ₂	Magnesium Chloride
TAE	Tris-Acetate-ethylenediaminetetraacetic
ddH ₂ O	Deionized distilled water
g	Gram
mg	Milligram
mL	Mililiter
mM	Milimolar
M	Molar
L	Liter
μL	Microliter
μM	Micromolar
mm	Millimeter
rpm	Rotation per minute
UV	Ultra violet

V	Volt
SCL	Straight Carapace Length
×g	Gravity
dNTP	Deoxy-nucleotide triphosphates
SCW	Straight Carapace Width
CCL	Curved Carapace Length
CCW	Curved Carapace Width
SFC	Sarawak Forestry Corporation
IK	Information Kiosk
WCS	Wildlife Conservation Society
SFD	Sarawak Forest Department
SEATRU	Sea Turtle Research Unit
SOP	Standard Operating Procedure
UNEP	United Nations Environment Programme
STIs	Sarawak Turtle Islands
MU	Management Unit
TIHPA	Turtle Island Heritage Protected Area
UNIMAS	Universiti Malaysia Sarawak
CCC	Caribbean Conservation Corporation
CBC	Community Based Conservation
FACA	Faculty of Applied and Creative Arts
LCD	Liquid Crystal Display
cm	Centimeter
RH	Relative Humidity

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Managing a species required an understanding of its basic biological characteristics such as life history traits, spatial and temporal distributions, demography, abundance, behaviour, growth and how humans affect these characteristics. Although many of these parameters have been studied in green sea turtle, they have been found to vary significantly among populations and possibly among foraging grounds (Eguchi *et al.*, 2010, 2012). These differences illustrate that these parameters must be determined at a smaller scale than at a species level to formulate suitable management strategies and planning for local populations (Ibrahim & Sharma, 2006).

Green sea turtle, locally known as ‘penyu agar’ are the major landers on Sarawak Turtle Islands. They could travel thousands of kilometres to the foraging grounds for food and usually adult females will return to mate and nest at their natal beach. The turtle could reach a maximum length of about 130 cm and maximum weight of approximately 204 kg (Spotila, 2004). Turtle studies in Malaysia largely focus on nesting biology and its management (Abd. Mutalib *et al.*, 2014; Abd. Mutalib & Fadzly, 2015), ecology (Tisen & Bali, 2000; Pilcher, 2010; Tinsung *et al.*, 2011; Bali *et al.*, 2012, 2014) as well as genetic studies (Joseph & Shaw, 2011; Yahya *et al.*, 2012; Joseph & Chong, 2014; Jensen *et al.*, 2016; Joseph *et al.*, 2016; Joseph & Nishizawa, 2016; Nishizawa *et al.*, 2016; Joseph *et al.*, 2017).

Headstarting is a management technique employed to enhance recruitment of turtles into diminished or extirpated turtle populations (Bell *et al.*, 2005). It is a technique

where newly hatched turtles are raised in captivity for a period of time, usually 9 to 12 months or until they reach a size that reduces their natural mortality factors (Woody, 1990; Mortimer, 1995; Heppell & Crowder, 1996). This is thought to increase their likelihood of survival to adulthood with the aim to subsequently raise their level of recruitment to foraging assemblages and ultimately their breeding populations (Heppell & Crowder, 1996; Bell *et al.*, 2005). Besides that, Heppell & Crowder (1996) stated that animals with little or no parental care, relatively high fecundity and low juvenile survival appear well suited for mass rearing programs.

There have been many issues regarding the use of the technique to help compensate the loss of the population; for example, Wood & Wood (1993) have documented cases of long term survival of released turtles together with their growth and movements suggesting that they could successfully adapt to the wild which is in contrast to claims by Shaver & Caillouet (1998) who mentioned that very few headstarted turtles joined a breeding population. Despite that, Burke (2015) mentioned that head starting wildlife is as old as wildlife conservation and management itself because it helps individuals survive vulnerable life stages which is an obvious and appealing way to increase population persistence.

Campbell (2003) suggested community-based conservation which has been increasingly popular in the culture of sea turtle conservation. One of the methods is through awareness program. A local community that is deprived of knowledge about their surrounding environment could become a strong force in the depletion of marine turtle populations and the destruction of their habitats (Marine Turtle Specialist Group, 1995). In Malaysia, the lack of enforcement and legislation on sea turtle egg harvest has led to the decline of nesting sea turtle (Tisen & Bali, 2000; Joseph *et al.*, 2014). The eggs of sea

turtles had been consumed without knowing those eggs are affected by toxic contaminants (Sakai *et al.*, 2000) thus further highlighting the importance of awareness program in safeguarding the public's health issue.

1.2 Problem statement and Objectives

Conservation effort on green sea turtle in Sarawak has started since the early 1950s (Leh, 1994) until present but the success has frequently been doubted. Anthropogenic factors such as incidental captures, excessive tourism activities and illegal poaching due to lack of awareness among the public on the issue has led to continuous decline in numbers of female nesters (Chan & Shepherd, 2002, Chan 2006; Senko *et al.*, 2011) as reflected in historical data (Tisen & Bali, 2000). Furthermore, lack of male turtles produced from the Sarawak Turtle Islands as claimed by Jensen *et al.* (2016) has worsened the situation.

Sea turtles are late maturing, migratory animals hence their life cycles tend to be complex therefore modelling their life history and population dynamics require information for several life stages, particularly those at sea (Hamann *et al.*, 2010) and since obtaining information on their 'lost years' is complicated and difficult to do, a headstart program could be another alternative in obtaining that piece of information. Moreover, the population size and trend estimated from this study is a much needed data as it could be used as proxies for other underlying population processes such as mortality patterns which are considered in the threats criteria score for overall assessments of conservation status of South Asia Regional Management Units (RMUs) (Wallace *et al.*, 2011).

There is a fundamental necessity for accurate data on factors such as age-at-maturity and age-based demographic parameters as model outputs are sensitive to variation in these

parameters (Heppell, 1998). Population models could be developed from these data and used as a basis for making decisions for sea turtle management and conservation in Sarawak. By conducting a headstart program, an age-at-length growth function could be developed using biometry data from captive turtles and the headstart facility could be used as a centre for information transfer for the sea turtle awareness program. The awareness program could be used to educate the public on sea turtles as well as to assess the level of knowledge and acceptance of sea turtles in the local community.

Hamann *et al.* (2010) have mentioned that little is known about the health status of oceanic turtles because of their difficult to study life history stage. However, in the case of headstarted turtles, all turtles are to be assessed in terms of health prior to release and preferably include a systematic post-mortem examination of all captive mortalities to determine the cause of death and minimize the risk of introducing new pathogens into release habitats (Hamann *et al.*, 2010). Therefore, the project has been carried out with the following objectives:

- i. To analyse the trend of female nesters, egg collected, egg incubated and hatched while providing an estimate of existing and future nesters at Sarawak Turtle Islands using 36 years' worth of past and recent data.
- ii. To elucidate the growth of post-hatchling green sea turtle in their first 18 months of life using headstart program in terms of behaviour and growth while establishing a Standard Operating Procedure for future headstart program and deducing a generalised age-at-length equation derived from growth data to estimate the age of turtles found in Sarawak waters.

- iii. To identify the type of disease, the possible causative agent and symptoms of the disease while documenting the steps taken to control the disease outbreak that causes the mortality of captive turtles during headstart program.
- iv. To instil a sense of responsibility and educate the local community of Sarawak to conserve green sea turtle population that nests along Sarawak coastal area.

1.3 Layout of the thesis

This thesis comprises of eight chapters. Chapter One of this thesis is the general introduction of the study inclusive of problem statements, objectives and a brief introduction to all the chapters. A flowchart (Figure 1.1) that summarizes all research activities carried out in this study is included in this chapter.

The following chapter comprises literature review on green sea turtle's biology, behaviour and ecology, the conservation status of sea turtle in Malaysia and the population dynamics and growth related studies of sea turtles.

Chapter 3 describes the current nesting trend, egg collected, egg incubated and hatched data for Sarawak Turtle Islands using data for the past 36 years. These data were obtained from Turtle Board and was used to estimate the number of nesters that are in Sarawak waters.

Chapter 4 discusses good husbandry practices for sea turtle headstarting, survival, growth curve and growth rate data of green sea turtle hatchlings for duration of 18 months during the headstarting project. This chapter also consists of an SOP, hatchlings' behaviour observation and a growth function deduced from growth data obtained from the headstart project.

Chapter 5 described diseases found in carcasses of green turtle hatchling and juvenile that died during the headstart project. The symptoms observed during the disease and the steps taken to overcome the disease were noted. In addition, the possible causative bacteria and fungi were detected using molecular biology technique.

Chapter 6 deals with awareness programs carried out to instil love for sea turtles among the public, especially to the younger generations who are going to be the earth custodians. At the end of the project, an educational tool kit comprising of manual, video and an information kiosk was developed and is ready for use in future conservation programs.

The second last chapter is the general discussion relating all chapter three, four, five, and six thus turning it into a bigger picture of the whole project.

Chapter 8 comprises of the conclusions and recommendations for future work related to sea turtle conservation in Malaysia.

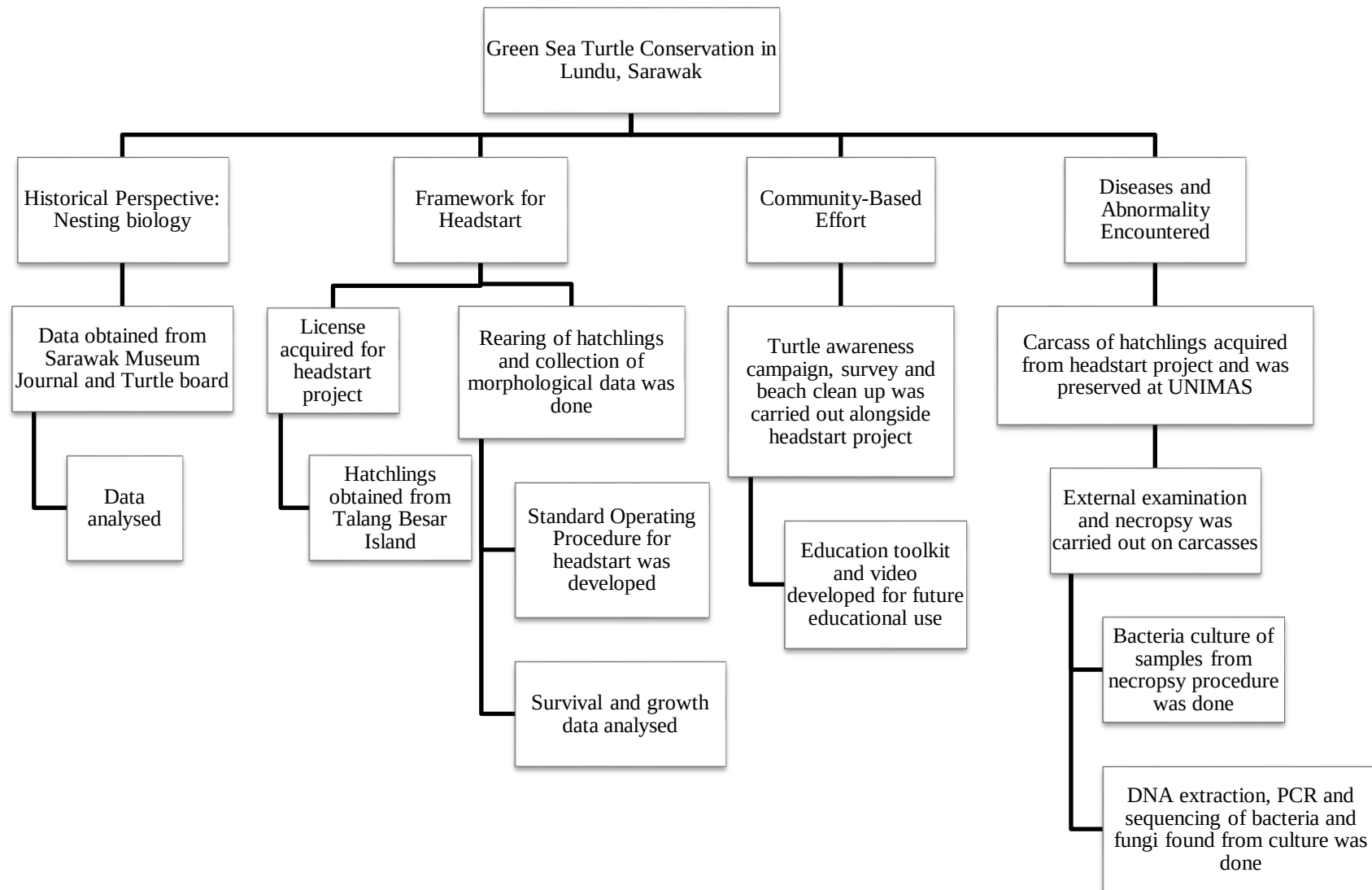


Figure 1.1: Flow chart of all research activities carried out in this study

CHAPTER 2

LITERATURE REVIEW

2.1 Green Sea Turtle Biology and Ecology

Worldwide, there are seven living species of sea turtles namely green sea turtle (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), olive ridley (*Lepidochelys olivacea*), leatherback (*Dermochelys coriacea*), flatback (*Natator depressus*), Kemp's ridley (*Lepidochelys kemp*i) and loggerhead (*Caretta caretta*). Green turtles are marine turtles with a worldwide tropical and subtropical distribution (Chan, 2006). Green sea turtles are classified under the Class Reptilia, Order of Testudines, Suborder Cryptodira and Family Cheloniidae (Seminoff, 2004). This truly international animal have been spotted in the Atlantic Ocean as far north as Massachusetts and far south as southern Brazil, throughout the Mediterranean Sea, over wide areas of the Pacific Ocean and in much of the Indian Ocean in which they were found to feed and swim in over at least 140 countries (Spotila, 2004).

The green sea turtle are distinguished from other sea turtle by a single pair of prefrontal scales, four pairs of lateral scutes and five vertebral scutes. The carapace is broad, low and heart-shaped. It is smooth, without keels and scutes are placed side by side. The carapace colour evolves with time as it is black in hatchlings, turns to brown and tan in juveniles and becomes olive or gray green with an occasional dust of yellow and brown in adults while the plastron is whitish to yellowish from hatchling to adulthood (Wyneken, 2001). Sexual dimorphism is present in adult animals and the male has very long tail and also a single claw which is markedly enlarged and strongly curved. The curved carapace length of adult green turtle ranges from 70 to 120 cm with an average of 99.5 cm in the Phillipines (Limpus & Chaloupka, 1997).

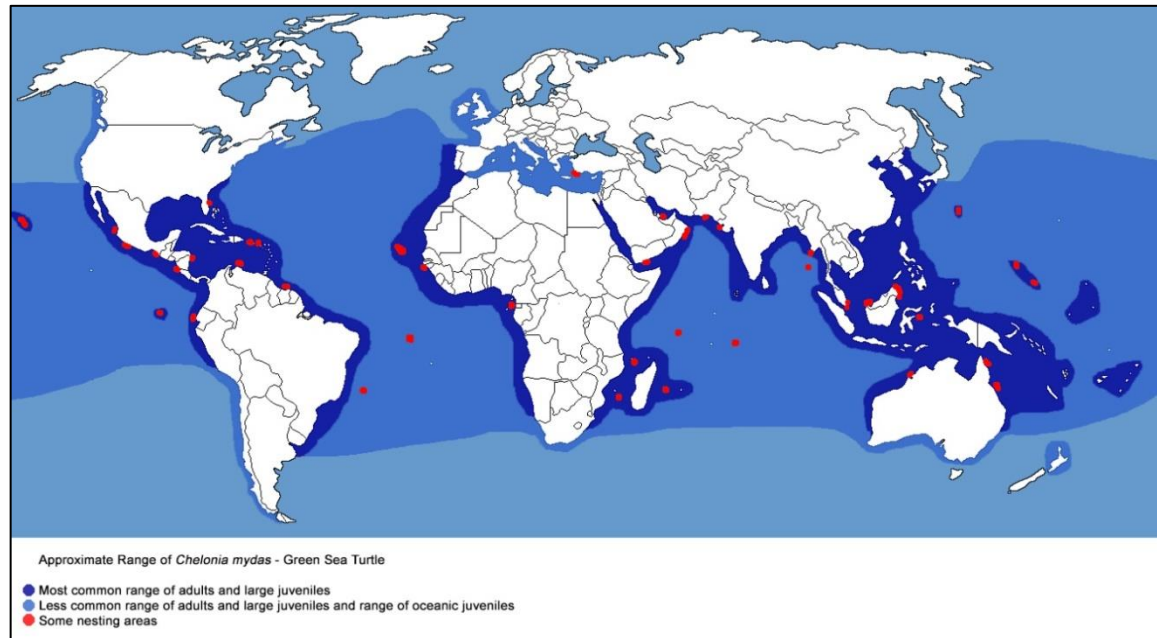


Figure 2.1: Map of common range of Green Sea Turtle in the world.
Map adapted from CaliforniaHerps.com

Green turtles nest on sandy beaches and exhibit natal homing behavior (Meylan *et al.*, 1990). The adults are herbivorous, feeding primarily on algae and seagrasses, and undertake extensive migrations between breeding and foraging grounds, which may span thousands of kilometers. Spotila (2004) had reported that green sea turtles are the only turtles that eat large amounts of plants namely seagrasses such as *Thalassia* (turtle grass), *Syringodium*, *Halophila*, *Posidonia*, *Halodule* and *Zostera*. Algae such as *Chaetomorpha*, *Sargassum* and *Hypnea* as well as other green and red algae, jellyfish, salps, sponges, mollusks, fish, polychaete worms and jellyfish are also eaten but to a lesser extent and with varying degree and intensity according to the turtles' foraging place (Spotila, 2004).

Hatchlings spend several years in pelagic environments (minimum of 1 to 2 years) before recruiting to necrotic habitats as juveniles (Formia *et al.*, 2006). There are few records of epipelagic hatchlings during their 'lost year' (Carr, 1987) however it is known that they occupy a series of foraging habitats dispersed over an extensive area. The plasma colour of pelagic stage green turtles is evidence of its carnivorous diet as it has been

observed in Bahamas that when green turtles first arrive on benthic foraging areas, their plasma is unpigmented. However, when they began feeding on seagrasses, their plasma has yellow pigmentation, a result of plant pigments (Bolten & Bjorndal, 1992).

2.2 Nesting Biology of Green Sea Turtle

The population status of marine turtles are measured by the number of nests produced by the various species per year, a figure that can be conveniently determined by counting the number of nests deposited on the nesting beaches (Chan, 2006). Nesting trends of the green turtles on the Sarawak Turtle Islands over the last 30 years appears to be in equilibrium, with two to three thousand nestings occurring per year. However, in the early 50's, nestings of over 20,000 per year were recorded, indicating a decline of over 90 % (Tisen & Bali, 2000). In Terengganu and Sarawak where hatchery programmes have been in place since the 50's and 60's, turtle egg harvesting among local people has led to the failure to protect population (Chan, 2006) maintenance. Loss of nesting habitats is also expected in Malaysia where prime beaches are being developed for tourism except in places where turtle sanctuaries have been established such as Sabah and Sarawak Turtle Islands. The establishment of turtle sanctuary/ national park named Talang-Satang National Park is aimed to secure all nesting sites of significance and to prevent them from further degradation (Chan, 2006). Reproduction is seasonal but extended throughout the year at several areas. The clutch size average is 104 eggs (SFD & WCS, 1996).

2.3 Threats and Conservation Status

Over the last 141 years, the green turtle populations are conservatively estimated to have declined by 37-61% and this have resulted in the classification of the species as globally endangered (Seminoff, 2004). One of the major factors that have contributed to the decline of the species in Malaysia is continuous egg harvest and loss of nesting habitats due to coastal development for tourism (Chan, 2006).

Worldwide, the populations of all these species are threatened by over exploitation, disease, incidental capture by fishermen and also destruction of critical nesting habitats (Wabnitz & Pauly, 2008). However, despite having existing laws and regulations to protect threatened sea turtles, law enforcement in certain areas of the world such as Liberia is still very weak or worse still, non-existent (IUCN, 2015).

The consumptive use of sea turtles documented by Thorbjarnarson *et al.* (2000) had listed countries that are using sea turtle meat as food, including the U.S. (Florida, Georgia, Louisiana, Mississippi, North Carolina, Texas and Virginia, Atlantic coast of central America, Ecuador, Peru, Madagascar, Seychelles, India, China, Sri Lanka, Japan (fishing in other waters), Indonesia, Australia, Torres Strait and Papua New Guinea in which the list actually does not stop there. It was also mentioned that in some of the countries listed, the use of sea turtles is illegal but still nevertheless continued without reference to why turtles are used and for what purpose (beyond economic profit).

For sea turtle egg, the list of both legal and illegal use of it is by countries on the Atlantic coast of central America, Mexico, Iran, Saudi Arabia, India, Thailand, Malaysia, Indonesia, the Philippines, Papua New Guinea and much more. All the study on egg use points to economic value and how it is regulated with exception to one legal, commercial egg collection project at Ostional, Costa Rica which is known to be the best example of

egg use (Thorbjarnarson *et al.*, 2000). In Malaysia, Terengganu permits collection and selling of turtle eggs (Chan, 2013) but not in Sarawak where total ban on any turtle products had been implemented according to Sarawak's Wildlife Protection Ordinance 1998.

In 1993, a long term marine turtle conservation project in Chagar Hutang Beach of Redang Island has been implemented under the Sea Turtle Research Unit (SEATRU) of the University Malaysia Terengganu (Chan, 2013). The once fully-funded project carried out *in-situ* egg incubation and a saturation tagging program to monitor the nesting activity of two species of sea turtles found there; green and hawksbill. Chan (2013) also mentioned that in 1998, the program lost its corporate sponsor and started to implement fund-raising activities that introduces turtle and nest adoption schemes and volunteer programs called STOP (Save our Turtles Outreach Program). The program is still an on-going program as the beach was accorded sanctuary status in 2005 in which the conservation site of 350 meter at the northernmost part of Redang Island became Chagar Hutang Turtle Sanctuary (Chan, 2013).

2.3.1 *In-situ* conservation of Green Sea Turtle in Malaysia

Some of the *in-situ* study on sea turtle nesting biology in Malaysia had been conducted by Sarahaizad *et al.* (2012) at Pantai Kerachut Turtle Conservation Centre in Penang Island where she had studied the nest site selection of green sea turtle. It was found that more digging attempts were aborted at the vegetation zone compared to the open beach or grassy areas which were most probably caused by the rough sand, rocks and roots. Moreover, it was also found that most turtles preferred to land and nest within the

vegetation zone of Merambong trees which could be due to the shelter, protection and dark environment to human vision (Sarahaizad *et al.*, 2012).

Another *in-situ* study was conducted by Sarahaizad & Shahrul Anuar (2015) which checks the behaviour of green turtles for one breeding season in Penang Island (from December 2012 and August 2013). They have found that from the eight green turtles landed, 106 were emergences, 38 were nests and 158 were digging attempts made over duration of one breeding season where the total clutch size deposited were from 170 to 979 eggs per turtle with total nests ranging from three to eight nests per turtle per season (inter-nesting interval of 9 to 29 days). Sarahaizad & Shahrul Anuar (2014) also stated that longer inter-nesting interval had been caused by disturbances caused by feral dogs, wild pigs, sea-coated otters and by the night activities of fisherman (use of wide torch light and sounds of boat engine). It was finally concluded that the Kerachut Turtle Conservation Centre should hire more staffs for beach patrolling to control human poachers and animal disturbances.

Yahya *et al.* (2012) had also mentioned that sea turtles in Talang-Talang and Satang Besar Island of Sarawak nests according to location, landscape similarity and also the behaviour of the turtles as they demonstrated the same preference in nesting place which was assessed through a mtDNA gene analysis. It was postulated that the turtles either exhibited natal homing or practiced social facilitation or chance encounter theory in determining their nesting beach (Yahya *et al.*, 2012).

2.3.2 *Ex-situ* conservation of Green Sea Turtle in Malaysia

An *ex-situ* study was conducted by Sarahaizad & Shahrul Anuar (2014) where 29 natural nests were relocated for incubation to test the effects of nesting depth and

temperature on hatching success and size of hatchlings. The hatchery that was not covered had a mean nest temperature of 29.5 °C while the covered nest was cooler at 28.0 °C. Sarahaizad & Shahrul Anuar (2014) mentioned that as the nesting depth increased, the nest temperature at 55, 65 and 75 also increased significantly with a higher success rate of 59.8 % in the uncovered nests. It was also reported that the warmer hatchery produces bigger sized hatchling and that eggs should be incubated in uncovered plots at nesting depth of 55 cm. Moreover, in an attempt to prevent poaching of turtle eggs, the Department of Fisheries, Penang have purchased sea turtle eggs from licensed egg collector to be incubated in hatcheries (Sarahaizad & Shahrul Anuar, 2014).

2.3.3 Laws and Legislation

Among the most relevant legislations to turtle conservation in Sarawak are the Turtle Trust Ordinance of 1957, the Land Code 1958, the Turtle Protection Rules of 1962 and the Wild Life Protection Ordinance of 1998 where under these rules, the management of sea turtles are aimed at three levels that is the protection of the nesting beaches, protection of the turtles in the sea and also the correct management to ensure that the animals complete their complex life cycle and breed (SFD & WCS, 1996). Yap (1997) had stated that green turtles in Sabah are protected under Wildlife Conservation Enactment 1997, Schedule I Section II, Totally Protected Species of Animals and Plants, Part I (Section 25(2)).

2.3.4 History of sea turtle conservation in Sarawak

According to Mrosovsky (1976), before the Japanese occupation, the turtle egg industry of the Sarawak Turtle Islands was placed in the hands of a few prominent Malay families where in 1941 it was later placed under a Turtle Trust Ordinance, administered by the curator of Sarawak Museum. In 1947, Tom Harrison became the curator of the museum where he took charge of the Turtle Islands, installed regulation of the exploitation and established a tagging program using monel metal cow ear tag as fin clip. In 1951, one of the world's oldest rookery management programs was established at the islands where some of the eggs from the natural nests were replanted in the hatchery while the rest were sold (SFD & WCS, 1996). Year 1956 was another milestone in sea turtle conservation of Sarawak as the sea turtle that had been tagged three years before came back to nest at the island and that was the first remigration of a tagged turtle (Mrosovsky, 1976).

Mortimer (1990a) mentioned that since 1927, most of the eggs laid on Turtle Islands have been harvested where between 1951 and 1962, only 2 % of eggs laid per year were replanted in hatcheries which later dropped to 1 % between year 1963 and 1975 (average egg planted per year: 3024). Therefore, to alleviate the decline in turtle nestings, the percentage of eggs replanted per year were increased to 12.8 % between 1976 and 1981 and was further increased to 45 % between 1982 and 1988 where Leh (1996) reported that about 90 % of eggs were replanted by 1995. Later, the official policy was changed to all eggs should be replanted for 9 months of the year and only taken for consumption during the 3 months of monsoon or 'landas' season. However, the members of the Turtle Board can still use their own egg ratios for consumption throughout the year (SFD & WCS, 1996). In the early 2000, the management of the Sarawak Turtle Islands were handed over to Sarawak Forestry Corporation (SFC) together with all matters related to sea turtle

conservation. Since then, the SFC team have been organizing turtle adoption program where the public helps by sponsoring a turtle and a nest and also by spending four days in Talang-Talang Besar Island at a reasonable price (RM 600 for locals and RM1200 for tourists) to help with the turtle conservation there by doing beach patrolling, transferring eggs to turtle hatchery, releasing hatchlings, recording data, doing nest analysis and also doing other research, education and conservation activities (SFC, 2009). The program aims to raise awareness on sea turtle among the public. The SFC has also been actively involved in the habitat conservation of sea turtle and also the local fisherman ground by deploying reef balls. Since 1998, 2284 units of reef balls have been seeded at the interesting areas of sea turtles which is around Talang-Satang National Park, Lawas, Bintulu and Kampung Buntal, Kuching (SFC, 2009).

2.4 Population Dynamics and Growth Related Studies of Sea Turtles

Ehrhardt & Witham (1992) mentioned that conservation programs are necessary to restore these populations and knowledge about the population dynamics of the species is required to successfully implement such programs and an important aspect of population dynamics of any species is the study of its growth rate.

Many sea turtle stocks are in decline worldwide due to over-harvesting and destruction of nesting and foraging habitats. Yet population abundance for many stocks can display non-deterministic fluctuations with no apparent long-term trend. What are the indications that the stock is at risk? Is the current management strategy appropriate for the stock protection? These are challenging questions that cannot be resolved without a thorough understanding of the sea turtle growth and population dynamics. The nesting

trend of the green turtles in Sarawak over the last 30 years appears to be in equilibrium with two to three thousand nestings occurring per year (Chan, 2006).

However, to enhance the conservation programme, the hatch rates of the turtle's eggs has to be monitored as there are a high percentage of unhatched eggs due to the monsoon season from November to April and also due to invasion of other turtles on the natural nests. Leh *et al.* (1985) recommended the use of *in situ* nests as a conservation strategy at a depth of 60, 56 and 52 cm where the hatch rates are 60.0 to 72.7 %, 47.2 to 76.9 % and 37.1 to 84.3 %, respectively. *In-situ* nests were recommended as there was no significant difference between hatch rates of *in-situ* nests and replanted hatcheries (Leh, 1994).

Somatic growth of wild stock green sea turtles is not well known (Chaloupka & Musick, 1997). The post-natal somatic growth for most species of sea turtle comprises of 2 different parts namely the epipelagic post-hatchling feeding phase (ca 5 to 35 cm CCL, curved carapace length) and benthic feeding phase (>35 cm CCL) (Limpus & Chaloupka, 1997).

According to Pilcher (2010), nesting turtle populations have been well documented in Southeast Asia but little information is available on the foraging populations of the region, and there is also no published information for Southeast Asian *C. mydas* on sex ratios in the wild or on the dynamics of turtle populations with regards to growth, survival and sex ratios or non-adult components of the populations. Therefore, Pilcher (2010) has gathered baseline information on foraging juvenile turtle population around Mantanani Island, Sabah, Malaysia in which he found out that the juveniles in the area ranged from 38-80 cm CCL and moves an average of 380 m between recaptures and exhibited site-

fidelity over several recaptures spanning up to two years. The growth rates among recaptures averaged at 3.6 cm year^{-1} and the laparoscopic examination of the gonads confirmed that all individuals were immature with a sex ratio of 1M:4F. It was also found that the growth rates of *C. mydas* in Mantanani Island were significantly higher than the $1.43 \text{ cm year}^{-1}$ for larger immature turtles in the Southern Great Barrier Reef (Limpus, 1979), also higher than the 2.3 cm year^{-1} to 2.7 cm year^{-1} for similar sized *C. mydas* in Florida (Bresette & Gorham, 2001) but much smaller than *C. mydas* in Florida (5.3 cm year^{-1}) (Mendonca, 1981).

Another study which attempted preliminary population dynamic study of residential *C. mydas* population in Sipadan Island, Sabah by Tinsung *et al.* (2011) reported that out of 147 individuals caught and measured, 77 were juveniles, 28 were sub-adults and 42 were adults with mean straight carapace length (SCL) of $557.7 (\pm 70.5) \text{ mm}$, $690.2 (\pm 40.7) \text{ mm}$ and $802.8 (\pm 60.6) \text{ mm}$ respectively. Mortimer (1991) reported that green turtles nest all year round in Sipadan Island with peak months between July and December. The extensive seagrass meadows with five species of seagrass in Sipadan Island may also contribute to the all year round nesting as food source is plenty.

Meanwhile, in southern Great Barrier Reef (sGBR), numerous long term studies on sea turtles especially *C. mydas* have been carried out with recorded growth data from as early as 1974. Norman *et al.* (1994) mentioned that all the rookeries in the sGBR comprise of the same panmictic interbreeding stocks and are genetically distinct from the northern Great Barrier Reef, Gulf of Carpentaria and Western Australian stocks. With the confounded time and cohort effect, Limpus & Chaloupka (1997) have modelled statistically the growth records using a non-parametric regression approach known as generalized additive modelling (GAM). The GAM regression analysis found that both

(maturity.group) and (mean.size nested) have a significant effect on green turtle growth rates as immature green turtles irrespective of sex grew much faster than adults (Limpus & Chaloupka, 1997). Other than that, it was also found that females grew faster than the males with a nonmonotonic growth rate function rising from recruitment size (>35 cm CCL) to maximum growth of 2.1cm year^{-1} at ca 60 cm CCL before declining to negligible growth approaching sexual maturity at ca 95 to 100 cm CCL (Limpus & Chaloupka, 1997). There seems to be significant regional differences in the magnitude of immature growth rates as Green (1993) stated that immature green turtles grow much slower in the Western, Central and Eastern Pacific regions than in western Atlantic-Caribbean waters. This statement is further supported by Bowen *et al.* (1992) as the growth patterns are consistent with the proposed phylogenetically derived population substructures for green sea turtles.

CHAPTER 3

SARAWAK GREEN TURTLE NESTING TREND

3.1 Introduction

Understanding the nesting behaviour and the ecology of marine turtles is important to ensure that the current conservation status and efforts are working. Knowledge of the age, size, growth rate and relative reproductive output of individuals is fundamental in understanding the demography of a population (Broderick *et al.*, 2003). An accurate assessment of population size, including a determination of whether a population are stable, increasing or declining is the foundation on which all management decisions should be based on (Eckert, 1999).

Monitoring beach nesting is by far the easiest and least expensive means to assess green turtle population abundance but surveys that are less than 10 years are inadequate (Chaloupka & Limpus, 2001) as green turtles are long-lived and the females have been observed to skip several nesting seasons due to nutritional constraints (Bjorndal, 1997). With respect to behaviour, nesting season of mature females could differ in timing and duration even among rookeries in the same region as some locales could support both nesting and feeding aggregates and non-migratory populations may exist at these sites (Pritchard, 1980). Furthermore, global warming has potential in negative impact to sea turtles as the increase in temperature could lower hatching success, lead to a gradual shift towards a feminisation of sea turtle populations (Hawkes *et al.*, 2009), affect internesting interval and nesting patterns of sea turtles (Patel *et al.*, 2016).

Sarawak Museum and Sarawak Turtle Board has recorded the number of turtle landings and the number of eggs collected yearly since 1927. However, most of the eggs

that have been laid since 1927 were harvested and Mortimer (1990a) had mentioned that over-harvest of eggs and meat at nesting beach as one of the most effective way to destroy a nesting population. Therefore, since 1950s, a rookery management program had been established to help sea turtles population thrive but the number of eggs replanted was not enough to ease the effect of decades of over harvesting thus resulting in the serious decline in nesting females (Mortimer, 1990a). After a nesting female decline of 87 % from 1949 to 1988, Leh (1989) have mentioned that the population has reached equilibrium as the conservation management of green turtles has been successful. Although the trend of female nesters was estimated to remain the same as the past 20 years, there are differences in the trend of turtle nesting as climate change could have easily disturbed the internesting and remigration interval of the nesters. Determining the number of times a turtle nests during a reproductive season is important, particularly if such data area is averaged and used in calculations to estimate the number of females in the population (Ehrenfeld, 1979). Based on data from Sarawak Museum Journal, the number of egg count has been documented since 1927 in Sarawak.

The objectives of this chapter are to: (1) analyse the trend of female nesters at Sarawak Turtle Islands (STIs) for the past 36 years thus determining the most recent trend of turtle landing that has been taking place, (2) determine the status of egg collected, incubated and hatched during the past 36 years and (3) provide an estimate of the number of future and existing nesters at Sarawak Turtle Islands.

3.2 Materials and Method

3.2.1 Study site and Data description

For this study, all data used were secondary data (Table 3.1). The annual turtle conservation record covers numbers of turtle landing, eggs collected, eggs incubated and eggs hatched, that were kept in total per year format for year 1980 to 2016 (latest) were obtained from the Turtle Board and Sarawak Museum. The landing data before 1980 were obtained from Mortimer (1990a).

Table 3.1: Type of data, year and source

Type of data	Year	Source of data
Turtle landings/ nesters	1949 - 1979	Mortimer (1990a)
	1980 - 2016	Turtle Board
Eggs collected, incubated and hatched	1980 - 2016	Turtle Board

The principal rookery for Sarawak's sea turtle stock is located of the coast of Southwest Sarawak on three islands known as Sarawak Turtle Islands (Figure 3.1).The three islands are Talang Talang Besar, Talang Talang Kecil and Satang Besar Island.

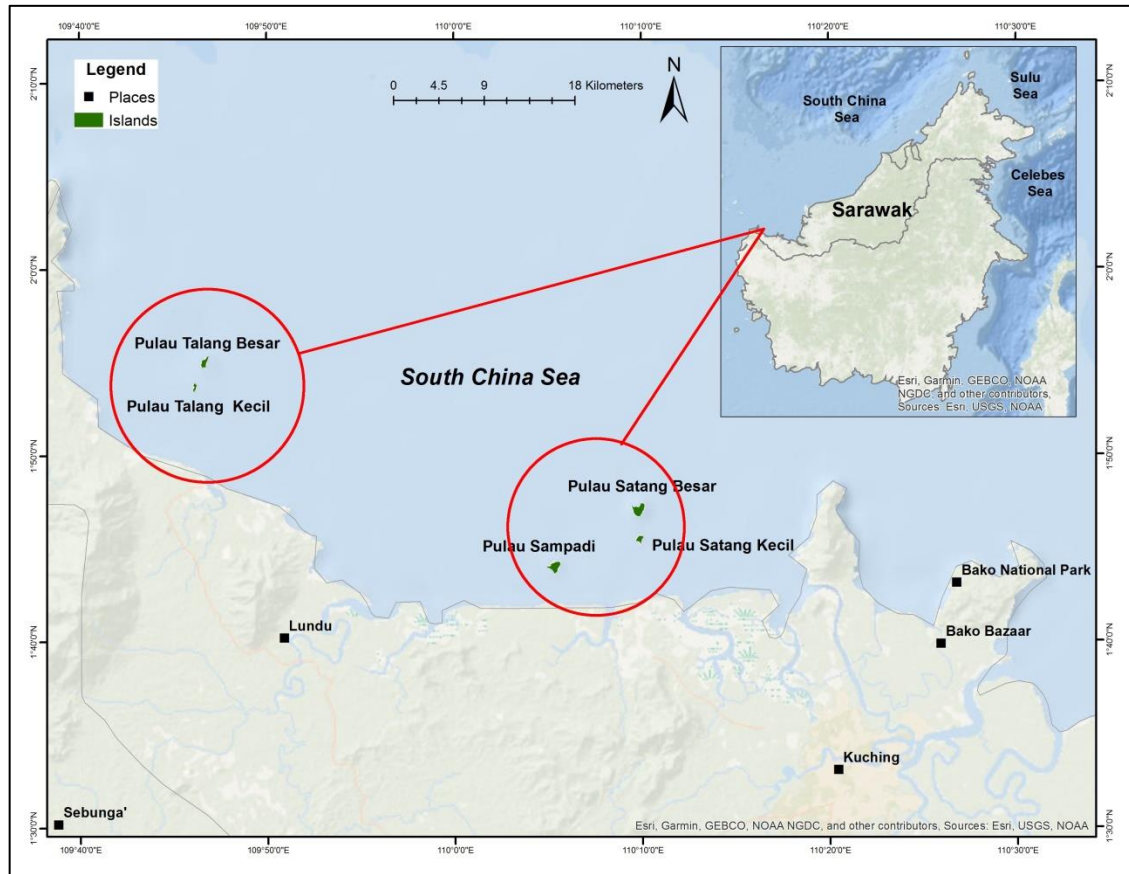


Figure 3.1: Location of Talang-Satang National Park (Sarawak Turtle Islands) and Sampadi Island (turtle foraging ground)

According to Leh *et al.* (1985), the Talang Talang Islands are approximately 2 kilometres apart at 109°46'E., 1°44'N., while Satang Besar Island is located at 110°9'E., 1°47'N., with the former being 9 kilometres off the mouth of Sematan River and the latter lie approximately 7.3 kilometres offshore and 18 kilometres away from Santubong (mouth of Sarawak River).

3.2.2 Estimating the Size of the Stock of Mature Female Turtles

Stock size of mature egg-laying female turtles was estimated with the turtle nesting or turtle landing data. The total number of nesting was assumed to be related to the number of

mature females depending on their remigration interval (r) and interesting frequency (i). The formula adapted for the estimation of mature (or egg-laying) female stock was adapted from Diong *et al.* (1999). The formulas used are as follows:

$$\text{For } r = 1, \text{ use: } \frac{1}{5i} \sum_{j=-1}^3 n_j$$

$$\text{For } r = 2, \text{ use: } \frac{1}{6i} \left[2 \sum_{j=-1}^3 n_j + (n_0 + n_2) \right]$$

$$\text{For } r = 3, \text{ use: } \frac{1}{2i} \left[\sum_{j=-1}^3 n_j + n_1 \right]$$

$$\text{For } r = 4, \text{ use: } \frac{1}{2i} \left[\sum_{j=-1}^3 n_j + \sum_{j=0}^2 n_j \right]$$

For the equations given, n_j denotes the total number of nesting turtles in a particular j^{th} breeding season where $j=1,2,3,4,5$. Since the estimation for the stock's latter number of mature females will be estimated every 10 years and the data used will be the data from the first half of the decade for that time (example: 2010 until 2014), n_{-1} , n_0 , n_1 , n_2 and n_3 indicates total number of nesters in year 2010, 2011, 2012, 2013 and 2014 respectively. From the data obtained, the stock size of mature egg laying females will be estimated from the data of year 2000 to 2004 (data A) and data of year 2010 to 2014 (data B).

Population of *Chelonia mydas* in Sarawak does not reproduce on a yearly basis; instead they exhibit a 2, 3 or 4 year breeding periodicity with the triennial cycle being the normal cycle (Diong *et al.*, 1999). According to Diong *et al.* (1999), the interesting frequency (i) of Sarawak's green turtle is from 2 to 5 times per breeding season with an average egg-laying of 3 clutches per female in a season. Hence making $2 \leq r \leq 3$ and $3 \leq i \leq 5$. The main

breeding activities on STIs have been determined to be from May to October each year although nesting takes place all year round.

3.1 Results

3.3.1 Nesting Turtles Annual Trend for Year 1949 to 1979

The nesting trend for mature nesters from year 1949 to year 1979 was in declining trend (Figure 3.2) where the highest number of nesters for Sarawak Turtle Islands was in the year 1950 at 23,576 nesters while the lowest was in year 1966 at 960 nesters. This shows a decline of 95.9 % from year 1950 to 1966. As for number of nesters in terms of decade, the average number of nesters for year 1950 to 1959 was 13,462 nesters, for year 1960 to 1969 was 4,527 nesters and the average for 1970 to 1979 was 2,339 nesters. The decrease in terms of percentage from the 1950s to 1960s was 66.4 % and from the 1960s to 1970s was 48.3 %.

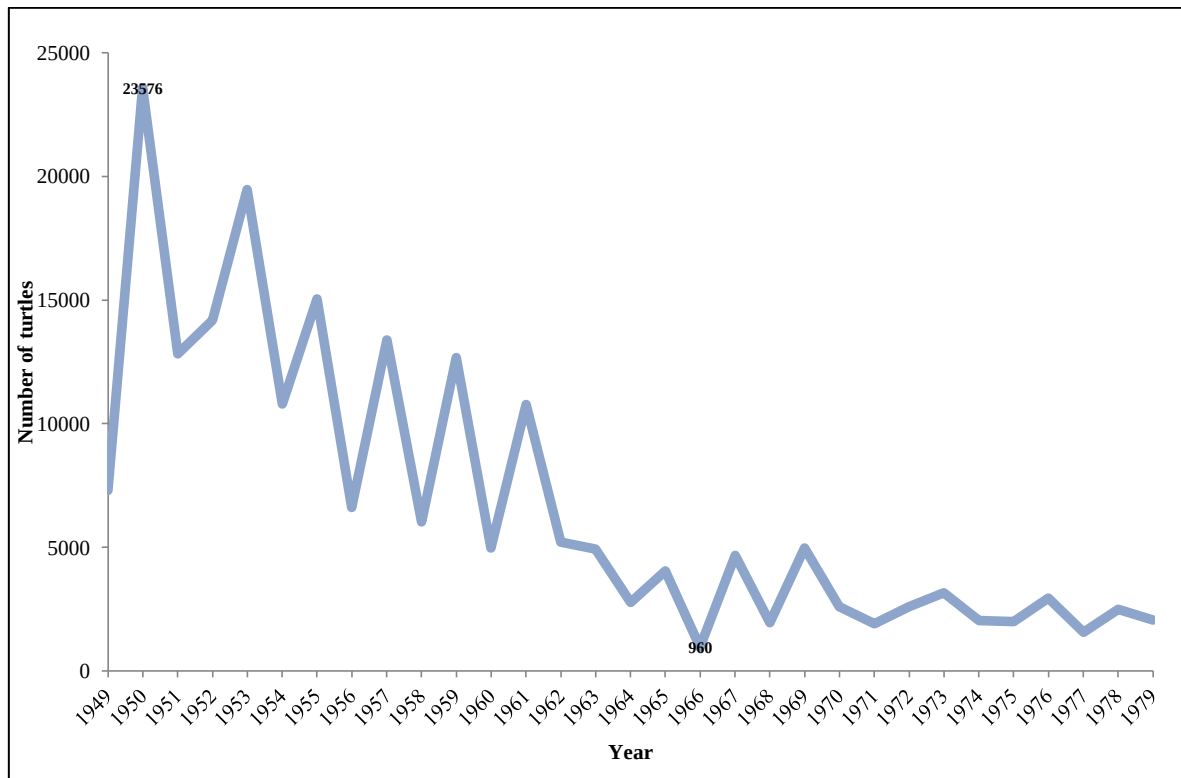


Figure 3.2: Figure of turtle nesting trend from year 1949 to 1979

3.3.2 Nesting Turtles Annual Trend for Year 1980 to 2016

From year 1980 to 2016, the highest number of nesters was in the year 1991 while the year with the lowest number of nesters was in 1987 (Figure 3.3). In terms of decades, the average number of nesters for year 1980 to 1989 was 2,071 nesters while the average for year 1990 to 1999 was 2,263 and the average for year 2000 to 2009 was 2,337 nesters. The average number of nesters for the past three decades is in equilibrium of approximately more than 2,000 nesters. However, this situation could also be interpreted as a plateau phase achieved after a sharp descend in terms of average nesters from the year 1950s and 1960s. Comparison of the average of the 1950s and 1960s to the year 2000's average yielded an 82.6 % and 48.4 % in decrease, respectively.

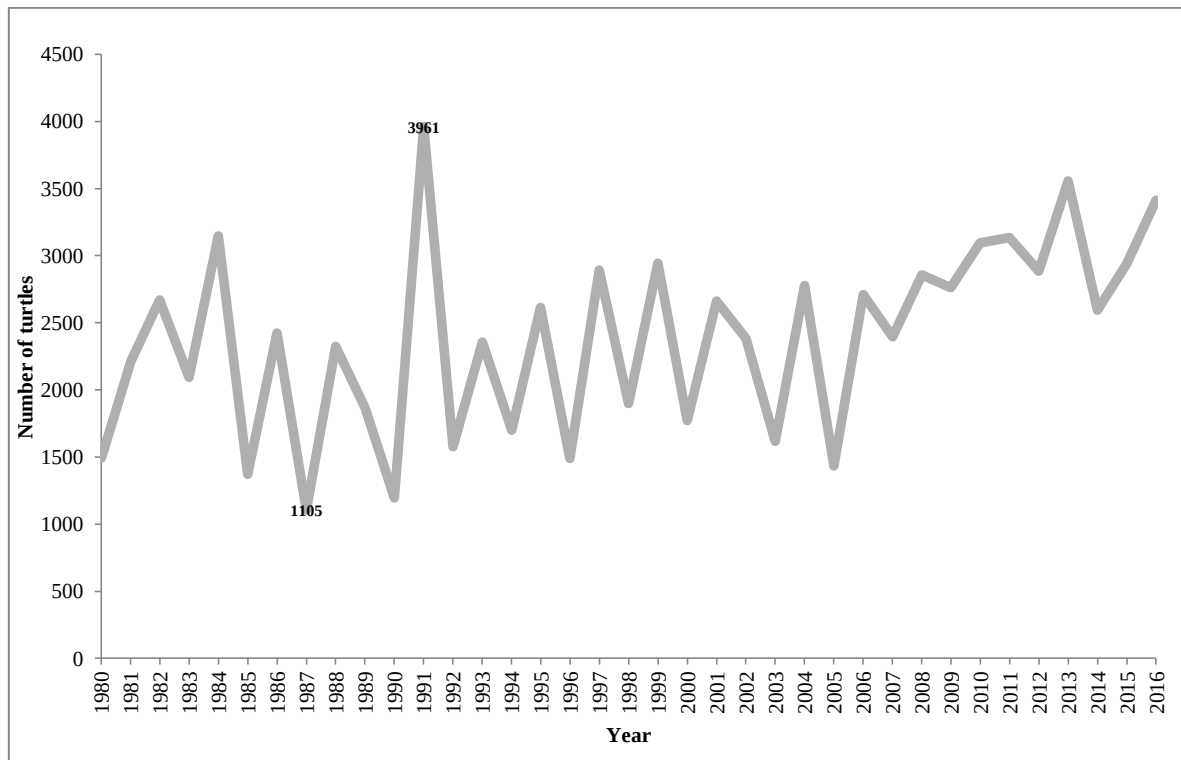


Figure 3.3: Turtle nesting trend from year 1980 to 2016

3.3.3 Estimation of Egg-laying Females

In this study, the estimation of the stock of mature female nesters was carried out using data from 2000 to 2004 (A) and data from 2010 to 2014 (B). The estimated number of mature female nesters as a function of remigration intervals (r) and internesting frequency (i) is as shown in Table 3.2 (data A) and Table 3.3 (data B).

1. Using data from 2000 to 2004 (data A)

Table 3.2: Estimation data of mature female nesters for decade of 2000

Interesting frequency (i)	Remigration interval (r)			
	1-year	2-year	3-year	4-year
2	1121	2225	3399	4469
3	747	1484	2266	2980
4	561	1113	1700	2235
5	448	890	1360	1788

2. Using data from 2010 to 2014 (data B)

Table 3.3: Estimation data of mature female nesters for decade of 2010

Interesting frequency (i)	Remigration interval (r)			
	1-year	2-year	3-year	4-year
2	1527	3102	4537	6210
3	1018	2068	3025	4140
4	763	1551	2269	3105
5	611	1241	1815	2484

Table 3.2 shows the estimated number obtained from using existing data of turtle landing from year 2000 to 2004 while Table 3.3 shows the data obtained from using the existing data from year 2010 to 2014. As the data were estimated from the same large group of turtles that is the stock of mature female turtles in Sarawak, if r is taken as 3, then the 2003, 2006 and 2009 turtle landings correspond to the same group of turtles which is 1 out of 3 equal parts of the total mature female group.

The stock of mature female nesters in the decade of year 2000 is estimated to be between 1,484 and 2,266 individuals. The value 1,484 is from $r=2$, $i=3$ while the value 2,266 is from $r=3$ and $i=3$. However, the average number of nesters for the 2000 decade was 2,337 which was slightly different from the estimated value. There are two possibilities for this situation; (1) the turtle's remigration interval was every 3 or 4 years with interesting frequency of 3 or (2) the turtles do practice remigration of every 2 or 3 years but have a lower interesting frequency of 2.

Therefore, from Figure 3.3, an average number of nesters from 2010 to 2016 was calculated and compared to data set B (Table 3.3). The average of 7 years of landing data was 3,088 which when compared to Table 3.3 corresponds to the remigration interval of every 3 to 4 years with interesting frequency of 3 ($3 \leq r \leq 4$, $i=3$).

3.3.4 Egg Collection and Incubation on Sarawak Turtle Islands (STIs)

Egg collection from year 1980 to 2016 (Figure 3.4) shows that the eggs were harvested annually with a 36 years average of 223,558 eggs. The highest number of eggs collected was in year 1991 at 384,579 eggs while the second highest collection was in 2013 at 314,552 eggs. The lowest number of egg collection was at 107,873 in the year 1987. Note that the eggs were collected from all three islands of STIs. The number of eggs incubated shows a rapid increment in which there is an overlap between the number of eggs collected and the number of eggs incubated from year 1991 onwards.

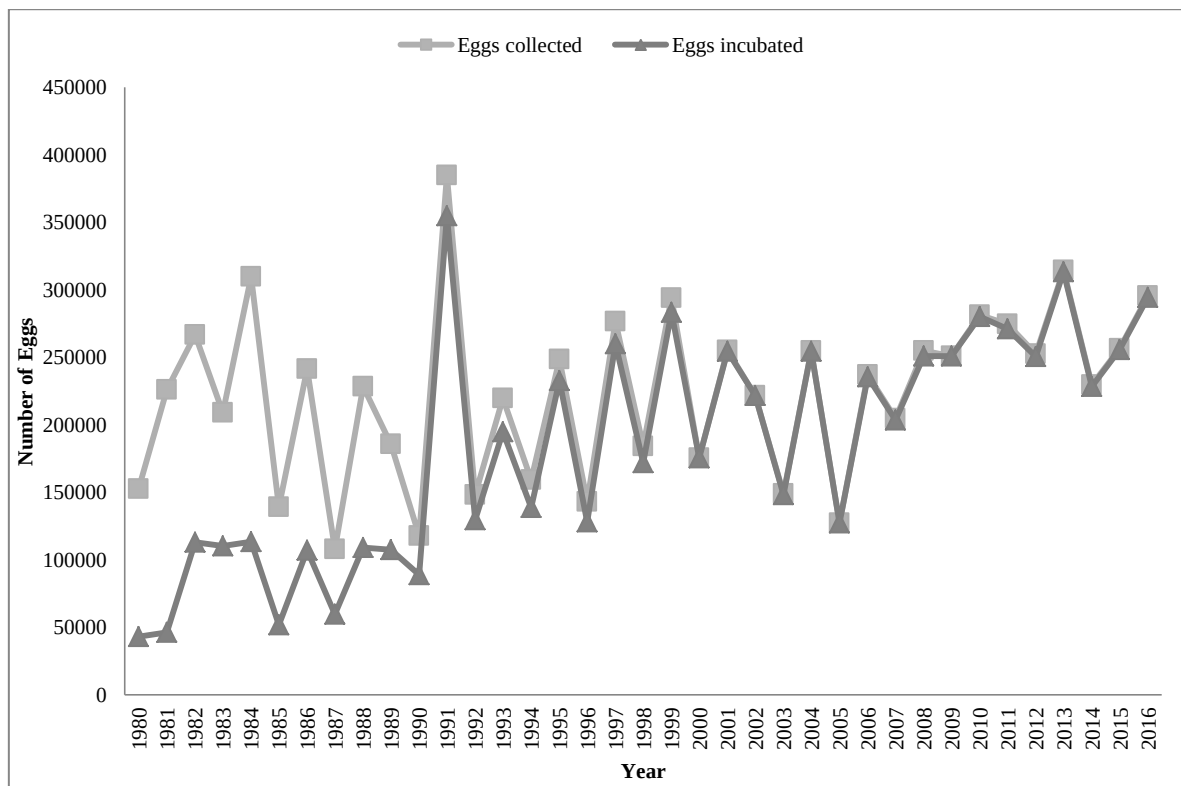


Figure 3.4: Total number of egg collected and incubated trend from year 1980 to 2016

From Figure 3.5, the lowest percentage of eggs incubated was in year 1981 at 20.4 % and year 1991 was the first time that egg incubation at STIs reached the 90 percent stage at 92.2 % thus causing the overlap between the egg collected and egg incubated graph.

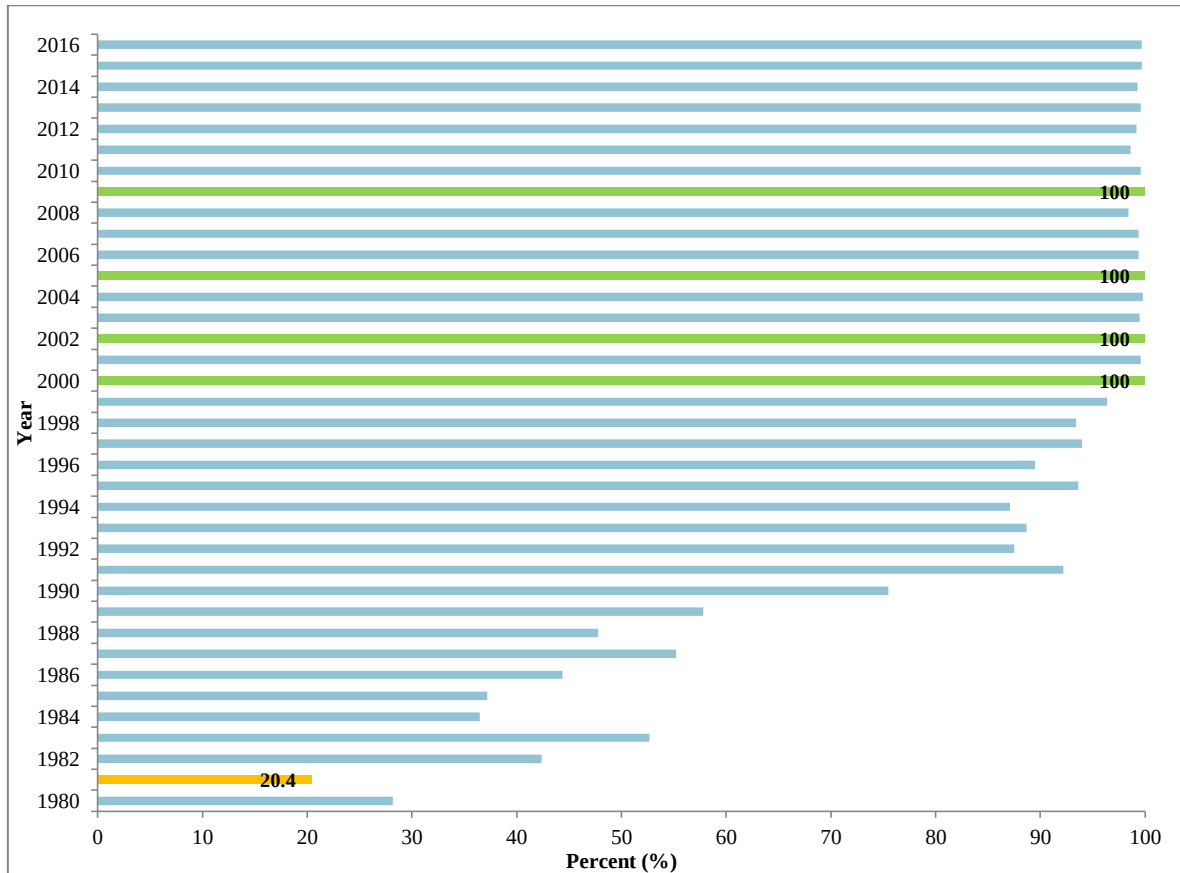


Figure 3.5: Number of eggs incubated in percentage from year 1980 to 2016

Moreover, 100 % incubation of eggs collected from STIs was recorded for year 2000, 2002, 2005 and 2009. Based on Figure 3.5, the percentage of eggs incubated on all islands for 2010 onwards was at least 99 % with an exception for year 2011 at 98.6 %.

3.3.5 Eggs Incubated and Hatched on STIs from 1980 to 2016

The hatch rate of turtle eggs on STIs is shown in Figure 3.6. The annual percentage of hatch rate was obtained using the number of eggs hatched over the number of eggs incubated. Over the course of 36 years, the year with the highest hatch rate was 2002 at 77.7 % and the year with the lowest hatch rate was 1998 at 33.4 %. The mean hatch rate between years 1980 to 1989 was 60.2 %, between years 1990 to 1999 was 53.1 % while the mean hatch rate for year 2000 to 2009 was 65.9 %. The 7 years mean average from 2010 to 2016 was 66.2 %. The trend of hatch rate for the past 36 years has been increasing especially from the 1990s to 2000s. The increasing trend could be correlated to the increase in numbers of eggs incubated thus ensuring a higher success in hatch rate on all 3 islands.

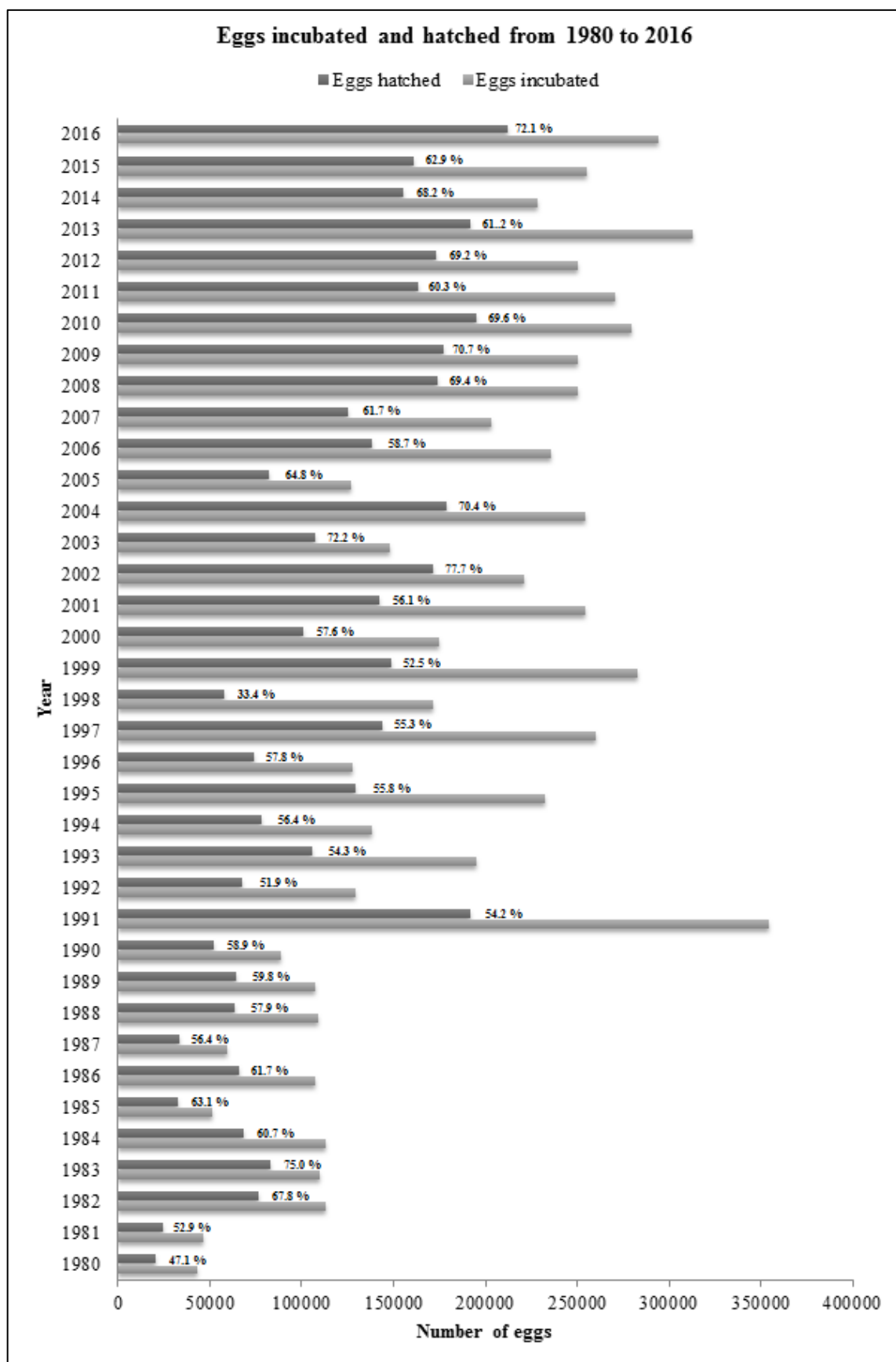


Figure 3.6: Percentage of egg incubated and hatched from year 1980 to 2016

3.4 Discussion

3.4.1 Nesting Turtles Annual Trend for Year 1949 to 1979

There was a sharp decline in terms of number of nesters from 1950 to the 1970s. It is hard to point out the cause of this pattern due to limited information on events happening during the 30 years period. The following paragraphs only discuss the possible causes based on published materials and should be read while bearing in mind that the possible causes are speculated based on other experiments and previous observations of STIs..

Diong *et al.* (1999), stated that the activity that contributed to the drastic decline in Sarawak's turtle population as the prolonged period of over-harvest of eggs prior to 1965 in which very few or no eggs were kept for incubation as the number of eggs harvested annually reached over 2 million eggs per year (Harrison, 1951; Banks, 1986).

Another factor that could have caused the 95.9 % decline from year 1950 to 1966 was the fact that turtles were actually slaughtered for food in the post-World War II period (1945 to 1948) which was something not practiced in the Borneo waters before the Japanese war (Harrison, 1962; Mortimer, 1990a). Harrison (1962) had also mentioned that the Japanese had worsened the situation by having a fighter airstrip opposite Satang Island *i.e* at Sibu Laut area and had used the 'Turtle Rock' located south of Talang-Talang Kecil Island as a bombing practise target. The sound and water pollution has negative impact on the turtle population in the area.

Moreover, Diong *et al* (1999) has also stated that there was chemical pollution in the marine environment caused by operations of the Sematan Bauxite Company from 1958 to 1965, consequently a rapid increase in the ocean traffic happened. Another possible reason that deterred the matured nesters laying eggs was the increased amount of lightings

along coastlines. Harrison (1962) mentioned that the Talang-Talang Islands were facing the mine; the lights from the mine's earth loading section was used day and night with the addition of Japanese steamers that anchor offshore and were visible from Talang Besar Island throughout the turtles active breeding months. Witherington *et al.* (2011) had emphasized that excessive light incidence can deter turtles from nesting areas.

Leh *et al.* (1989) added that the introduction of trawl and drift net fishing in the 1960s as another contributing factor to the reduction in turtle populations in Sarawak. According to Harrison (1962), the whole tempo of Sarawak's development was stepped up in the mid-fifties with the increase in summer disturbance by vessels especially the Chinese motor fishing *kotak* fleet. To make matters worse, turtles that returned to nest at STIs at the time were found to be wounded or evidently escaped from being tied up after catching. Harrison (1962) stated that this situation had never occurred before 1955 and suspected the mainland inhabitants, Indonesian boats and the Chinese *kotak* fleets as the culprit to this crime.

3.4.2 Nesting turtles Annual Trend for Year 1980 to 2016

From 1980 to 2016, the annual nesting trend of turtles on STIs fluctuated (Figure 3.3). It is noted that the nesting turtle stock showed a slight recovery but with dependency to the remigration interval of the turtles. The remigration interval of the turtles is dependent on the food availability, weather, pollution, location, behaviour of the turtles as well as human disturbance factors.

Nesting abundance of sea turtles is greatly affected by the variability in primary and secondary production in sea turtle foraging areas as female turtles depend on specific

amount of food for vitellogenesis or formation of yolk in eggs before migrating to nesting beaches (Wallace *et al.*, 2006). Therefore, lack of food could be directly related to the delayed return which means that sea turtles will nest less frequently with limited food availability (Broderick *et al.*, 2001). The physiology and overall survival of sea grasses are affected by water temperature; therefore, the rising temperature due to climate change will have a profound effect on sea grass meadows (Duarte, 2002).

In Sarawak, seagrass beds were discovered at the end of 2008 by Sarawak Forestry Corporation (SFC) at Sampadi Island which was located at about 5 km from Satang Island section of the Talang-Satang National Park (Bali *et al.*, 2012). A field work was carried out by SFC in 2009 where it was discovered that Needle Seagrass (*Halodule pinifolia*) was present in abundance within the estimated 15 hectare of seagrass bed at Sampadi Island. The seagrass beds comprised of parts that were exposed during low tide; however, immature green turtles were observed feeding at deeper parts of the seagrass areas (Bali *et al.*, 2012). Other than Sampadi Island, seagrass beds of Kuala Lawas have been confirmed as foraging ground for green turtles in Sarawak (Bali *et al.*, 2014).

There are many potential impacts of climate change on sea turtles for example loss of nesting habitats due to sea level rise and effect of sea surface temperature on nesting patterns of sea turtles (Weishampel *et al.*, 2004; Katselidis *et al.*, 2014). Mazaris *et al.* (2009) reported that increment of 1.5 °C in sea surface temperature at a foraging ground had resulted in decline of almost 500 nests per season in Zakynthos, Greece. Patel *et al.* (2016) calculated a similar trend in Rethymno, Greece and added that the nesting declines could be an effect of deterioration of foraging grounds or could correspond to the strong anthropogenic impact on these beaches as the downward nesting trend did not affect a nesting beach with limited human presence. Hamann *et al.* (2002) had supported this by

mentioning that the conditions of a beach influences its suitability for nesting and hatchling emergence thus affecting the chances of female marine turtles nesting there. Although climate change concept is generally accepted, there is still on-going debate on the magnitude of its effects.

The other factors that could affect the nesting behaviour of the turtles are the characteristics of sand of the landing beach, the coastal vegetation, the offshore approach and the shoreline composition. Ehrenfeld (1979) mentioned that sand characteristics such as its salinity and grain size coupled with other conditions such as smooth slopes, sandy beaches and the humidity level could be factors forcing females to change their nesting behaviour. Abd. Mutalib *et al.* (2014) supported this as their research showed that the temporal distribution of nesting turtles in Setiu, Terengganu could be affected by the northeast monsoon that causes erosive waves and habitat alterations from November until February. Mortimer & Carr (1987) had suggested that rainfall affects the frequency of nesting of marine turtles as damper sand encourages nesting. A study conducted by Sarahaizad *et al.* (2012) showed that a greater number of nests were found within the vegetation zone as *Merambong* trees provided shelter and protection against disturbance from humans or feral dogs. A low percentage of nests were found at the open beach (nearest to the high tide line) at Pantai Kerachut and Telok Kampi as Mortimer (1990b) had mentioned that it was prone to collapse and destruction by wave wash. Mortimer (1982) had also shown that there is an overall decrease in the number of clutches laid on beaches characterized with obstacles and that turtles seems to prefer the deepest approach to the beach. The main reasons for this are rocky shores coupled with heavy surf makes coming ashore dangerous, not to mention the heightened predation pressure on hatchlings as more predators occur at rock strewn bottom than over sand (Mortimer, 1982). He argued

that as for the deepest approach to the beach or the gradient of the beach, it is what determines the distance the turtles must crawl over land in order to reach a nest site. Similarly, big leatherback usually chooses a nesting beach with a steep slope is to reduce the distance between the water line and the nest site (Pritchard, 1971).

Natal homing is the dominant paradigm for sea turtle migrations, although the geographic specificity of homing varies widely (Bowen & Karl, 2007). A matured adult female usually practices natal homing but could switch to social facilitation if their birth place was deemed to be unfit for nesting. Yahya *et al.* (2012) had demonstrated that there were two clades in terms of nesting females of sea turtles of the STIs based on D-loop region gene analysis; namely (1) clade of Talang-Talang Island nesters, (2) clade of mixture between the Talang-Talang and Satang Besar Islands. The presence of the first clade shows that natal homing dominates as the nesting population possess a unique genetic signature in terms of female transmitted mtDNA (Bowen & Karl, 2007) while the presence of the second clade suggested the social facilitation hypothesis. Carr *et al.* (1978) have stated that climatic fluctuations that could have altered the availability of the green turtle habitats promotes strays and wandering that are both advantageous and adaptable change necessary for colony proliferation. The change in nesting habitat of Sarawak's egg laying female stock from Satang Island to Talang-Talang Island could be attributed to the active eco-tourism activity being carried out in Satang Besar Island (Mortimer, 1990a). However, both islands of Talang-Talang are strictly for turtle conservation activities led by Sarawak Forestry Corporation since 1999. Talang-Talang Besar Island is home to a sea turtle egg hatchery and a nesting beach for most green turtles that visit STIs. The six islands namely Talang-Talang Besar, Talang-Talang Kechil, Satang Besar, Satang Kechil, Tukong Ara and Tukong Banun were gazetted as a Marine Protected Area in 1999 for

protection of marine turtle critical habitats and ecosystem (Bali *et al.*, 2012). Besides that, since 1999, the Sarawak State government had also enforced a total ban on selling of turtle egg to public besides commencing the reef ball project, which among many of the objectives is to help maintain population of green turtles in Sarawak (Bali *et al.*, 2014).

3.4.3 Estimation of Egg-laying Females

The estimation of mature egg-laying female turtles was done to estimate the current mature turtle populations in Sarawak. Based on this estimation, the interesting and remigration interval of the mature turtles could be predicted and the factors that affect the turtles could be identified. Moreover, the number of new females could be estimated as the new females would make up the rest of the number after deduction of the existing stock number from a particular year. A hatchling could take a minimum of 19 years to 30 years before it grows to a mature adult female and once reaching maturity, it could continue laying eggs for 30 or 40 more years (Bjorndal & Bolten, 1988).

The number of mature females needs to be estimated so that the future stock could be maintained as turtles that mature in the 1970s could possibly not lay eggs anymore in 2020. Mortimer (1990b) stated that 40 to 70 years or more is the time duration of when a female first hatches out of her egg and when she stops laying eggs, which means that the current population is composed of individuals that would have hatched from their eggs anytime between 1947 and 1997.

From the quasi-periodicity in estimated nester abundance suggests that female green turtles migrate to nest at STIs every 3 or 4 years, major ocean climate anomaly had been named as an environmental forcing function which could have synchronized multi

stock nesting at Southeast Asian green turtle rookeries (Chaloupka, 2001). If this situation is proven to be true, there might be a downward trend in the near future if new juvenile females are not cultivated from now.

Apart from that, another possible reason for this is females nest more frequently, which means that the return period between successive nesting seasons is shorter rather than there being more nesters. This could be a plausible reason as there seems to be a fluctuating trend in nester abundance which occur every 2 or 3 years since 1980. The increment in sea surface temperature could have been the causal factor for the shorter remigration interval. This fact was supported by Balazs & Chaloupka (2004) and Solow *et al.* (2002) which have shown that there was an association between sea surface temperature with annual fluctuations in the nesting of green turtles at the Hawaiian and Tortuguero rookery.

Bjorndal *et al.* (1999) pointed out that if clutch frequency varies between years, the increase in nesting does not necessarily reflect an increase in the females. In addition, environmental variables may influence the number of turtles ready to nest in a given year (Chaloupka, 2001). If the mean remigration interval has decreased as a result of environmental change, an increase in nesting would occur without an increase in female (Broderick *et al.*, 2001).

3.4.4 Egg Collection and Incubation on Sarawak Turtle Islands (STIs)

Mortimer (1990a) has described the current population of nesting females on STIs as critically endangered due to the long period of egg harvest as prior to 1976; fewer than 2 % of the eggs laid annually were placed in the hatcheries. She stated that a serious decline in

number of nesters should be expected in the next couple of decades as only a small number of eggs were protected between 1963 and 1975; taking into account the current estimate of survival from egg to adult range from 1 female produced per 1000 eggs to 1 female per 10000 eggs. To protect the green turtle population of Sarawak, Mortimer (1990a) has recommended implementation of 100 % protection of eggs collected following the suggestion that at the very least 70 % or more eggs need to be protected when the population has a history of over-harvest (Limpus, 1987).

There is positive increase in terms of number of eggs incubated from 1980 to 2016. The number of eggs collected was fluctuating over the course of 36 years, similar to the number of turtle landings. The effort made by the turtle custodians of Sarawak in order to help the turtle population grow was translated in the increased number of eggs incubated. This effort also shows that the implementation of Wild Life Protection Ordinance 1998 (Laws of Sarawak, Chapter 26) which prohibits exploitation and trade in all marine turtles, their eggs and any derivative or their parts (Tisen & Bali, 2000), is working.

3.4.5 Eggs Incubated and Hatched on STIs from 1980 to 2016

Despite the commendable effort of the local Sarawak agency which is mirrored through the number of eggs incubated, the result would still be the same if the hatch rate of eggs is low. Therefore, to ensure the success of the egg incubation, factors that could affect the hatch rate should be considered and studied. The major nest factors that could affect embryonic development of turtle eggs are gas exchange, moisture and temperature (Mortimer, 1990b). In this study, average percentage of successful hatching from year 2010 to 2016 was 66.2 %. This is lower compared to the hatching success rate in Setiu, Terengganu (Abd. Mutalib & Fadzly, 2015).

The sand's grain particle size and the water content of the sand are said to have a direct impact on the gas exchange rate of the nest environment. A study by Mortimer (1982) shows that there is an optimum range of grain sizes for hatching success, as nests can fail if the sand is too fine or too coarse. Ackerman (1981) had mentioned that inadequate gas exchange could slow growth and increase embryonic mortality. As the same colony of green turtles were observed to utilize a wide range of sand types (from fine sand to coral pebbles), it was concluded that the grain size is probably less important to a turtle in her choice of a nesting beach (Hughes, 1974; Mortimer, 1982).

Another major factor that could affect the hatching of turtle eggs is climate change. Turtle eggs hatchability rate and the gender of the turtles depend on the temperature of the nest: within the range of 26 to 32 °C, a change of 1 °C will result in addition or subtraction of 5 days to the incubation period (Mrosovsky, 1980). The pivotal temperature varies among species and between populations (Limpus *et al.*, 1985); however, the general idea that cooler temperatures produce males while warmer temperatures produce females holds for all species (Georges *et al.*, 1994). These eggs are highly sensitive to temperature as eggs that are incubated at temperatures lower than 23 °C for the last third of incubation seldom hatch while eggs that are held at 33 °C for extended period do not hatch. Other than temperature, the eggs are also sensitive to desiccation and excessive moisture as the egg mortality rate is higher in drier condition and during inundation of eggs for extended period of time (hours) (Kaufman, 1968). Therefore, if the amount of rainfall were to increase and the temperature of the sand at the nest site decreases, the hatch rate will be lowered. This has been reported by Leh (1994) in which the percentage of unhatched eggs was as high as 82 % during the wet monsoon months of November to April at STIs.

A study by Broderick *et al.* (2003) has shown that the clutch size of green and loggerhead turtles that were nesting in Cyprus increases with body size. The body size factor, however, does not apply to the number of clutches laid. Broderick *et al.* (2003) have also mentioned that in contrast to loggerhead turtle that lays smaller clutches as it becomes resource limited at the fifth clutch, green turtles usually lays smaller clutches at the start of the laying season which resulted in gradual increase in efficiency or capacity in the process related to vitellogenesis. Besides that, the green turtles in Cyprus were reported to lay larger clutches in subsequent nesting season compared to previous ones after a long remigration interval which could be the result of an increase in efficiency in breeder with time (Broderick *et al.*, 2003). It should be noted that the energy and chemical components contained in the egg follicles were derived from food eaten in the foraging area thus proving that fluctuations in food availability will affect reproductive output (Wikelski & Thom, 2000).

Another factor that could affect the hatch rate of sea turtle eggs in Sarawak is the fertility of the eggs. Diong *et al.* (1999) shows that the depth of nest had no significant effect on hatch rate and incubation period while clutch size did. It was mentioned that infertile eggs could have a dampening effect on viable fertile eggs as those are not able to generate metabolic heat to create optimum thermal environment for nest incubation. The examination of unhatched eggs done by Diong *et al.* (1999) revealed that infertility was the cause of low hatch rates instead of embryonic mortality. However, it should be noted that their research had been conducted towards the end of the breeding season which could have contributed to the low fertility rate of the eggs. Meanwhile, in Setiu, the 73.9 % of eggs incubated had been successfully hatched with only 19.7 % of unhatched eggs showing no embryonic development (Abd. Mutalib & Fadzly, 2015). In addition to that, Chan

(1991) suggested that the high proportion of infertile eggs could be due to fewer males being available to mate with females.

A recent study by Jensen *et al.* (2016) had caused renewed concern over this matter. Jensen *et al.* (2016) had conducted a mixed stock analysis using molecular markers from samples of green turtles at Mantanani and Layang Layang Island, which both areas are known as foraging ground for the immature turtles. They claimed that more than 80 % of the juvenile turtles originated from genetic stocks of Sarawak Management Unit (MU), Peninsular Malaysia MU and Sulu Sea MU which includes the TIHPA (Turtle Islands Heritage Protected Area) (Dethmers *et al.*, 2006). They found that the estimated mean contribution of males and females from STIs were only 2 and 25 %, respectively; which contradicts with Peninsular Malaysia's contribution of 62 % of males and 30 % of females. Lack of contribution from STIs suggests that conservation efforts carried out in STIs need to be stepped up to ensure sustainable population of *Chelonia mydas*.

Besides that, San *et al.* (2004) had estimated that hatchery-raised hatchlings for Sarawak rookery was highly female biased (80- 96 % females) in comparison to nests left *in situ*. Although Wright *et al.* (2012) claimed that the higher breeding frequency of males may counteract female biased sex ratio, it should be noted that the highly skewed sex ratio will eventually impact clutch fertility as males become a limited resource especially when considering predictions of future increment in temperature which could lead to female bias hatchling recruitment (Witt *et al.*, 2010; Fuentes *et al.*, 2010; Fuentes *et al.*, 2011). Moreover, Polaczanska *et al.* (2009) had suggested that concern should be raised if male: female (M: F) sex ratio of hatchlings across all nesting beach for a particular stock approaches 1M: 4F. However, it is still not known how much a female bias population can

sustain the overall population as green turtles takes 20 to 50 years after hatching to reach sexual maturity thus making female bias condition undetectable until decades later.

3.5 Conclusion

Changes in the legislation and the immense conservation efforts that had been carried out in Sarawak since the 1970s are shedding some light into the seriously declined population of nesting female turtles of Sarawak. This situation is however still at an unstable phase and future trend should be observed as it could be an effect of climate change which shortens the remigration interval between nesting seasons. Therefore, the future nesters should be continually cultivated for the future of turtle population of the Sarawak rookery. Moreover, the average 60 % of hatch rate of turtle eggs on STIs should be noted, however, as the cause behind the relatively low percentage is still unknown. In order to achieve a healthy and balanced turtle population, the fertility percentage of the eggs laid should be made known and a new research on turtle nest temperature should be conducted on the island's hatcheries on a yearly basis so that the effect of climate change could be countered with proper hatchery management measure to ensure that a healthy sex ratio of sea turtle hatchlings will be produced.

CHAPTER 4

STANDARD OPERATING PROCEDURE FOR HUSBANDRY, BEHAVIOUR AND GROWTH OF GREEN TURTLES DURING HEADSTART PROGRAM

4.1 Introduction

In sea turtle life history, the post-hatchling period or the period after hatchlings leaves their natal beach for the water is not well known and that is why that period of time is called their 'lost years' (Witham, 1980). Many attempts have been made to discover the mystery behind their 'lost years'; however, lack of suitable and durable tags for hatchlings data acquisition have disturbed the process (Witham, 1980). According to Dodd & Seigel (1991), conservation strategies involving reintroductions, repatriations or relocations of amphibians and reptiles are increasing worldwide. However, the most elaborate technique that has been attempted might have been headstarting (Huff, 1989; Fontaine *et al.*, 1990; Wood & Wood, 1993; Caillouet, 2000; Bell *et al.* 2005).

Witham (1980) reared green turtle hatchlings and released them as yearlings in the 1960s to check the dispersal, growth rate and movement of the yearlings. Many authors have suggested that the factor influencing sea turtle dispersal is ocean currents. Witham (1980) stated that his experiment supports dispersal of sea turtles by ocean currents, by detail analysis of the tags attached to yearlings. Witham (1980) also mentioned that turtles exhibit a wave orientation response by unhesitatingly swimming towards surf with sufficient wave action but hesitates when the waves are very small or absent. However, green turtle growth appears to be slow in nature, although food resources are available for sea turtles long term survival while following the ocean currents. He strongly suggests that

“the lost year” is a period of oceanic existence when turtles opportunistically use oceanic currents and food resources for dispersal and survival.

Bell *et al.* (2005) have described a 22 year old headstarting effort which was started by Cayman Turtle Farm Limited in the Cayman Islands, Atlantic Ocean, to check the capability of headstarted turtles in surviving the wild environment and recruiting to breeding populations. Approximately 16,422 captive–raised hatchlings and 14,347 yearlings of green turtles had been released from the Cayman Turtle Farm between 1980 and 2001 in public events aimed to increase public awareness and educating the public. Approximately 80 % of the turtles released were subjected to tagging in the form of notching, flipper tags, living tags or the combination of living and flipper tags. In a 19 years interval, a total of 392 tagged turtles have been recaptured in which 160 individuals were captured in the Cayman Islands and the other 232 were from other locations within the wider Caribbean and south–eastern USA. Data suggested that growth rates are comparable to those of wild green turtles in the Great Inagua, Bahamas (Bjorndal & Bolten, 1988), Mosquito Lagoon, Florida (Mendonca, 1981), US Virgin Islands (Boulon & Frazer, 1990), Culebra, Puerto Rico (Collazo *et al.*, 1992), and Hutchison Island, Florida (Bresette & Gorham, 2001). There were at least six living tags returned (4 released as yearlings and 2 released as hatchlings) which indicated an age at maturity of 15-19 years, placing age at maturity towards the lower range of that previously estimated for green turtles in the wild (Frazer & Ehrhart, 1985; Zug & Glor, 1998; Mrosovsky & Godfrey, 2003).

Culture trials of green turtle hatchlings were also conducted on Yorke Island, Northern Australia by Kowarsky (1977). He assessed the feasibility and acceptability of a proposed commercial turtle culture venture. The paper describes the details of the culture

condition under which turtles have been raised over the first 5 months of the hatchlings life and tested on effect of water quality and stocking density on the survival of the hatchlings. Kowarsky (1977) had demonstrated the importance of water change schedule in the culture of young green turtles, poor water quality rather than the degree of physical crowding was the major adverse influence on the turtles.

Research related to growth rate of turtles are done based on the wild rather than on captive populations. For example, Casale *et al.* (2009) had carried out study on the age at size and growth rates of early juvenile loggerhead sea turtles (*Caretta caretta*) based on length frequency analysis in the Mediterranean. They stated that growth rates in the first years of life are particularly difficult to obtain due to: (i) low availability of very small turtles in the sea, and (ii) sea turtles show great variability in growth rates even within the same population which may be caused by genetic, sexual and/or environmental factors. Casale *et al.* (2009) had managed to obtain 88 turtles ranging from 6.4 to 30 cm curved carapace length (CCL) from the Italian waters and further analysis shows that the juvenile loggerheads in the Mediterranean takes one to four years to grow to 30 cm CCL; with average growth rate ranging from 11.8 cm/year in the first 6 months of life to 3.6 cm/year at the age of 2.5 to 3.5 years. Results indicated that the Mediterranean loggerhead turtles in the first four years of life display growth rates that are very similar to the Atlantic turtles. The results obtained also suggested that a secondary increase in growth rate among larger turtles recruiting to neritic habitats, which supports a polyphasic growth pattern that being reported happened in other populations and other sea turtle species.

In Culebra, Puerto Rico, Collazo *et al.* (1992) had carried out assessment on growth rate for green sea turtles from 1987 to 1989. The samplings involved seven areas, with 167 individuals that were measured, tagged and employed catch and release technique using

nets. Out of 167 turtles, 25 were being recaptured 32 times, thus the estimation for recapture rate at 19 %. Mosquito Bay and Culebrita had the highest abundance of turtles with 2.16 and 2.04 caught per net set. Collazo *et al.* (1992) stated that the observed size class composition was indicative of a juvenile population with carapace length from 24.6 to 75.3 cm, with an average of 46.34 cm. The age of the turtles captured was in the range of 2 to 14 years old according to the US Virgin Islands age-size model (Boulon & Frazer, 1990). Research by Collazo *et al.* (1992) also showed that the juveniles grew at an annual rate of 5.08 cm carapace length (CL) with the highest growth rates recorded in the 40 to 50 cm CL interval.

In Malaysia, attempts were carried out to keep sea turtles in captivity in Sarawak Museum (Harrison, 1955). However, no long term growth data were documented. The turtle hatchlings were only documented on their food, feeding behaviour and stimuli as well as their learning behaviour. The average weight of hatchlings that were fed and not fed were also recorded which showed no differences in weight after a week (Harrison, 1955). A study conducted at Mantanani, Sabah by Pilcher (2010) claimed that the average growth rate of green sea turtles at the area was 3.60 cm year⁻¹. To get this data, turtle rodeo capture techniques were used with interval between initial and subsequent recapture ranging from 47 to 661 days. However, the growth data was influenced by success of recaptures. His data was not based on the post-hatchling period, but rather on the juvenile phase (turtles involved had average carapace length of 47.4 cm).

Chapter 3 described conservation efforts for sea turtles in Sarawak over the duration of 36 years. The efforts have been successful at different levels, due to many challenges faced after a rapid decline from the 1950s. A contributing factor to the challenges faced is lack of information on the growth and behaviour of post hatchlings

during their period of ‘lost years’. This information is important in order to carry out sustainable conservation of this national heritage.

Therefore, this study was conducted to (i) elucidate the growth of post-hatchling green sea turtles in their first 18 months of life during a headstart effort hoping to shed lights on green turtle general behaviour during their ‘lost year’; (ii) estimate the age of turtles that are found in Sarawak water based on a generalised age-at-length equation derived from growth data of headstart turtles (iii) establish a standard operating procedure (SOP) or framework which could serve as guidelines for future headstart programs.

4.2 Materials and Methods

This section is divided into 3 sub-sections namely, (i) Standard Operating Procedure (SOP), (ii) Observation on behaviours of post-hatchlings in captivity and (iii) Survival and growth performance of green turtles in captivity.

4.2.1 Standard Operating Procedure (SOP) Methodology

For green turtle rearing study methods were adapted from Higgins (2003), with several modifications. Permit to carry out this study had been applied to Forestry Department, Sarawak 8 months prior to this project. Permit number NCCD.907.4.4(Jld.10)-107 and Park Permit Number: 61/2014 was granted. The permit stated that a total of 200 hatchlings may be taken from Talang-Satang National Park to be used in the headstart project. A Standard Operating Procedure (SOP) or framework was established detailing steps and

guidelines for a headstart program. The summary of work related to green turtle headstart project as in Figure 4.1

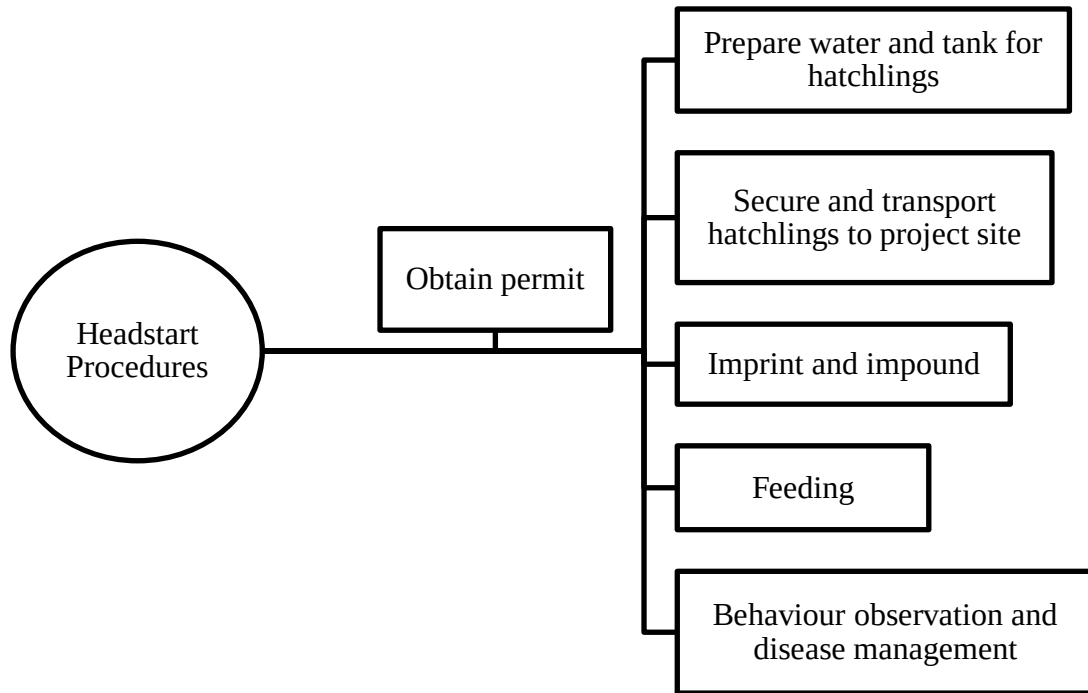


Figure 4.1: Summary of headstart project

4.2.1.1 Procedures to Secure and Transport Hatchlings

A total of 120 hatchlings were obtained on 2nd June 2014 from Talang Besar Island for the first headstart (Batch 1). For the second batch, a total of 50 hatchlings were taken on 3rd September 2014 (Batch 2). The hatchlings chosen for the project were checked for physical deformity, activity, weight and size before they were kept in four plastic boxes filled with approximately 1.0 cm (depth) of sea water. The hatchlings were then transported back to the research site, Pandan GoldCoast Holiday Villa, Lundu, Sarawak for

imprint and impound activity on the beach and sand of Pantai Pandan, Lundu, Sarawak following Manzella *et al.* (1988) method with several modifications to suit the local setting.

The hatchlings were then reared for 18 months before they were released back to the sea on December 2015 and May 2016. In this thesis, ‘hatchling’ refers to day 1 until 2 months old; ‘juvenile’ refers to green turtle aged 3 until 12 months old while ‘yearling’ refers to those of 13 months old and above, following Glazebrook & Campbell (1990).

4.2.1.2 Tank and Water Supply Preparation

The hatchlings were kept in 2 tanks, namely Tank A and B, with length of 2.25 m, width of 0.90 m and water depth of 0.05 m (Figure 4.2a). There were 120 hatchlings involved in Batch 1 and 50 hatchlings in Batch 2 during this study. Therefore, each tank was home for approximately 60 hatchlings at a given time. This provides each hatchling with estimated individual space of 33.75 cm³. The water was changed on a daily basis. pH was measured daily using ECOTestr pH 1. Two aeration pumps were installed in both tanks to facilitate the water movement and to encourage the hatchling’s ‘wave surfing’ activity. The aeration pumps were equipped with mechanical filters that were washed daily to filter out large particles such as turtle faeces. The humidity level was measured using a digital thermo-hygrometer (Model: SPI188).

The sea water that was used throughout the project was unfiltered sea water obtained directly from the sea as the study site was located by the sea side. The water was pumped in using submersible sea water pump (Brand: Romakua, Model:RM0103) into two pools of 4.03 m long, 1.50 m wide and 0.65 m depth (Figure 4.2b) located by the holding

tanks during high tide. The freshly pumped water was allowed to settle overnight before it was transferred to the turtle holding tanks.

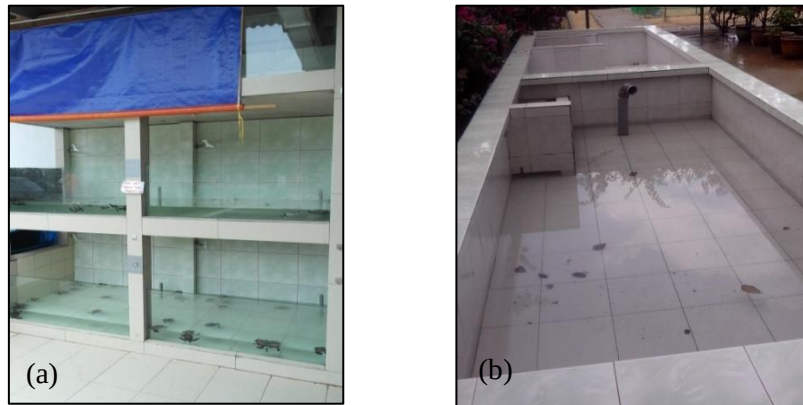


Figure 4.2: (a) Turtle holding tanks, (b) Tank used to store sea water and house juveniles and yearlings

4.2.1.3 Feeding

The turtles were fed twice a day, at 8 am and 4 pm. The turtle's food consists of fish, shrimp, squid and cucumber. The food was cut into small pieces of approximately 1 to 2 cm, to facilitate their feeding when they were hatchlings. Food amount to be given was weighed according to their appropriate food requirement throughout the 18 months. As the turtles grew, the food given was no longer cut into small pieces, to suit feeding behaviour. The amount of food given to hatchlings was calculated following Higgins (2003), with approximate 2 to 5 % of the body weight.

When they were still hatchlings and until they were juveniles (up to 7 months old), the turtles were taken out from their holding tanks and divided into separate sea water-filled basins during each feeding time. Initially, 4 hatchlings were allocated to each basin. The number was gradually reduced as the turtles grow, to allow adequate space for the

hatchlings and then juveniles to eat. The leftover water was discarded after each feeding activity to ensure cleanliness was maintained at the highest level possible.

A slightly different method was used to feed the turtles in their late juvenile until yearling phase. The turtles were by then housed in the bigger tank (Figure 4.2b) to accommodate their size and space requirement. During each feeding time, 1 whole fish were given to them in their holding tank to encourage diving and natural feeding habits. The amount of fish given was weighed to approximately 5 % of their body weight.

4.2.2 Survival and Growth Monitoring

4.2.2.1 Morphometric Data Collection

Morphometric data collection was carried out once every fortnight (Kowarsky, 1977) for duration of 18 months. Each turtle was weighed using analytical balance (Brand: A&D, Model: EW150i) up to 0.01 g, numbered using paint marker (written on carapace) and measured using vernier calliper (Brand: Mitutoyo) up to 0.01 mm for Straight Carapace Length (SCL). All turtles were then photographed using personal hand-phone camera (Brand: Samsung, Model: GT-N7100) to facilitate future identification.

Other morphometric data obtained were in curved carapace length (CCL), therefore, the data were later converted to straight carapace length (SCL) to standardize it so the data could be easily compared to other datasets. CCL was converted to SCL using an equation by Peckham *et al.* (2008) for *Chelonia mydas* :

$$(1) \text{ SCL} = 0.932 * \text{CCL} + 0.369$$

4.2.2.2 Data Analysis

All morphometric data obtained in this study were analysed using Excel (version 2010) and IBM SPSS Statistics version 22. The weight-length relationship of both batch were analysed using Excel and SPSS. The length-weight relationship was calculated using the formula

$$(2) \quad W = aL^b \quad (\text{Pauly, 1984})$$

‘W’ is weight in gram

‘L’ is length in millimetre

‘a’ and ‘b’ are parameters with ‘b’ being called the rate of allometric.

By taking logarithmic transformation on both sides of the equation, the linearized model:

$$(3) \quad \log W = \log a + b \log L$$

was achieved. The linear regression test was carried out to test the weight length relationship using IBM SPSS Statistics version 22. In this analysis, weight is the dependent variable whereas SCL is the independent variable following Wabnitz & Pauly (2008). Wabnitz & Pauly (2008) mentioned that R square value greater than 0.95 indicates no remaining outlier or inclusion of extreme individuals such as early juveniles or aberrant adults (Froese *et al.*, 2011). Paired t-test was done to check whether there is a significant different between weight and length of the two batches using IBM SPSS Statistics version 22. Hypothesis tested as follow:

H_0 : There is no significant difference in weight and length of turtles in Batch 1 and 2

H_a : There is significant difference between weight and length of turtles in Batch 1 and 2

Graphs were constructed to illustrate growth curves, whereby growth rates were calculated accordingly.

The survival percentage and the graph for survival rate for the 2 batches involved in this study were calculated using Excel. Paired t-test was used to check the level of significance between the survival rate of the 2 batch using IBM SPSS Statistics version 22.

Hypothesis tested as follow:

H_0 : There is no significant difference in survival rate of turtles in Batch 1 and 2

H_a : There is significant difference in survival rate of turtles in Batch 1 and 2

To estimate age at length, Frazer & Ladner (1986) has used a von Bertalanffy equation by Fabens (1965) that allows fitting of data from animals of unknown age to estimate growth curves which requires measurements at capture and recapture and the time intervals between measurements. This equation has been used previously to provide growth curves for both *Chelonia* and *Caretta* in Florida waters (Frazer & Ehrhart, 1985). The von Bertalanffy equation used by Fabens (1965) was:

$$(4) L_t = a (1 - be^{-kt})$$

Where L_t is length at age, a is asymptotic length (L_∞), b is parameter related to length at birth, e is base of natural logarithms, k is constant growth rate and t is time.

The carapace measurements and the time interval between measurements were fitted into FiSAT II program (Gayanilo *et al.*, 2005) where 'a' is the equivalent to L_∞ which equals to 110 cm (maximum size of adult green turtles found in Malaysia) following SEATRU (2010).

4.2.2.3 Behavioural and Physical Observation

The hatchlings were observed daily for any changes in physical or behaviour. Behaviour observation was done during feeding and after feeding (when they were released into a bigger tank) once a day. Common behaviour of turtles were recorded based on Wyneken (1997). Any signs of lethargy or changes in behaviour would be noted. If the condition of the hatchling became worse, the hatchling was isolated and kept in a quarantine tank to avoid harming as a weak turtle is usually prey to other healthy turtles. The hatchlings were regularly checked for any injury or signs of infection.

4.3 Results

This section is divided into (i) Standard Operating Procedure of green turtle headstart project, (ii) General observation of green turtles behaviour and (iii) Survival and growth of green turtles in captivity.

4.3.1 Standard Operating Procedure (SOP)

This SOP has been established to aid future headstart projects. It comprises of human-turtle interaction for the public, good husbandry practices, behavioural and disease management tactics.

4.3.1.1 Human-Turtle Interaction for the Public

(a) At hatchery (egg hatching facility) (for handling of hatchling /juvenile)

1. Wash hands with soap and clean water before touching juvenile turtles or hatchlings (not recommended).
2. Listen to instructions and explanations of the researcher or head facilitator before attempting interaction with turtle.
3. Be careful when holding turtle to avoid injuring it (do not let it fall down).
4. When holding a turtle, please ensure that the turtle's flippers have been folded to the back of its body before lifting up the turtle (Figure 4.3a).
5. Please be sure to place one hand on the turtle's carapace and another hand on the turtle's plastron to stabilize the handling of the turtle (Figure 4.3b).
6. Hold turtle at medium strength (do not squish or squeeze the turtle).

7. The turtle handler and facilitator should pay extra attention to the turtles being handled. If the turtle starts to show stress signals (salivating or struggling), please slowly lower the turtle back into the pool and let it go.

***Note:** Children should not try to hold or lift up turtles without close monitoring from an adult.

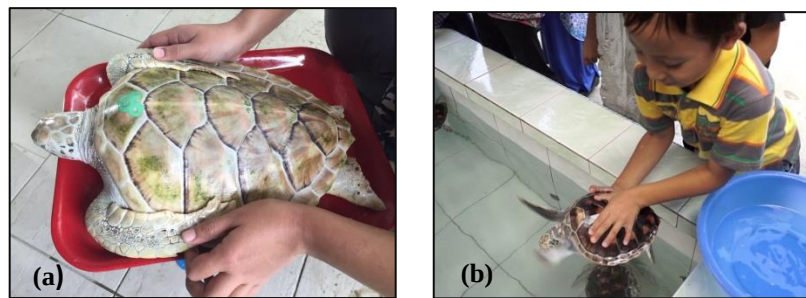


Figure 4.3: (a) Turtle with folded flippers, (b) The correct way to handle a turtle

(b) At turtle nesting area (for handling of nesting female turtle)

1. Do not flash your light towards the sea or towards turtles that are trying to come ashore to nest. It is highly recommended to use light that emits yellow coloured light (not LED or white light) while beach combing at night.
2. If you happen to see a turtle that is trying to come ashore to nest at the beach, immediately turn off all light sources or reduce the use of light. In addition, please limit your movement there.
3. Inform the officer in-charge of the turtle landing.
4. Do not go near the turtle until it has finished digging and laying eggs.
5. When interacting with the turtle:
 - (i) Do not step on the shell or any other part of the turtle's body as the turtle could feel any light touch on its carapace.

- (ii) Do not flash light to the turtle's eyes.
 - (iii) Minimize selfies with the turtles (especially when using camera flash)
6. Please cooperate and do not disturb or hinder the measurement, weighing and tagging process done by the officer in-charge.
 7. Give way to the turtle when it is heading back to sea after nesting.

4.3.1.2 Husbandry Practice

WARNING: All headstart projects must be with proper license or permit from the Wildlife Department or any relevant agencies.

(a) Egg Management

1. Turtle eggs can only be handled by experienced and well-trained Park Rangers. Volunteers should be properly trained prior to involvement with such work.
2. The eggs should not be moved or shaken.
3. Egg handlers should be extra careful when they are relocating or moving the turtle eggs.
4. The eggs should be placed in a hatchery within 2 to 5 hours after the turtle finish laying its eggs.

(b) Hatchling Management

1. The hatchlings that are needed for the headstart project should be taken from a registered hatchery (with permission) to avoid negative criticisms regarding change in gender ratio of the turtles via temperature manipulation.

2. Be sure to check the health condition of the hatchlings that are going to be used in the headstart project (make sure there is no obvious physical deformity or disease).
3. Weigh and measure the hatchlings. Choose hatchlings that are uniformly sized.
4. Steps to transfer hatchlings:
 - (i) Choose the right container (no sharp edges or harmful substance).
 - (ii) Pour some sea water into the container (about 1 cm in depth).
 - (iii) Place the hatchlings into the container.
 - (iv) Carefully transport the hatchling filled container back to the headstart facility.
5. At the hatchery, the hatchlings should be allowed to imprint and impound on the beach of the headstart facility.
6. After 30 minutes of imprint and impound, the hatchlings should be caught and brought back to its holding tank.

(c) Imprint and Impound Activity

1. For this activity, a custom-made trap is needed. During this project, a rectangular netting of 3.50 m long, 1.50 m wide and 0.75 m tall, with net mesh size of 1 mm had been constructed using PVC pipes.
2. The imprint and impound activities should be conducted at approximately 7.30 pm (right after sunset) to imitate the natural condition.
3. The net should be placed 25 % in water and 75 % on sand (Figure 4.4) to allow the hatchlings to imprint upon the sands
4. The hatchlings should be allowed crawl on the sands and swim for about 5 minutes in the sea, before being brought back to the rearing facility.

5. Imprint and impound activities should be carried out every week for the first 3 months of the hatchlings life to teach them about the structure of the local beach, where they are supposed to return to nest.

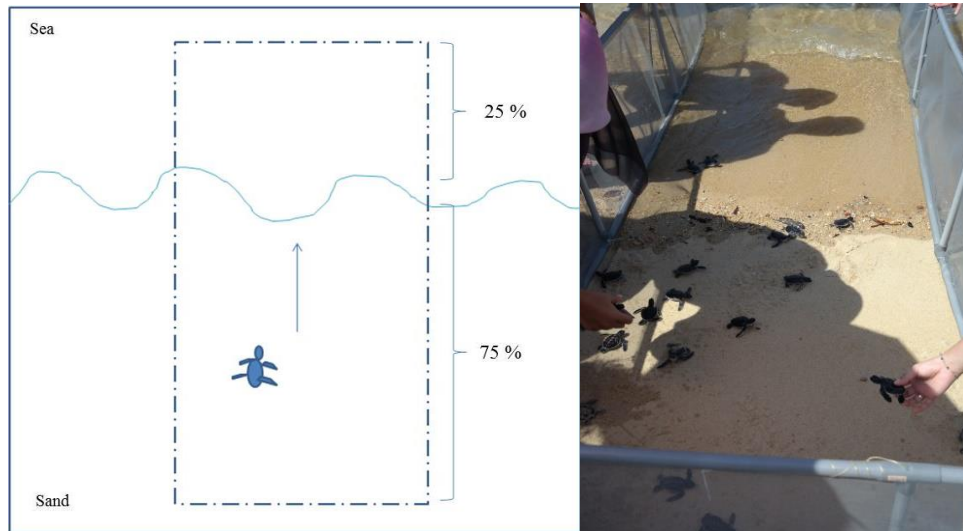


Figure 4.4: Picture and illustration of the imprint and impound process

(d) Water Management and Tank Preparation

1. A week before the hatchlings arrive at the facility, the turtle holding tanks need to be properly cleaned (hand scrubbed) before being rinsed with fresh water and left soaked in mixture of fresh water and bleach for 24 hours to disinfect the tank.
2. The tanks were then drained and rinsed with fresh water again before being refilled with sea water and left to soak for another 24 hours.
3. After 24 hours, the tanks were drained and left to dry under the sun.
4. The tanks should be filled with sea water of approximately 5.0 cm deep.
5. Density of turtle in each tank should not be more than 60 in a 2.25 m long and 0.90 m wide tank (allow surface area of at least 0.03 m^2 per turtle). To avoid over-crowding in only 1 side of the tank, it is recommended to partition the tank and to divide the number of hatchlings equally in each partition.

6. Avoid placing furnishings like corals and pipes or other items or surfaces that could lead to the hatchling's injury in the holding tank.

*Note: The hatchlings will try to eat the furnishings so it would be better to stock the tanks with only water.

7. The optimum water temperature is from 25 to 30 °C with salinity of 29 to 31 ppt.
8. The water that is used in the holding tank has to be changed daily with sea water.
9. The water change frequency is dependent on the size of the turtles. The water may have to be changed more frequently if the size of the turtles increases.
10. Turtle rearing or holding tank:
 - (i) Should be sheltered from direct sunlight to avoid unfavourable algae growth. However it has to be illuminated during the day.
 - (ii) Equipped with filtration system to filter the turtle waste or the food excess in the tank.
11. An extra water keeping tank is needed to ensure the continuous water supply for the turtles.
12. Water level in the holding tank:
 - (i) 5 cm or lower for the first week (so that the hatchlings will not be too tired to surface for air)
 - (ii) The water level has to be increased every 2 weeks to promote the natural behaviour of the hatchlings such as diving and swimming habits.

(e) Feeding process

1. The hatchlings should not be fed for approximately 3 to 4 days after hatching as there is still food (yolk sac) in its stomach.

2. Observe the hatchlings daily. When it starts to bite other hatchling, then it is sign of hunger indicating exhaustion of yolk sac.
3. For the 'biting' hatchlings, feed them with food of about 1 % of its body weight for the first week.
4. During feeding time, the hatchlings need to be taken out from its holding tank and fed separately or in pairs to ensure that all hatchlings received equal amount of food.
5. The hatchlings early food has to be cut into small size (as big as a peanut) to suit their mouth size.
6. After a few months, a juvenile turtle can be fed with a bigger sized food such as a normal sized sardine fish that has been cut in half.

(f) Daily Care

1. After eating, the turtles should be allowed to spend some time to swim in a deeper tank (not in the holding tank).
2. During their swim time, turtles should be cleaned and observed for any signs of lethargy or disease.
3. A sick turtle should be kept in a separate container, away from the rest of the hatchlings to avoid disease transmission between the turtles.
4. Summary of a headstart operational program is in Figure 4.5.

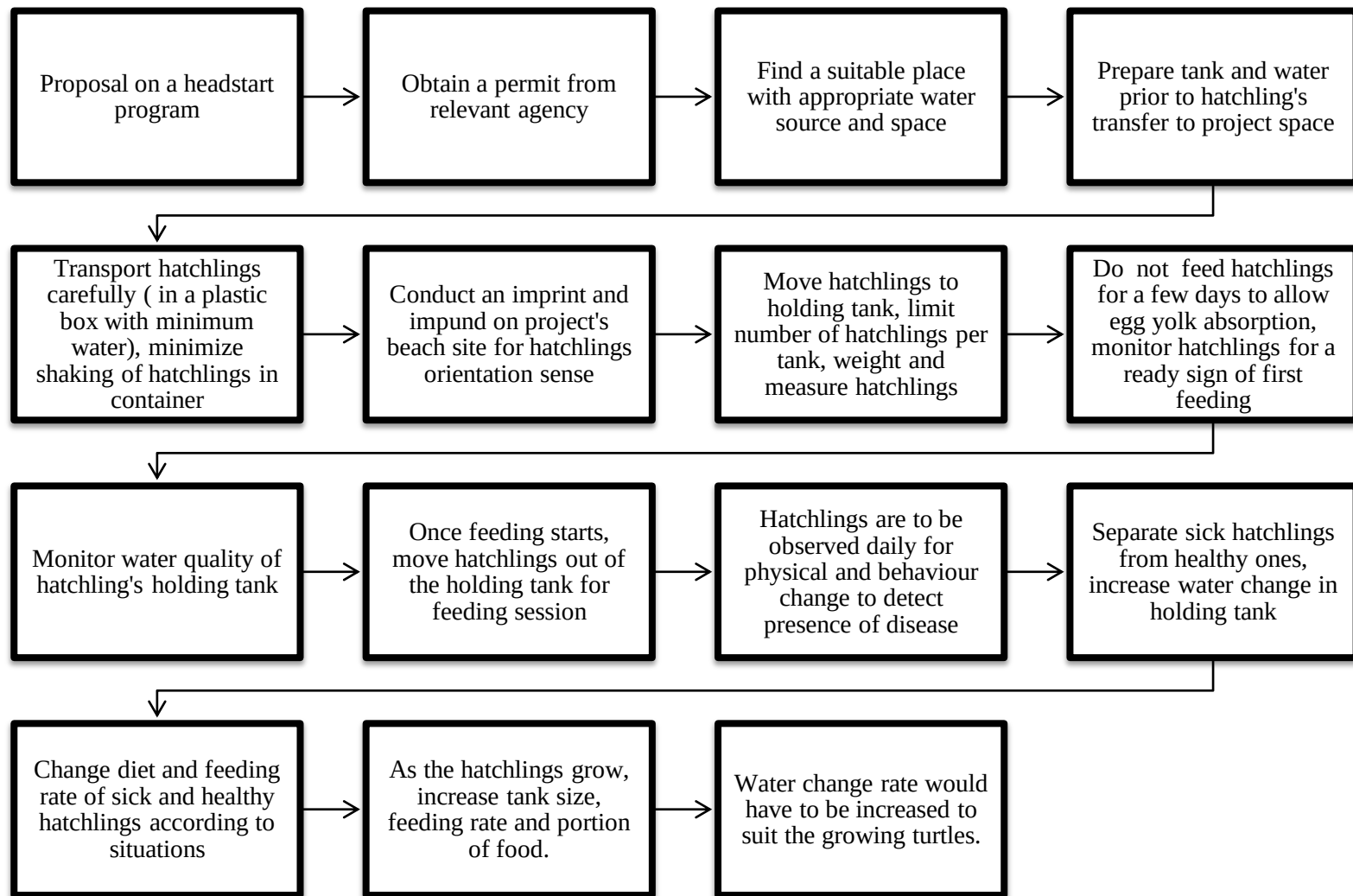


Figure 4.5: Summary of headstart program operation

4.3.1.3 Disease and behavioural management during headstart process

There are two types of behavioural management tactic and two types of disease management tactic described in this section.

4.3.1.3.1 Behavioural Management Tactic 1: Floating Cucumber Treatment

To avoid a widespread disease among hatchlings, the commonly observed biting behaviour of the hatchlings will have to be first controlled. One of the methods to curb the biting habits of the hatchlings is to provide each turtle with adequate space or by placing each turtle in its own captive space. However, to do so, the facility will have to be big to accommodate the growing turtles. During this headstart program, budget and space are both limited entities, therefore, approach to solve the problem went to its root, namely the individual turtle *i.e.* improve each turtle behaviour. To start this behaviour management process, a few important points were noted:

1. The turtles have to be acclimatized to the treatment from the first day they start eating.
2. The amount of cucumbers given must be closely monitored. For example, during their first week of feeding, only $\frac{1}{2}$ a slice of cucumber should be given to each turtle but the amount should be increased gradually as time goes by.
3. The floating cucumber treatment serves as a distraction tactic for the turtles.
4. The turtles still have to be observed daily for signs of aggression or fatigue as some turtles will not respond well to the cucumber treatment at first.
5. Daily exercise still has to be carried out for the turtles to practice their natural behaviour.

4.3.1.3.2 Behavioural Management Tactic 2: Seagrass, small fish and shrimp fry treatment

1. To promote the natural behaviour of turtles, it is encouraged for headstart program to carry out this treatment on all turtles at least once a month.
2. During their regular exercise routine, release small fish or shrimp fries into the tank where the turtles are to encourage their natural scavenging behaviour.
3. To help familiarize them to their benthic life, it is recommended to add patches of seagrass, placing them at the bottom of the tank.
4. The addition of coral like structures should be considered to help the turtles familiarize themselves with corals. However, for turtles under the age of 1 year old, such structures is not necessary as the young turtles have been observed to drown when they are caught in confined space as well as injuries from abrasion with such structure is likely to happen with hatchlings or yearlings.

4.3.1.3.3 Disease Management Tactic 1

1. It is highly recommended to identify the nearest veterinary clinic (familiar with wildlife especially Reptiles) to the headstart program facility to help whenever the turtles are sick.
2. The hatchlings have to be observed daily for any behaviour or physical changes.
3. Any signs of lethargy or abnormal behaviour would have to be noted.
4. If the condition of the hatchling became worse, the hatchling has to be kept in a quarantine tank to avoid commotions, as weak turtle is usually prey to other healthy turtles.

5. The hatchlings must be frequently checked for any signs of injury or infection.
6. For sickly turtles, it would be better to isolate them from the other turtles to stop the spread of the disease.
7. It is important to pay special attention to the water quality (WQ) parameters therefore the monitoring must be done on daily basis.
8. All external injury caused by biting should be treated with yellow lotion (Brand: Betadine) while all eye infection were treated using specialized eye drops (Brand: Colircusi Gentadexa), whenever required. Consult the vet to avoid mistreating the turtles.
9. For sick turtles, injectable antibiotics in early stage of infection could greatly help to increase the chances of recovery.

4.3.1.3.4 Disease Management Tactic 2

1. In the case of disease spreading among the turtles, the water in the holding tank has to be changed at least twice daily.
2. If the disease continues to spread, a small amount of bacteria controlling solution should be mixed into the holding tank of the turtles for a few days to help alleviate the problem.
3. The solution TMS™ which contains alkyl dimethyl benzyl ammonium chloride (usually used for prawn culture) could be used to control the situation.
4. The solution has to be used at a small dosage as to not harm the turtle's normal bacterial flora (1g of TMS per litre of water will result in 100 % bacteria die off).
5. It is very important NOT to use the solution for a long time and at a high dosage.

6. As 1 g of TMS per litre of water is equivalent to 400 ppm, the dosage that had been used before was 20 to 120 ppm per litre of water.
7. Water in the holding tank need to be changed daily and it is recommended to only use the solution for the quarantine tank.

4.3.1.4 Feeding Regime of Turtles in Relation to Growth

The amount of food given to turtles showed increasing trend (Figure 4.6). The increase of food taken by individual per meal for batch 2 was more stable, coinciding with good growth as in Figure 4.10 and 4.12. In contrast, the food intake of Batch 1 fluctuated especially during the early phase when there was a disease outbreak due to unknown reason and hatchlings were not eating well. This was the reason behind a significant difference in growth between the 2 batches. Moreover, Batch 1 took a longer time to reach the exponential phase in food intake compared to Batch 2 (approximately 10 weeks later than Batch 2). For Batch 1, food intake showed fluctuating trends: increased from week 1 until week 16, before it dropped in week 18 and went through a plateau phase until week 22 before showing a declining trend until week 28 and gradually increase from there until week 66, in which rapid increase in food intake at week 68 could be observed.

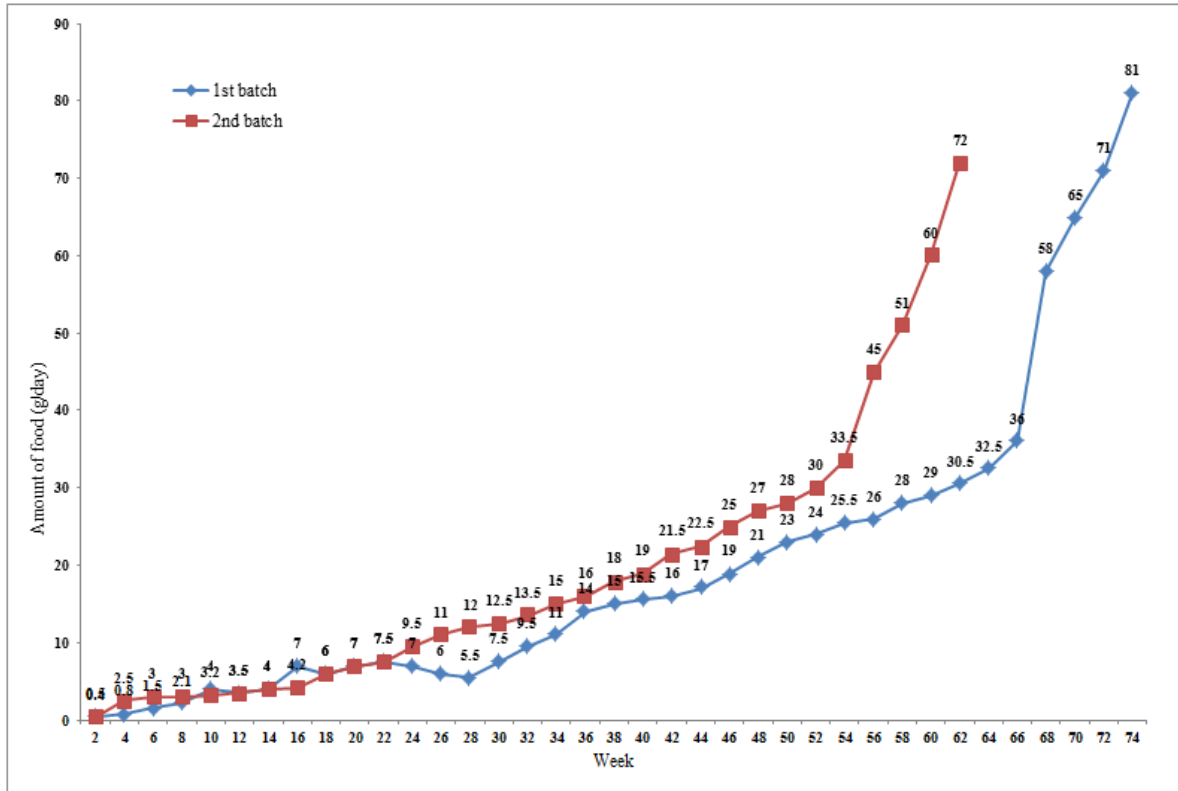


Figure 4.6: Amount of food intake for Batch 1 and 2 (gram/individual per meal)

4.3.2 General Observation of Sea Turtle Hatchling's Behaviour

The following subsections describes two common behaviours of the turtles namely (i) locomotion and (ii) sleeping habits. In addition, the imprint and impound procedure is included in this section as behavioural adjustment procedure.

4.3.2.1 Locomotion

The followings are locomotion modes that were common to both hatchlings and juveniles:

- (a) Dogpaddling is a drag-based locomotion which involves the use of all four limbs, acting as oars or paddles. Drag produces thrust during the dogpaddling technique, rear flipper kicking and in the limb retraction phase of the powerstroke technique.

For this type of movement, the flipper blade was swept ventrally and posteriorly and was then protracted with the anterior edge being forward oriented to minimize surface area.

- (b) Rear flipper kicking was observed to be used mostly when the turtles were in their resting mode. The rear flipper kicking consisted of paddling or rowing using the rear flippers alone in which the fore flippers were flexed and folded back over the carapace of the turtle.
- (c) Power stroking was mostly observed when the turtles were brought out to a bigger pool once a day (when they were less than one year old) for their daily exercise routine. Power stroke is a highly efficient gait that produces propulsion during all phases of the stroke (upward-outward, then downward-inward) in which the hind limbs does not contribute to thrusts.

4.3.2.2 Sleeping Habits

The turtles tend to fold and flex their flippers close to their body as they rest (Figure 4.7a). ‘Crowding’ was common behaviour shown by hatchlings and yearlings during sleeping and resting. Hatchlings were observed floating while sleeping but gradually changed the habit to ‘underwater sleeping’ (Figure 4.7b). Other than that, basking activities were also observable as the turtles crowded at a place where there was sand and sun. Furthermore, they often slept at the same convenient spot during the night (Figure 4.7c).



Figure 4.7: (a) turtle in resting or sleeping position, (b) turtles crowding together while sleeping while some turtles had a tendency to stay underwater longer, (c) Turtles basking at a sunny spot in the tank

4.3.2.3 Imprint and Impound Procedure

As a part of behavioural adjustment based on experience of the turtles, the imprint and impound procedure had been carried out for Batch 1 for 2 months. The results could not be verified yet, because this headstart project lasted for only 18 months.

4.3.3 Survival and Growth of Green Turtles in Captivity

This section comprises of survival rate, growth curves comparison between Batch 1 and 2 based on weight and SCL, growth curves of both batch and the von Bertalanffy growth function.

4.3.3.1 Survival Rate

The survival of green sea turtle hatchlings (Batch 1) dropped rapidly from 92.5 % to 69.2 % from week 6 to 8. Then, another decrease in survival could be observed (60.8 % to 42.5 %) from week 10 to 12 (Figure 4.8). There was a small reduction from week 12 to 14 (5.0 % difference), week 14 to 16 (7.5 % difference), week 16 to 18 (5.8 % difference) and lastly from week 22 to 24 (1.7 % difference). The survival stabilized at week 24 at 22.5 %. The original number of hatchlings in Batch 1 was 120 (n=120). Overall, the survival of Batch 1 was 22.5 %.

For the second batch (n=50), the survival (Figure 4.8) did not drop rapidly but only showed small reductions in a stable manner from week 2 to 4 (2.0 % difference), week 4 to 6 (4.0 % difference), week 6 to 8 (4.0 % difference), week 8 to 10 (2.0 % difference), week 10 to 12 (2.0 % difference), week 12 to 14 (2.0 % difference) and lastly from week 50 to 52 (2.0 % difference). For Batch 2 (n=50), up to 15 months the survival is at 82 %.

Paired t-test (Appendix 1) revealed that the mean survival of batch 1 was 1.5457 (SD=0.21442) while the mean for batch 2 was 1.6293 (SD=0.02123). The p value obtained for this test was $p=0.023$ which is smaller than 0.05 at confidence interval limit of 95 % ($t(31)=0.023, p<0.05$). Therefore, it can be concluded that there is a significant difference between the survival rates of the 2 groups. Batch 2 has a higher survival rate when compared to Batch 1, further discussion on this will be in Section 4.4.4.3.

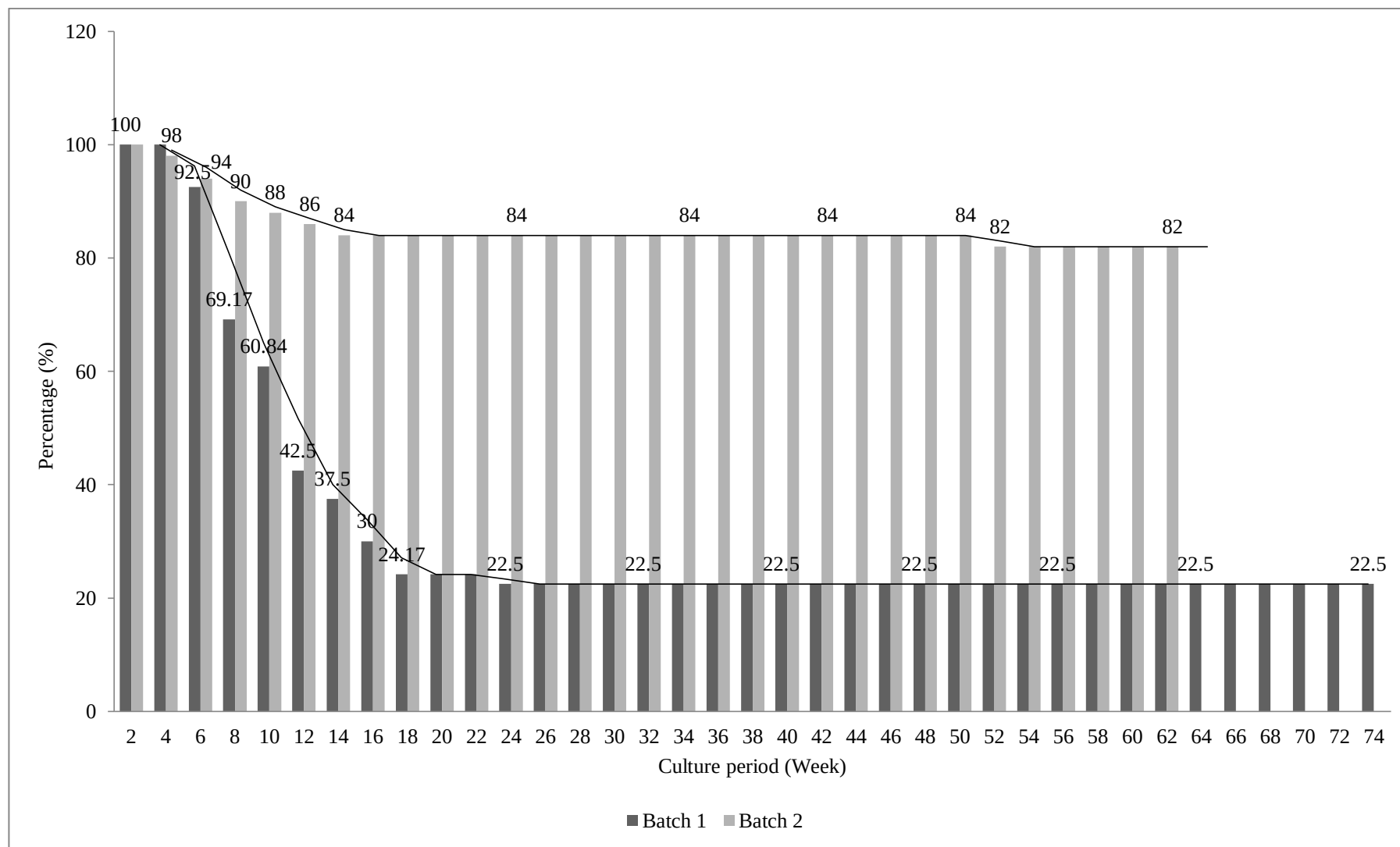


Figure 4.8: Survival of hatchlings in Batch 1 (n=120) and Batch 2 (n=50), in percentage (%)

4.3.3.2 Growth Curve Comparison between Batch 1 and 2 Based on Weight

Figure 4.9 showed that there was a prolonged lag phase of up to week 40 as the first batch experienced a sudden epizootic situation in the facility. After that, Batch 1 experienced a steady increase in weight and finally reached exponential phase at week 44. There was no sign of growth slowing down at week 72 or 74, as the turtles are still in their juvenile phase of life.

Batch 2 shows a gradual increase in weight over time. The lag phase of Batch 2 was observed until week 36 before the exponential phase started at week 44 (Figure 4.10). There was no sign of the turtle weight slowing down after week 60 and 62 as the turtles were only approximately 15 months old. This suggests the growth curve is still at exponential growth.

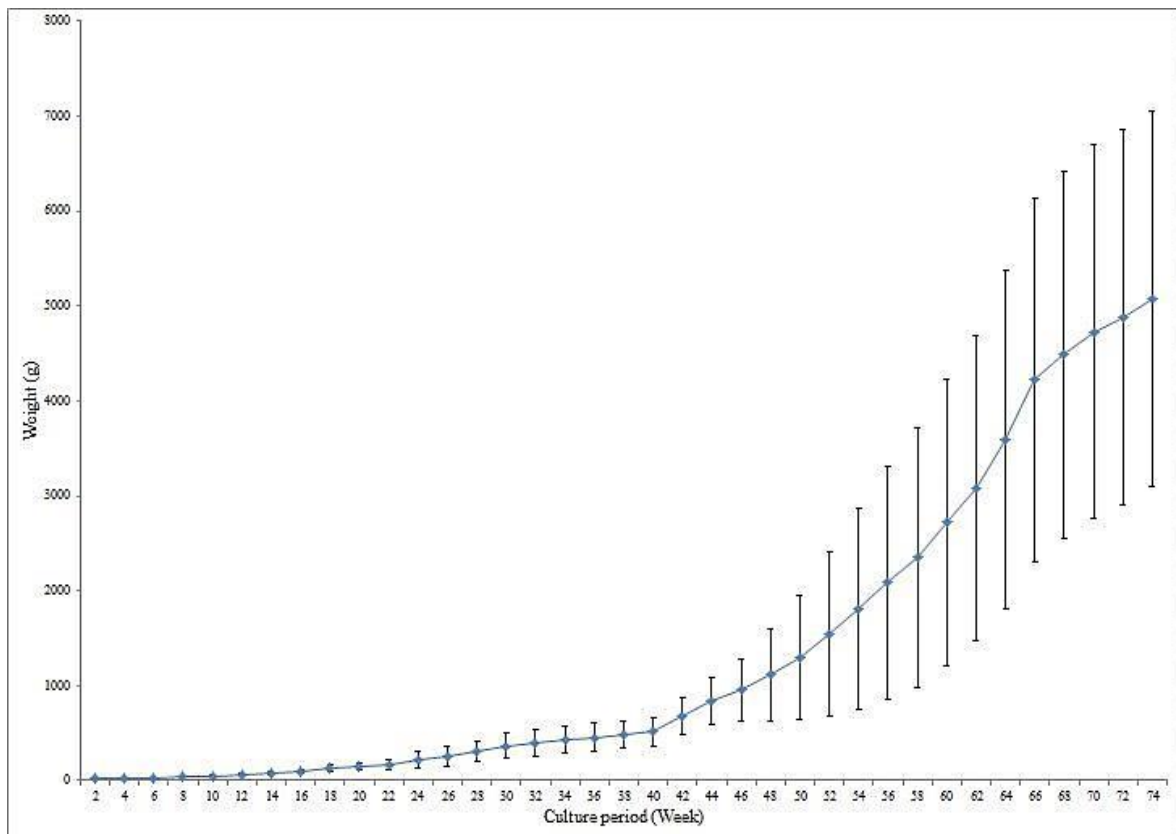


Figure 4.9: Growth curve of Batch 1 based on weight (g)

The paired t-test results (Appendix 1) showed that there is significant difference in mean weight between batch 1 (mean=2.5109, SD=0.63218) and 2 (mean=2.6512, SD=0.63545) as $t(31)=0.000$, $p<0.05$. Batch 2 is heavier or much sturdier than batch 1 as the mean weight and SCL of batch 2 was higher and showed a steadier increase over time (Figure 4.10). The possible reason for Batch 2 having a better weight when compared to Batch 1 is as discussed in Section 4.4.2.

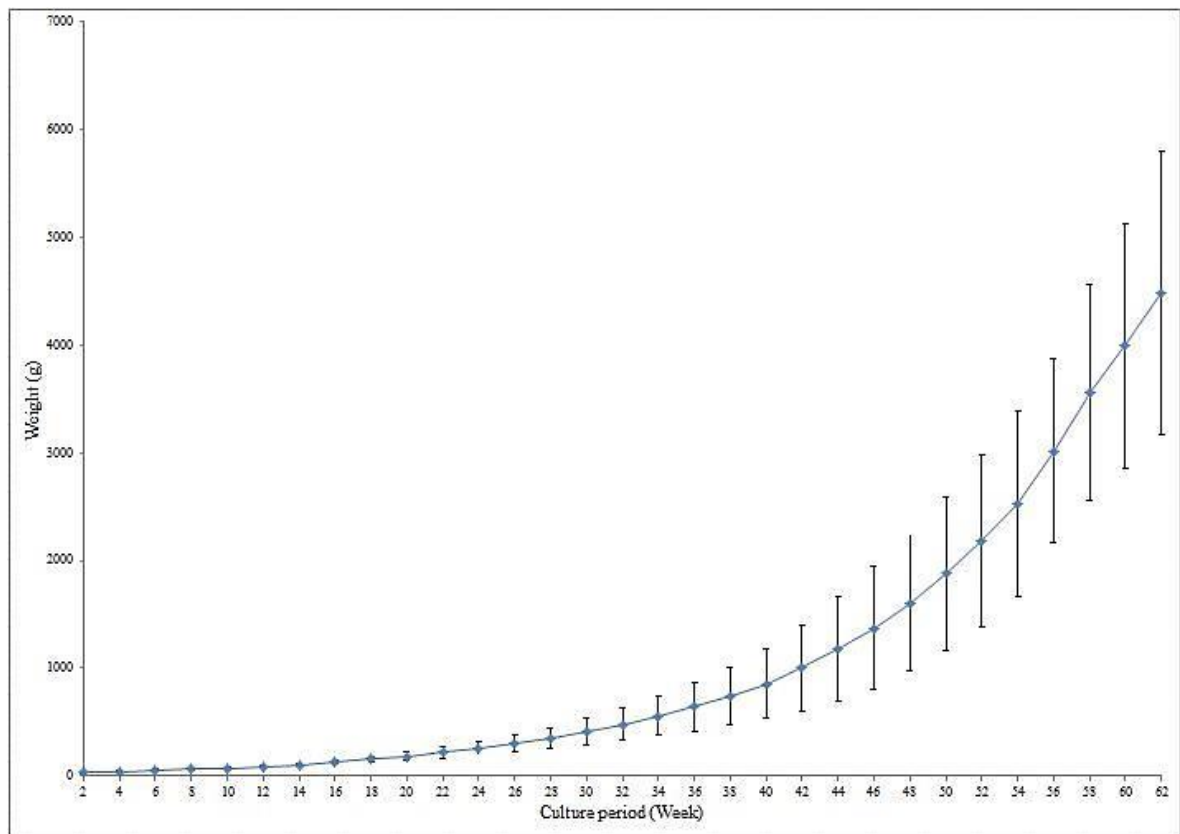


Figure 4.10: Growth curve of Batch 2 based on weight (g)

4.3.3.3 Growth Curve Comparison between Batch 1 and 2 Based on SCL

Figure 4.11 shows an upward fluctuating trend with a sudden sharp increase in week 26 before gradually increased until week 50 before reaching exponential phase at week 52. The graph is still rising exponentially after week 72, suggesting the turtles are

still growing fast during juvenile phase. The SCL graph of Batch 2 (Figure 4.12) shows a rapid upward trend with no observable lag phase. The SCL of the second batch seems to be linear with a small transition phase at week 28. The growth line shows no sign of stopping as it continuously grew after week 60. t-test result showed that the mean SCL for Batch 2 (mean=2.1593, SD=0.22865) was higher than batch 1 (mean=2.0795, SD=0.19199). The sample size used in the T-test was n=31 while the value of p was 0.000. The value of p is smaller than 0.05 at confidence interval limit of 95 % ($t(31)=0.000, p<0.05$). SCL of Batch 2 was bigger or longer compared to Batch 1. Possible reasons leading to a bigger sized Batch 2 is in Section 4.4.4.3.

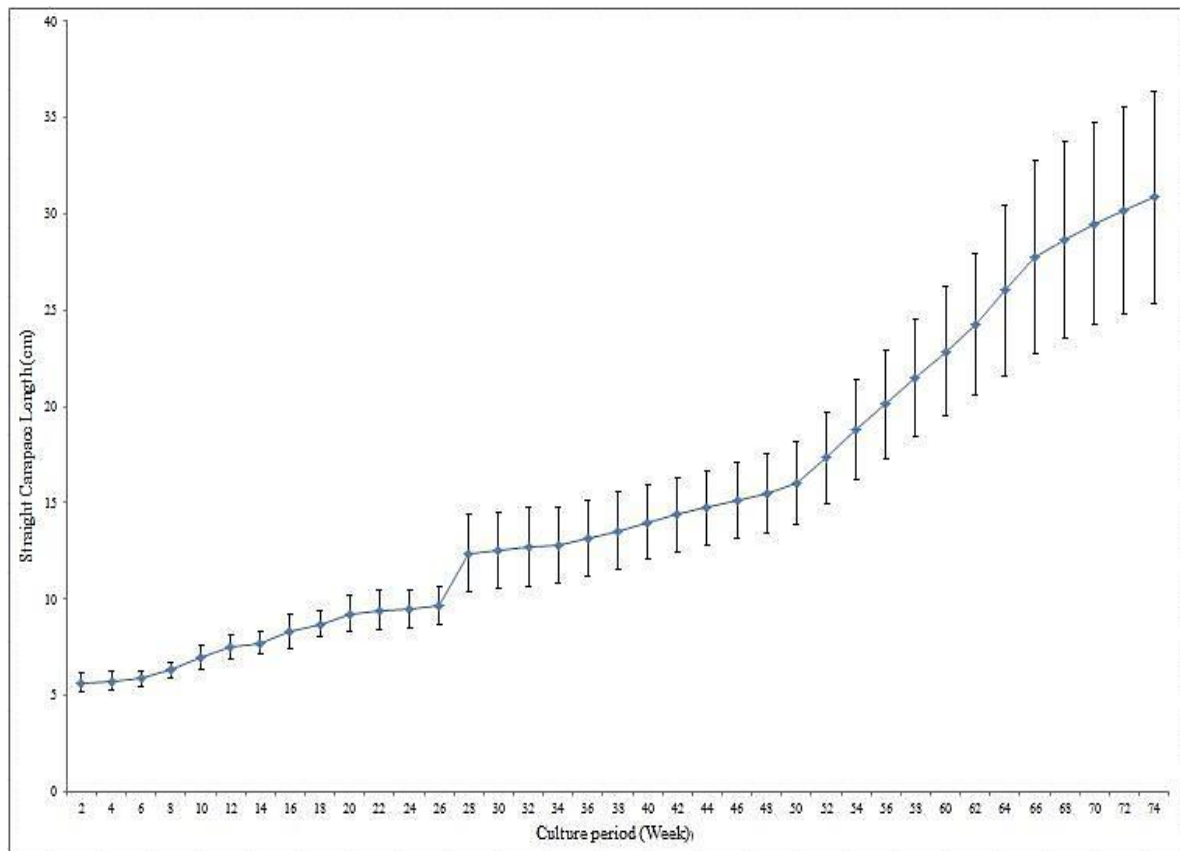


Figure 4.11: Straight carapace length (cm) of Batch 1 (n=120)

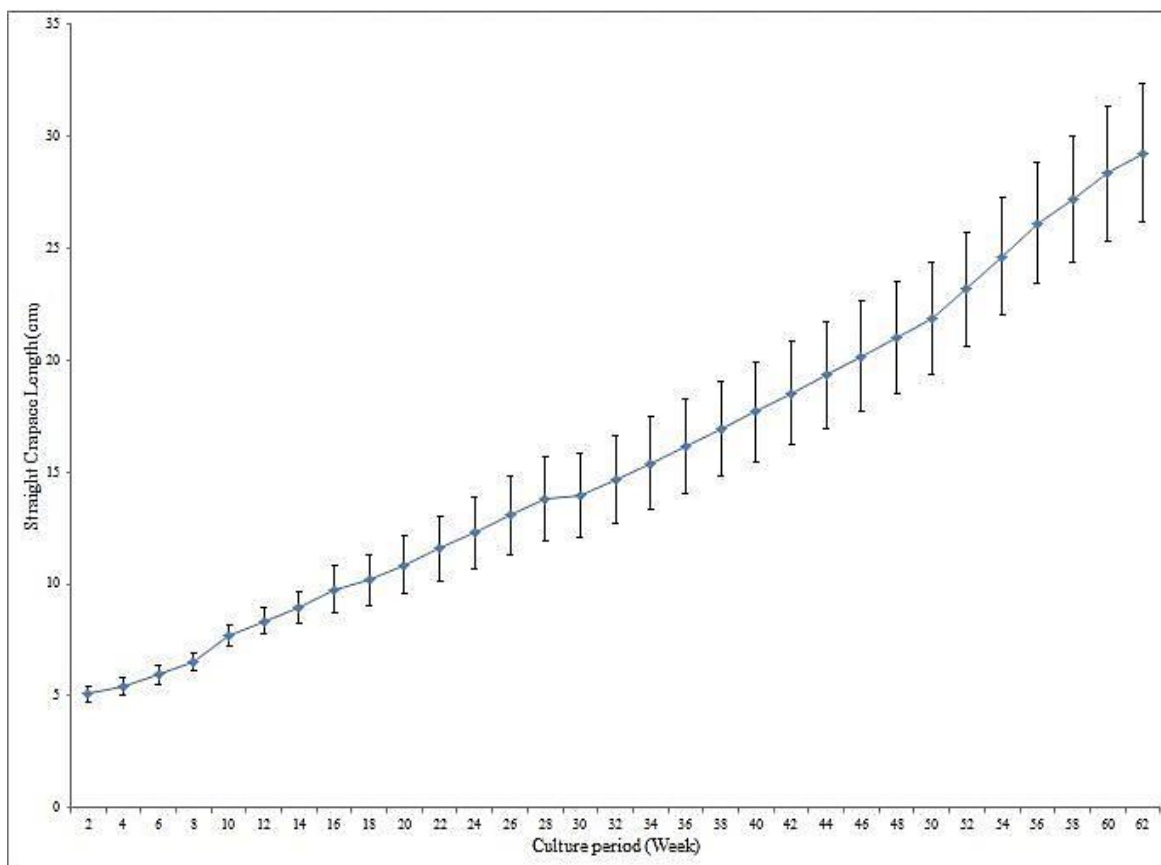


Figure 4.12: Straight carapace length (cm) of Batch 2 (n=50)

4.3.3.4 Growth Rate of Batch 1

The growth rates of individuals from both Batch 1 and 2 are expressed in terms of cm/day and g/day (Table 4.1 and Table 4.2). The data was divided into 3 phase as follows: (i) phase 1: week 2 to 24, (ii) phase 2: week 26 to 48 and, (iii) phase 3: week 50 to 74. For Batch 1, the weight showed a tremendous increment, namely 1.27 ± 1.05 g/day for phase1, 5.32 ± 4.13 g/day for phase 2 and 21.77 ± 9.68 g/day for phase 3.

Similarly, the SCL data showed increasing trend: phase 1 was 0.025 ± 0.016 cm/day, increased to 0.036 ± 0.051 cm/day during phase 2 and lastly increased to 0.084 ± 0.029 cm/day during phase 3 (Table 4.1).

Table 4.1: Growth of Batch 1 in weight (g/day) and SCL (cm/day)

Week	Sample size	Mean Weight $\pm$ SD	Growth (g/day)	Growth every 6 months (g/day)	Mean SCL $\pm$ SD	Growth (cm/day)	Growth every 6 months (cm/day)
2	120	23.71 $\pm$ 3.27	-		5.65 $\pm$ 0.51	-	
4	120	27.12 $\pm$ 3.57	0.24		5.76 $\pm$ 0.51	0.0078	
6	111	29.96 $\pm$ 3.95	0.20		5.87 $\pm$ 0.43	0.0078	
8	83	43.81 $\pm$ 7.39	0.99		6.32 $\pm$ 0.43	0.0321	
10	73	48.40 $\pm$ 7.22	0.33	Phase 1:	6.95 $\pm$ 0.62	0.045	Phase 1:
12	51	67.27 $\pm$ 14.48	1.35	1.27 $\pm$ 1.05	7.51 $\pm$ 0.59	0.04	0.025 $\pm$ 0.016
14	45	78.02 $\pm$ 20.79	0.77		7.71 $\pm$ 0.60	0.0143	
16	36	90.69 $\pm$ 20.52	0.91		8.30 $\pm$ 0.91	0.0421	
18	29	129.48 $\pm$ 30.06	2.77		8.70 $\pm$ 0.69	0.0286	
20	29	144.55 $\pm$ 33.14	1.08		9.24 $\pm$ 0.97	0.0386	
22	29	169.79 $\pm$ 47.35	1.80		9.43 $\pm$ 0.99	0.0136	
24	27	218.78 $\pm$ 90.71	3.50		9.49 $\pm$ 0.99	0.0043	
26	27	261.74 $\pm$ 107.80	3.07		9.65 $\pm$ 1.02	0.0114	
28	27	308.11 $\pm$ 114.43	3.31		12.38 $\pm$ 2.01	0.195	
30	27	365.85 $\pm$ 135.22	4.12		12.51 $\pm$ 1.96	0.0093	
32	27	398.67 $\pm$ 141.21	2.34		12.70 $\pm$ 2.06	0.0136	
34	27	427.41 $\pm$ 144.45	2.05	Phase 2:	12.82 $\pm$ 1.97	0.0086	Phase 2:
36	27	454.89 $\pm$ 145.34	1.96	5.32 $\pm$ 4.13	13.17 $\pm$ 1.99	0.025	0.036 $\pm$ 0.051
38	27	484.33 $\pm$ 148.62	2.10		13.56 $\pm$ 1.99	0.0279	
40	27	513.26 $\pm$ 147.77	2.07		13.98 $\pm$ 1.92	0.03	
42	27	680.22 $\pm$ 196.89	11.93		14.37 $\pm$ 1.93	0.0279	
44	27	839.19 $\pm$ 245.34	11.36		14.73 $\pm$ 1.95	0.0257	
46	27	953.15 $\pm$ 333.27	8.14		15.11 $\pm$ 1.99	0.0271	
48	27	1112.70 $\pm$ 489.35	11.40		15.51 $\pm$ 2.05	0.0286	
50	27	1294.11 $\pm$ 652.55	12.96		16.00 $\pm$ 2.16	0.035	
52	27	1546.41 $\pm$ 862.93	18.02		17.34 $\pm$ 2.37	0.0957	
54	27	1805.56 $\pm$ 1054.68	18.51		18.78 $\pm$ 2.62	0.1026	
56	27	2082.93 $\pm$ 1230.27	19.81		20.12 $\pm$ 2.83	0.0957	
58	27	2347.59 $\pm$ 1369.19	18.90	Phase 3:	21.46 $\pm$ 3.06	0.0957	Phase 3:
60	27	2718.33 $\pm$ 1501.50	26.48	21.77 $\pm$ 9.68	22.85 $\pm$ 3.36	0.0993	0.084 $\pm$ 0.029
62	27	3080.41 $\pm$ 1612.26	25.86		24.25 $\pm$ 3.67	0.1	
64	27	3594.63 $\pm$ 1781.40	36.73		26.03 $\pm$ 4.45	0.127	
66	27	4224.93 $\pm$ 1914.74	45.02		27.76 $\pm$ 5.04	0.1236	
68	27	4489.74 $\pm$ 1932.74	18.92		28.62 $\pm$ 5.10	0.0614	
70	27	4722.22 $\pm$ 1968.47	16.61		29.47 $\pm$ 5.24	0.0607	
72	27	4879.59 $\pm$ 1971.68	11.24		30.16 $\pm$ 5.38	0.0493	
74	27	5074.78 $\pm$ 1984.58	13.94		30.86 $\pm$ 5.53	0.05	

A simple regression analysis for the weight and SCL of Batch 1 is as shown in Figure 4.13. There is a positive relationship between weight and SCL (Figure 4.13).

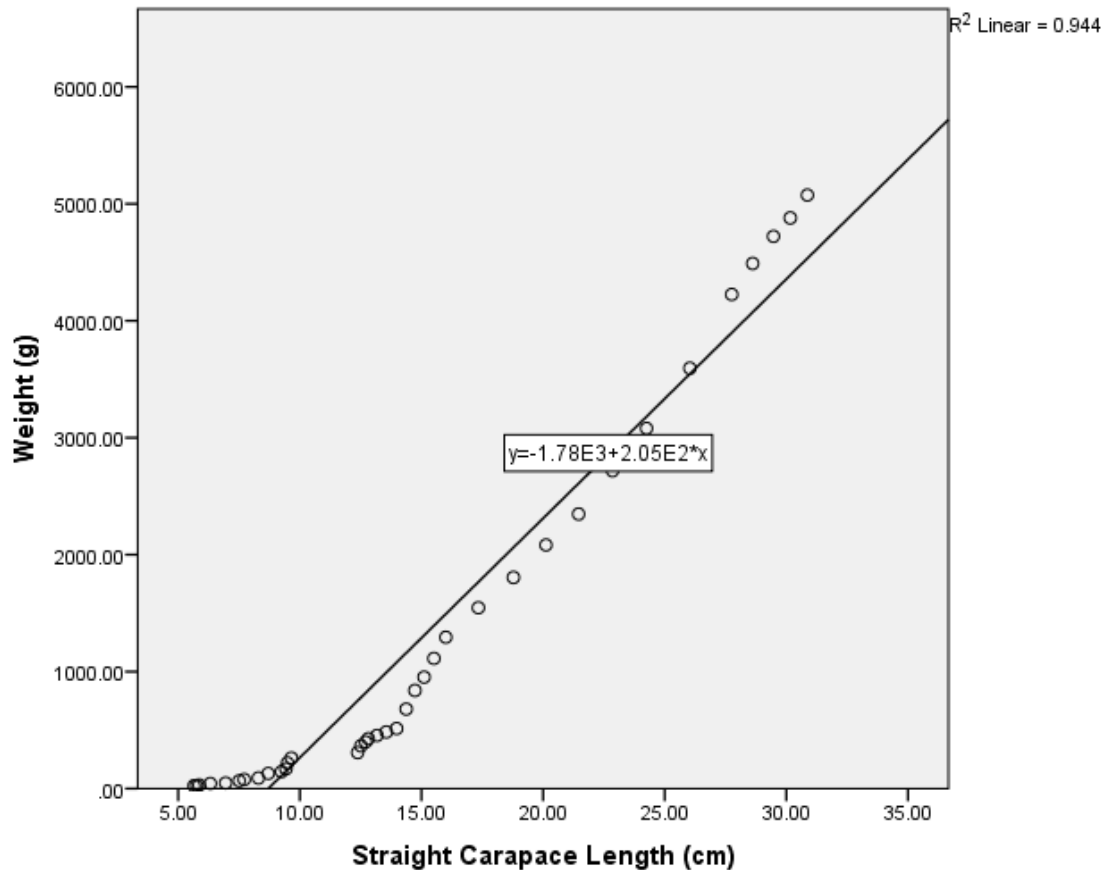


Figure 4.13: Simple regression analysis of weight and SCL of Batch 1

Both the data used for this study (for Batch 1 and Batch 2) was transformed to $\log_{10}$ before it was analysed for simple regression. The analysis done shows that the value of 'b' that was used to assess the condition of the turtles was 3.189. This value falls slightly above the normal range as value of 'b' spanned between 2.495 and 3.134 for each species of sea turtle. The value of 3.189 obtained for Batch 1 shows that the turtles in this Batch were 'stout'. The value of 'a' was 0.1239 while the R square value obtained was 0.984 (N=36). With all the values obtained, the relationship between weight and length of green turtles of Batch 1 is:

$$\text{Weight} = 0.1239 * (\text{SCL})^{3.189}$$

4.3.3.5 Growth Rate of Batch 2

For Batch 2, in Table 4.2, Phase 1 had recorded growth rate of 1.44 ± 0.75 g/day, Phase 2 was 8.03 ± 4.39 g/day whereas Phase 3 was 29.39 ± 7.53 g/day. For Batch 2, Phase 3 only involved 12 weeks (3 months) of data compared to the normal duration of 24 weeks as the second batch was taken 3 months later than the first batch. The weight gained was high among turtles in Batch 2, compared to Batch 1 which may be due to improved husbandry practices as well as increase in skill of rearing the turtles. For the SCL of Batch 2, the first phase was 0.047 ± 0.015 cm/day while the second phase was 0.052 ± 0.013 cm/day and Phase 3 was 0.084 ± 0.019 cm/day.

A linear regression test (Figure 4.14) for Batch 2 resulted in value of 'b' as 2.873 which is within the range that qualifies Batch 2 to be deemed as a fit batch. The value of 'b' being generally close to 3 also implies that the shape of the animal is remaining the same as they get older and gain in size (isometry). The value of 'a' was 0.232 while R square value obtained was 0.986 (N=30). . With all the values obtained, the relationship between weight and length of green turtles of Batch 2 is:

$$\text{Weight} = 0.232 * (\text{SCL})^{2.873}$$

Table 4.2: Growth of Batch 2 in weight (g/day) and SCL (cm/day)

Week	Sample size	Mean Weight $\pm$ SD	Growth (g/day)	Growth every 6 months (g/day)	Mean SCL $\pm$ SD	Growth (cm/day)	Growth every 6 months (cm/day)
2	50	34.34 $\pm$ 11.12	-		5.08 $\pm$ 0.38	-	
4	49	42.12 $\pm$ 11.45	0.56		5.40 $\pm$ 0.39	0.023	
6	47	52.53 $\pm$ 10.40	0.74		5.94 $\pm$ 0.41	0.039	
8	45	62.07 $\pm$ 10.13	0.68		6.54 $\pm$ 0.38	0.043	
10	44	72.80 $\pm$ 11.06	0.77	Phase 1:	7.68 $\pm$ 0.46	0.081	Phase 1:
12	43	86.81 $\pm$ 10.25	1.00	1.44 $\pm$ 0.75	8.34 $\pm$ 0.59	0.047	0.047 $\pm$ 0.015
14	42	105.00 $\pm$ 13.62	1.30		8.96 $\pm$ 0.70	0.044	
16	42	128.26 $\pm$ 18.41	1.66		9.75 $\pm$ 1.05	0.056	
18	42	155.10 $\pm$ 27.15	1.92		10.17 $\pm$ 1.15	0.03	
20	42	184.33 $\pm$ 36.29	2.09		10.86 $\pm$ 1.29	0.049	
22	42	218.33 $\pm$ 50.27	2.43		11.58 $\pm$ 1.42	0.051	
24	42	255.50 $\pm$ 62.08	2.66		12.29 $\pm$ 1.62	0.051	
26	42	301.00 $\pm$ 78.40	3.25		13.06 $\pm$ 1.74	0.055	
28	42	349.31 $\pm$ 95.77	3.45		13.80 $\pm$ 1.90	0.053	
30	42	407.36 $\pm$ 123.03	4.15		13.94 $\pm$ 1.87	0.01	
32	42	477.90 $\pm$ 150.30	5.04		14.67 $\pm$ 1.94	0.052	
34	42	554.40 $\pm$ 181.64	5.46	Phase 2:	15.40 $\pm$ 2.06	0.052	Phase 2:
36	42	643.88 $\pm$ 225.04	6.39	8.03 $\pm$ 4.39	16.16 $\pm$ 2.10	0.054	0.052 $\pm$ 0.013
38	42	740.07 $\pm$ 270.87	6.87		16.93 $\pm$ 2.14	0.055	
40	42	857.83 $\pm$ 323.34	8.41		17.71 $\pm$ 2.23	0.056	
42	42	1002.00 $\pm$ 395.08	10.30		18.53 $\pm$ 2.30	0.059	
44	42	1182.90 $\pm$ 483.75	12.92		19.34 $\pm$ 2.39	0.058	
46	42	1373.12 $\pm$ 565.67	13.59		20.16 $\pm$ 2.47	0.059	
48	42	1605.19 $\pm$ 636.32	16.58		21.00 $\pm$ 2.48	0.06	
50	42	1878.95 $\pm$ 716.81	19.55		21.83 $\pm$ 2.51	0.059	
52	41	2178.27 $\pm$ 794.20	21.38		23.18 $\pm$ 2.55	0.096	
54	41	2526.07 $\pm$ 855.95	24.84	Phase 3:	24.61 $\pm$ 2.62	0.102	Phase 3:
56	41	3016.34 $\pm$ 849.02	35.02	29.39 $\pm$ 7.53	26.13 $\pm$ 2.67	0.109	0.084 $\pm$ 0.019
58	41	3559.76 $\pm$ 1005.37	38.82		27.17 $\pm$ 2.82	0.074	
60	41	3990.24 $\pm$ 1137.88	30.75		28.34 $\pm$ 3.01	0.084	
62	41	4485.37 $\pm$ 1319.81	35.37		29.24 $\pm$ 3.09	0.064	

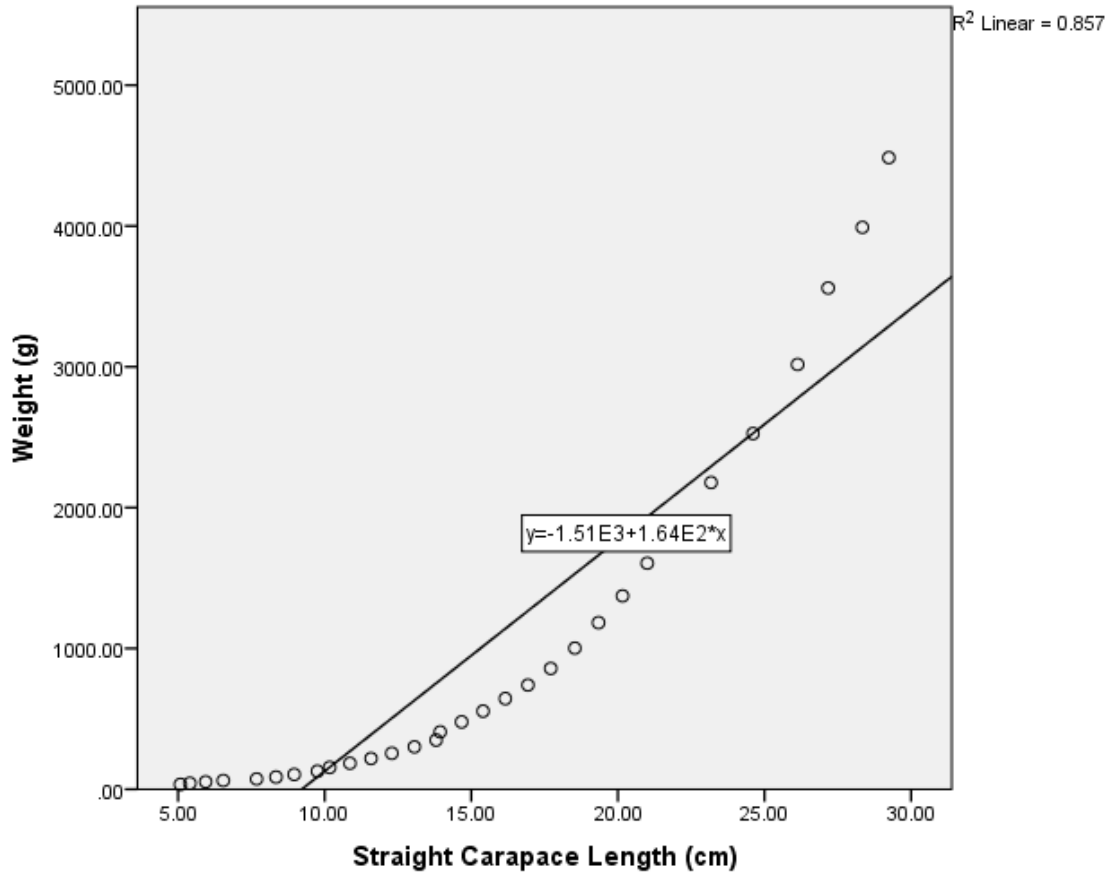


Figure 4.14: Simple regression analysis of weight and SCL of Batch 2

4.3.3.6 Von Bertalanffy Growth Function

According to Shepherd's K Scan in FiSAT II, when L_{∞} was 110 cm, k was 0.190. $L_{\infty}=110$ cm was used as the length at which green turtle in Sarawak grows indefinitely as it is the one recorded.

At hatching ($t=0$), equation becomes

$$(1) L_0 = a(1-b) \text{ in which } b = 1 - (L_0/a)$$

When L_{∞} or $a = 110$, $k = 0.190$ and length at birth (L_0) = 5.08,

The value of $b = 1 - (5.08/110)$ which is 0.9538 or when corrected to 2 decimal point = 0.95.

When the original equation by Fabens (1965) is rearranged according to the obtained values, the equation becomes:

$$(2) L_t = 110(1 - 0.95e^{-0.190t})$$

Other than using FiSAT II, k can be calculated using $L_r = a - (a - L_c) e^{-k t}$

where L_r is length at recapture, L_c is length at first capture, t is time interval.

Using one example of a turtle with a significant time interval and growth (for example: turtle average length between week 2 and 62 with time interval of 1.25 years), k could be calculated using formula: $29.24 = 110 - (110 - 5.40) e^{-1.25k}$

When re-arranged, $k = 0.190$, the equation obtained will be the same as Equation (2).

With this equation, the length of Sarawak's turtles could be estimated by age (in years) when substituted into the equation. Therefore, Table 4.3 provides an estimation of Sarawak's turtle length at age.

Table 4.3: Estimation of age at length for Sarawak's green turtle population

Age (years)	SCL (cm)	Age (years)	SCL (cm)
6.0	76.58	13	101.16
6.5	79.61	14	102.69
7.0	82.36	15	103.96
7.5	84.87	16	105.00
8.0	87.14	17	105.87
8.5	89.21	18	106.58
9.0	91.10	19	107.17
9.5	92.81	20	107.66
10.0	94.37	21	108.07
10.5	95.79	22	108.40
11.0	97.07	23	108.68
11.5	98.25	24	108.91
12.0	99.31	25	109.09
12.5	100.28	26	109.25

From this equation by altering time (t) in years, the length at age can be calculated and plotted to graphically present a length at age graph (Figure 4.15) for green turtle in Sarawak.

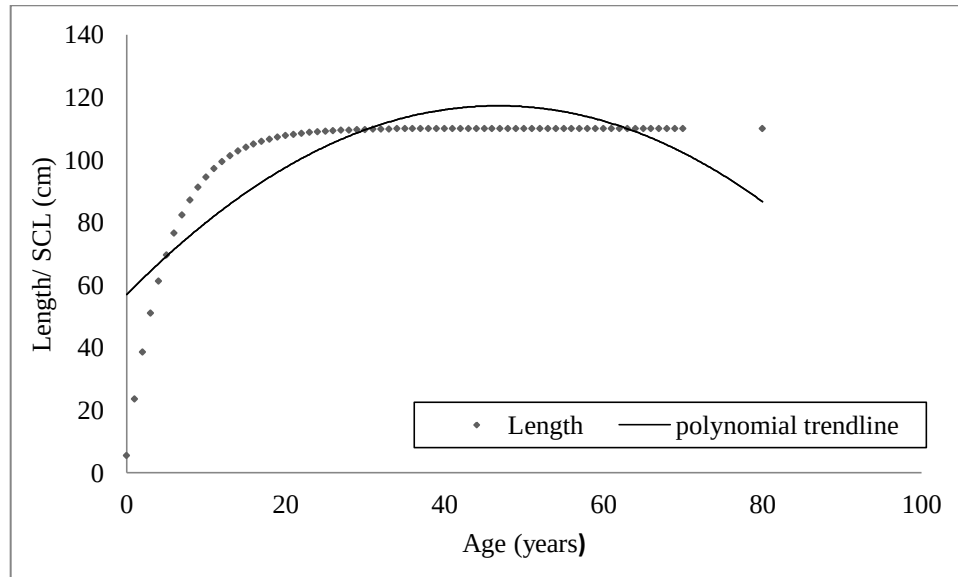


Figure 4.15: Estimated length at age for green turtles in Sarawak based on the Von Bertalanffy growth function

According to Sarawak Museum data, in 1982, the SCL for the smallest first time nester was 81.45 cm while the biggest one was 101.03 cm. Following Figure 4.15 and Table 4.3, at 81.45cm SCL, the first time nester's age was estimated to be within 6.5 to 7 years old while at 101.03 cm, the nester's age is estimated to be between 12.5 to 13 years old. Despite this, these values are only estimation of the corresponding age and length of green turtle in Sarawak, Malaysia. This estimation should be taken in with caution as the original data used to generate the growth parameter (k) was obtained from a headstart or captive project.

4.4 Discussion

4.4.1 Standard Operating Procedure (SOP)

This Standard Operating Procedure (Section 4.3.1) is the first small scale headstart procedure that had been produced in Malaysia. Similar to this SOP are manual for sea turtle husbandry techniques by Bluvias & Eckert (2010) and Higgins (2003). Bluvias & Eckert (2010) had produced a 'Marine Turtle Trauma Response Procedures: A Husbandry Procedure' that focuses on basic guidelines and procedures for individuals who rehabilitate sick and injured sea turtles. The manual by Bluvias & Eckert (2010) was designed to meet the needs of the Sea Turtle Trauma Response Corps organized by the Wider Caribbean Sea Turtle Conservation Network (WIDECAST). The manual helps prevent common problems faced with sea turtle rehabilitation specifically by providing guidance on handling and transport, facilities requirement, diet and feeding , emergency procedures, release etc. This comprehensive manual however requires extensive number of professional personnel with experience plus a complete rearing facility with massive space for tanks and an enormous amount of budget to be in operation.

The 'Sea Turtle Husbandry' chapter in Sea Turtle Biology (Volume II) by Higgins (2003) is better fit of husbandry manual for headstart program as it was based on the National Marine Fisheries Service, Sea Turtle Facility (NMFS STF) that was established in 1977 by the U.S Federal Government to reduce sea turtle by catch in the U.S. commercial fisheries. The facility that was the basis for this manual production had been rearing hundreds of sea turtles of different species including loggerheads and Kemp's ridley each year. Therefore, this manual was based on a large scale headstart program with a lot of experience and space for tanks with unlimited budget.

Both manuals (Bluvias & Eckert, 2010; Higgins, 2003) described good husbandry techniques which were followed during this project. However, several modifications to suit the local and small setting was done. For example, the tank preparation was cleaned following Higgins (2003) and the environmental parameters that are monitored daily namely the temperature, salinity and pH were based on recommendations of Higgins (2003) where the pH range in the tanks were maintained at 7.5 to 8.5 and the range of humidity was measured to be 73 to 89 RH daily. The salinity of the water was constant at 29 to 31 ppt daily. Moreover, the criteria for hatchling selection and routine cleaning were adapted from Higgins (2003) with modification to suit the local setting and situation.

During the early stages of this headstart program, the hatchlings were isolated following Higgins (2003) and Bluvias & Eckert (2010) when aggression occurred in the turtle's holding tank. However, it was found that this method did not help improve the situation unless the turtles were permanently isolated as the turtle would readily display aggression again after being isolated in the first place. Therefore, this study introduces behavioural management solutions namely floating cucumber treatment and seagrass, small fish and shrimp fry treatment. This treatment enabled aggression among turtles to be controlled successfully without the need to isolate them in separate containers. The aggression could be reduced up to an estimate of approximately 85 %. After the implementation of the cucumber treatment on turtles of Batch 2, it was evident that the mortality rate had been reduced. Therefore, it could be safe to say that trauma through injury incurred by biting was one of the main reason for a reduced survival rate of Batch 1.

Besides that, it was discovered that water quality plays a major role in controlling the outbreak or spreading of disease. Kowarsky (1977) has supported this by stressing that the daily water change rate affects the turtle's survival rates much negatively compared to

the turtle's stocking density. Techniques commonly used in aquaculture for example addition of TMS solution also helped in disease control. Therefore, headstart program in future should explore combinations of techniques in order to be more successful.

4.4.2 Feeding of Turtles in Relation to Growth

The inconsistent in food intake of turtles for Batch 1 was most probably caused by the sudden epizootic which affected the turtles as they became lethargic and anorexic. Therefore, to stop food wastage at that time, the amount of food was reduced accordingly and the turtles were only fed twice for every 2 days. This newly applied feeding regime was one form of disease control, and it was continued until week 54 for both batches. After that, the disease was no longer observed among turtles and no re-occurrence of disease was recorded in the facility. At the start of the project, the hatchlings of Batch 1 were fed with cockles, shrimp or squid. After approximately 6 weeks, the hatchlings lost their appetite, refused to eat the food offered and started to develop eye infection and lethargic condition was later observed.

George (1997) mentioned that juvenile captive turtles that were fed diets consisting of solely fish and squid were prone to life threatening anemia while hatchlings that were fed peneid shrimps were noted to develop gastrointestinal obstruction. Therefore, the food was changed to oily fishes that are rich in vitamin A such as sardines and mackerel tuna. After nearly 8 weeks, the hatchlings had a change in food preference again so the food had to be mixed between sardines and squid or shrimp. A condition called 'soft shell disease' similar to those reported by Leong *et al.* (1989) affected 3 % of the turtles population, suggesting the food given was probably very low in calcium and other vitamins, therefore the additional pellet food or turtle sticks (containing high quality fish meal, soybean meal,

yeast powder, fish oil, soy lecithin, vitamins and minerals) were administered thrice a week, as replacement of the normal food given during feeding time.

The 2 - 5 % of body weight of food given to turtles is adequate but may have to be modified according to situations especially the health of the turtles.

The turtles were all placed in the same holding tanks, thus they had a tendency to bite and show aggression, which led to further disease outbreak due to traumatic biting injury. Therefore, to divert their attention finely sliced cucumbers were added. The presence of sliced cucumbers floating in the tank facilitated calmer situation (less aggressive behaviour) among the turtles. To add to that, as reported in previous animal studies, cucumbers have anti-oxidant, anti-inflammatory and anti-cancer properties that could help turtles system against diseases (Nutrition Society of Malaysia, 2010).

4.4.3 Behavioural Observation

4.4.3.1 Locomotion and Sleeping Habits

During the study, the dogpaddling method (Wyneken, 1997) was observed to be used by hatchlings when they were surfacing for air. During that time, they continued progressing forward while elevating the anterior part of the body, with their head surfacing out of water for air intake. Frick (1976) had observed the same method where he mentioned that at 5 to 10 seconds interval, the swimming hatchling would rise to the surface while treading water movements momentarily with all four limbs.

Meanwhile for the rear flipper kicking technique (Wyneken, 1997), hatchlings was observed to be using this method of swimming whenever they were cornered by nets, in which they tend to swim backwards to escape it. This backward (reverse) swimming was mostly using their hind flippers where the fore flippers were straightened out to the front

while their hind flippers were moved simultaneously like oars on a boat to get them out of the nets.

Another swimming technique observed was power stroke (Wyneken, 1997) which could be observed on a daily and sudden basis when they had been permanently placed in the bigger sized pool. The power stroking technique was mostly used by the turtles when they were surprised, which suggests that this technique is most likely an escape strategy for the turtles.

In terms of sleeping habits, folding and flexing of flippers to body was a common habit which was observed in this study and by Frick (1976). He mentioned that the hatchlings floated on the surface of water with the flippers were pressed flat along the carapace in the sleeping position after swimming for about 5km. This suggests that the technique is a resting position used whenever the turtles are in migration.

As for the turtle's basking behaviour, Whittow & Balazs (1982) had mentioned that green turtles of both sexes and stages (adults as well as immature turtles) were observed to crawl ashore to bask on oceanic beaches of remote north-western Hawaiian Islands (U.S.). Therefore, the basking behaviour could be claimed as one of the common behaviour of turtles.

In relation to behaviour, aggressive individuals had been observed throughout the 18 months of rearing period and these individuals are most likely alpha males as suggested by Wyneken (1997). However, it was observed that the level of aggressiveness displayed had become less as the turtles age increased. The turtles were observed to be much calmer as time goes by, making it easier to do the biweekly morphometric measurement. Turtles are smart creatures that are able to adapt to situations and learn. After 3 months of

undergoing the same feeding routine, the turtles actually know when it is feeding time as they gather at the same spot where the researcher is standing when it is near feeding time. Harrison (1951) had supported this statement after observing the same behaviour from a turtle kept in Sarawak Museum.

This study has shed some light on how turtle juveniles behave in the first 18 months of their life. It is likely those in the wild have similar behaviour, therefore keeping the ocean free from pollution is important as Bolten & Balazs (1995) claimed that turtles spent 10 or more years in the open sea.

4.4.3.2 Imprint and Impound Procedure

Green sea turtles is said to nest at the age of 15 to 19 years (Bell *et al.*, 2005) thus approximately in year 2031, revisit of Pandan Beach, Lundu and adjacent beaches should be carried out to determine the success of this experiment. All the turtles that were released in December 2015 and May 2016 had been tagged for future tracking. Mrosovsky & Godfrey (2003) suggested a condensed maturation time because headstarted animals has been past the pelagic phase which helps to speed up the growth process. However, there is always a possibility of slowing of growth as the yearlings released adjust to life in the wild which could partially negate the headstart that they received as Wood & Wood (1993) have observed decrease in weight gain from average of 6.6 kg per year in captivity to 3.0 kg per year in the wild.

4.4.4 Survival and Growth of *Chelonia mydas* in Captivity

The survival rate for Batch 1 was 22.5 % after an 18 months rearing period which was longer and better compared to Leong *et al.* (1989) who reported the survival of 9 % of loggerhead after 10 months of rearing period. On the other hand, the survival rate of Batch 2 was 82 % which is consistent with subsequent finding of Leong *et al.* (1989) in which the survival rate later improved to 68 until 95 % for headstarting of different batches of Kemp's ridley after gaining experience from rearing of loggerhead hatchlings.

In order to compare this study's growth data to other studies, the data obtained concerning growth increment was converted to growth per year. For this data, Batch 1 showed an increment rate of 9.86 cm/ year while Batch 2 has a rate of 15.92 cm/year. Eguchi *et al.* (2012) reported the growth rate of wild 22 green turtles from San Diego Bay, California that are smaller than 90cm to be in the median of 4.89 cm/year up to 11.35 cm/year. Bjorndal *et al.* (2000) had reported increment of median 4.3 cm/year up to 10.8 cm/year in southern Bahamas. In other studies, Casale *et al.* (2009) reported loggerhead turtles in the wild grew at 11.8 cm/year (approximately 0.32 mm/day) while Pilcher (2010) claimed that wild green turtles in Sabah grew at 3.60 cm/year (approximately 0.09 mm/day). The comparison of growth rate between this study and previous studies should be noted with caution where previous studies reported growth in the wild whereas this study reported data only for captive turtles. Eguchi *et al.* (2012) had stated that the rapid growth of green turtles from San Diego Bay was attributable to the physical environment where the turtles were exposed to warm effluent from a power plant that enabled the turtle to be active during winter months. Differences in growth rates among foraging habitats have been reported for the Great Barrier Reef, Australia (Chaloupka *et al.*, 2004) and the Hawaiian Archipelago (Balazs, 1995; Balazs & Chaloupka, 2004).

4.4.4.1 Von Bertalanffy Growth Function

Carr & Goodman (1970) had mentioned that individual green turtles mature at different sizes and they grow very slowly, if at all, after reaching maturity. Therefore, it is normal to doubt that turtles mature at 81.45 cm SCL, or at approximately 6.5 to 7 years old compared to the turtle that were found to first nest at 101.03 cm SCL (age of 12.5 to 13 years old) as it would be a better estimate of the age of maturity for nesting female turtle population in Sarawak. This estimated maturity time is relatively similar to Seminoff *et al.* (2002) who estimated turtles to take 9 to 21 years in Mexico to reach maturity after recruitment, and 9 to 24 years for estimated age at first maturity (Ehrhardt & Witham, 1992).

Moreover, Wood & Wood (1980) had mentioned before that captive reared green sea turtles may reach maturity in only 8 to 11 year at sizes that are similar to mature females in their natural population thus proving that the estimated length at age as given in Table 4.3 to be biased towards the captive population. However, the use of the captive turtle data was inevitable as no mark recapture data of sea turtles in Sarawak were made available during the time of analysis. In addition to that, turtles experience strong ontogenic habitat shifts thus making green turtles enter the oceanic pelagic habitat as post-hatchlings and then turn into coastal benthic feeders as juveniles resulting in their diet shift from omnivorous to herbivorous diet (Bjorndal & Bolten, 1988). The change in green turtle's habitat and diet from post hatchling to juvenile stage was postulated to be the reason behind marine turtle's somatic growth polyphasic stage (Hendrickson, 1980; Chaloupka & Musick, 1997).

This lifestyle change in green turtle could possibly be the reason of lower maturity estimation time for green turtles from this study as the data used to estimate the length at age of first time wild nesters of Sarawak's green turtle population was obtained from the post hatchling to yearling stage only where the turtles could still be in the oceanic pelagic habitat thus highlighting the importance of the mark recapture data with recorded time between recapture in providing a more robust estimation of length at age data for an accurate estimation of matured female's age at length.

4.4.4.2 Possible Reasons for Differences in Growth between Batch 1 and 2

One of the main reasons that caused Batch 1 to be significantly different to Batch 2 is the handling and management technique. Batch 2 was taken from Talang-Talang Besar Island after the main researcher had experienced various difficulties with Batch 1 and subsequently had improved the husbandry techniques. Among the factors that had been modified are the daily water change rate (Kowarsky, 1977), the space allocated to each hatchling, the security and safety of turtles, the behavioural management, disease management and the feeding regimes.

The sea turtle holding tanks were cleaned once a day when the turtles were less than 4 months old. However, as they grew, the tanks had to be cleaned twice daily and water changed according to the availability of the sea water following the water tide level. Turtles that are out of the water had a tendency for carapace desiccation. Therefore, even during cleaning, the turtles had to be sprayed with water to avoid peeling of the scutes. According to Higgins (2003), damage to carapace from desiccation may provide an entry

path for bacterial and fungal infection. The daily cleaning routine of the sea turtle holding tank are as the following:

1. The hatchlings were moved out of the holding tank and transferred into the spare sea water tank.
2. However, during juvenile stage, they were not moved out of the tank.
3. The water of the holding tanks was completely drained.
4. All inner surfaces of the holding tank were scrubbed with a brush and hosed off using fresh water to ensure cleanliness of the tank was maintained at the highest level possible.
5. The tank was refilled with fresh sea water (pumped straight from nearby sea).

Higgins (2003) mentioned that captive reared turtles are susceptible to heavy and rapid biofouling since they lack access to a substrate on which to scrape away accumulating epibiota. Epibiotic build up may create a medium for bacterial and fungal infection therefore periodic removal had been carried out during this study. The daily cleaning routine of the sea turtle are as follows:

1. After feeding, the hatchlings were checked for algae or any other abnormal growth.
2. For juveniles, they were checked after the water of their holding tank had been drained.
3. The dirty turtles were then gently scrubbed with a sponge or a soft tooth brush and rinsed off with clean water before they were released back into their holding tanks.

4.5 Conclusion

In conclusion, the growth rate of the turtles that were reared for the headstart project was higher compared to those living in the wild. A growth function was able to be produced, thus an estimate of length at age for the Sarawak's green turtle population was established. Moreover, a detailed and public friendly SOP on human turtle interaction and sea turtle husbandry was produced alongside new behavioural and disease management procedures that could be adapted for future headstart programs. In addition, the commonly observed behaviour of post hatchlings and juveniles that were observed during the headstart program was documented for future references.

CHAPTER 5

DISEASES AND ABNORMALITY ENCOUNTERED DURING HEADSTART PROGRAM

5.1 Introduction

Stedman's Medical Dictionary (2001) had defined health as the overall condition of an organism at a given time and as freedom from disease or abnormality. Other than health threats, there are many other naturally occurring threats such as predation and environmental pollution which could exacerbate conditions leading to infection caused by pathogens and parasites. Both types of threats need to be studied (Broderick & Hancock, 1997) as they have negative impacts on the survival of the species.

Infectious diseases are one of the major causes of morbidity and mortality in all reptiles (Alfaro *et al.*, 2006). Infections in sea turtles are almost always the result of immunosuppression and associated with the stress of captivity (Mader, 2006). According to George (1997), the adrenal glands of a stressed sea turtle releases corticosterone which reduces the humoral and/or cell-mediated defense mechanism of the turtle, thus inhibiting the ability of the immune system to respond to infectious agents.

George (1997) also mentioned that stresses can be environmental (salinity, pollution, temperature etc.), nutritional, or physical (trauma). According to Alfaro *et al.* (2006), most of the infections that are caused by bacteria and viruses happened due to opportunistic circumstances where the pathogenic organism attacks when the immune system had already been compromised, meanwhile protozoa and macro-parasites requires a high level of specialization to rule the mode of infection.

Leong *et al.* (1989) had described various diseases, malformations and injuries observed in captive reared sea turtles namely Kemp's Ridley (*Lepidochelys kempii*) and loggerhead (*Caretta caretta*). Both species were reared for one year or less (for headstarting) in an attempt to prevent the extinction of Kemp's ridley population that declined from an estimated 40,000 in 1947 to fewer than 600 nesting per day during the 1987 nesting season (Manzella *et al.*, 1988). To gain experience, Leong *et al.* (1989) had reared loggerhead for about 10 months, in which only 9 % had survived. This situation had provided new insights on turtle pathology and suitable health care methods. As a result, information on diseases was documented in detail. The diseases of turtles were then classified into 27 major categories, in which one of the most common causes of hatchlings mortality was described as the sudden hatchling death syndrome.

Various diseases affects sea turtles; one of the most prevalent is fibropapillomatosis (FP), a globally distributed neoplastic disease of marine turtles which causes external and internal tumours and also debilitation that affects immature animals (Herbst, 1994). According to Work *et al.* (2003), the prevalence of FP in immature green turtles fluctuates between 40 and 60 %, depending on where and how the animals were sampled. Their study was carried out in Hawaii, where the disease was reported to be present on all main Hawaiian Islands except for Kona/Kohala. Common theory accepted by many scientists is infection occurs as the result of immunosuppression due to stress, and this had been supported by Aguirre *et al.* (1995) who concluded that turtles with severe FP were stressed and immunosuppressed, based on haematology and blood cortisol level evidences.

Moreover, Work *et al.* (2003) also reported that a free-ranging green turtles are likely being bacteraemic as the severity of FP increases, because they are most vulnerable to infection with bacteria, as a result of the collapse of defence against foreign agents with

severe FP. Dow (1995) had also mentioned that bacteraemia in animals could result in metabolic changes such as altered electrolyte balance, hypoglycaemia, altered clotting profiles and toxaemia. In addition, Novak & Siegel (1986) also mentioned that bacteraemia can result in anorexia and lethargy in reptiles.

Glazebrook *et al.* (1993) described three bacterial diseases namely ulcerative stomatitis, obstructive rhinitis and pneumonia which have been linked as associated complexes and were together responsible for mortality rates of up to 70 % in farmed and oceanarium reared turtles in Sue, Badu, Yam, Yorke and Coconut Islands in the Torres Strait and from an oceanarium on Magnetic Island near Townsville, Australia. After examining 140 affected turtles, results showed that individual diseases occur more frequently than complex forms of a disease with ulcerative stomatitis appearing in hatchlings as early as 3 weeks old while the other condition arises from caseous material being lodged in the upper and lower part of their respiratory tract. Hatchlings and juveniles were found to be susceptible to ulcerative stomatitis (58.6 % of the cases diagnosed) and bronchopneumonia (58.3 %) while obstructive rhinitis was found to be present in 70 % of hatchlings with mouth rot or ‘canker’. Focal pneumonia however occurred more frequently in juveniles (81.8 %). In the same study, three bacteria namely *Vibrio alginolyticus*, *Aeromonas hydrophila* and *Flavobacterium* sp. were repeatedly isolated from cases of ulcerative stomatitis and obstructive rhinitis. Four genera of fungi (*Paecilomyces* sp., *Penicillium* sp., *Aspergillus* sp. and *Fusarium* sp.) were also recovered from caseous material in the trachea and bronchi of turtles with bronchopneumonia.

Apart from bacterial infection, some diseases are also associated with fungi that were pathogenic to green turtles under cultured conditions. Jacobson *et al.* (1979) had described a pulmonary infection which was histologically shown to be granulomatous

lesions containing branching septate hyphae on turtles with buoyancy abnormality which was later diagnosed as mycotic pneumonia. Jacobson *et al.* (1979) recorded that several fungal species such as *Sporotrichium* sp., *Cladosporium* sp. and *Paecilomyces* sp. were present in the lungs of the affected turtles. He also mentioned that although several mycotic agents were isolated, other infectious agents may have been involved and that it might have initiated the process which could ultimately lead to fungal involvement. In addition, Smith *et al.* (1972) reported that mycotic pneumonias are well documented for mammals and birds, with aspergillosis (brooder pneumonia) commonly found in domestic Avian species, which are similar to green sea turtle mycotic pneumonia.

The presence of parasites in sea turtles had been documented. For example, larval anisakid nematode *Sulcascaris sulcata* that matures in sea turtles (*Caretta caretta* and *Chelonia mydas*) had marine molluscs as first host. It was also claimed that sea turtles are accidental hosts for *Anisakis* spp. larvae (Alfaro *et al.*, 2006) but the parasites are not linked to any human disease. Glazebrook & Campbell (1990) reported that *Anisakis* larvae was found to be responsible for a form of parasitic gastritis-serositis that affected 14.4 % turtles in Darnley and Murray Island. The yearlings of the two islands were found to be more heavily infested as the food (fresh sardines and hardyhead fish) fed routinely to them were infested with the worms. In the same study, the ulceration of the gastric, intestinal mucosa and the visceral adhesions were caused by the migration of the larvae from the lumen (of the gut) to the pleuroperitoneal cavity. They also mentioned that gastric and intestinal flukes could occur in farmed turtles, but most commonly found in wild turtles.

During headstart project in Lundu (Chapter 4), it was noted that diseases were spread among hatchlings. For the first 4 months of this project, the number of dead hatchlings was approximately 5 per day because of an unknown illness. Therefore, this part of the project

aims to carry out profiling of bacteria and fungi that may have caused the diseases in hatchlings reared in captivity, which in many cases resulted in mortality and morbidity of hatchlings.

The objectives of this chapter are to: (1) identify and describe the type of disease that causes the mortality of turtle hatchlings and juveniles during headstart program, (2) to identify the possible causative agent and symptom of the diseases and (3) to document steps taken to control disease outbreaks for future programs.

5.2 Materials and Methods

Necropsy of turtle hatchlings and juveniles were first carried out on carcasses as the disease identification process could not be carried out based on external appearance of carcasses alone. Several abnormal patches and caseous materials were found in the carcass which leads the study into two approaches namely microbiology and DNA sequencing in order to identify the identity of possible pathogens. Standard procedures were carried out following Glazebrook & Campbell (1990).

5.2.1 Collection of Samples

A total of 101 samples were collected from the headstart program that took place from June 2014 until December 2015 (Chapter 4). Carcasses of hatchlings were frozen in a - 20 °C fridge immediately after death, labelled then transported back to Molecular Aquatic Laboratory, UNIMAS. During transportation, all specimens were kept in cooler box with ice cubes before it was deposited into the – 80 °C Ultrafreezer. No deliberate sacrificing or

injuring of normal live sea turtle was carried out in this study. All the hatchlings and juveniles died naturally, and were never euthanized for scientific purpose. For each specimen, records of unusual signs and symptoms were available as observations on behaviour of the turtles were carried out continuously during the project.

5.2.2 External Examination and Necropsy Procedure of Carcass

The necropsy procedures were performed in Molecular Aquatic Laboratory, UNIMAS. Prior to the procedure, hatchlings (day 1 to 8 weeks old) and juveniles (2 to 12 months old) were weighed individually using an electronic balance (Brand: Shimadzu). Size was determined by measuring the curved and straight carapace length and width as well as the length of the plastron (Figure 5.1a). The carcass was also photographed at different angles of body parts with scale for documentation (Figure 5.1b-d). All external injuries or observable bite marks or lesions were noted in a form, following Flint *et al.* (2009) and the area of lesions was sampled appropriately. Further internal examination of sea turtle hatchlings carcasses was conducted following Wyneken (2001) which involved detailed examination on the organs (intestine, stomach, liver, heart, and lungs), body cavity and oral cavity of the carcasses. During the post mortem, any suspicious growth or gross lesions were collected and kept in saline solution for use during bacteria plating, or in 70 % ethanol for parasite inspection using stereo microscope (Brand: Motic). The number of samples obtained from the headstart program is in Table 5.1. Classification of diseases into infectious or non-infectious category was done following Mader (2006).

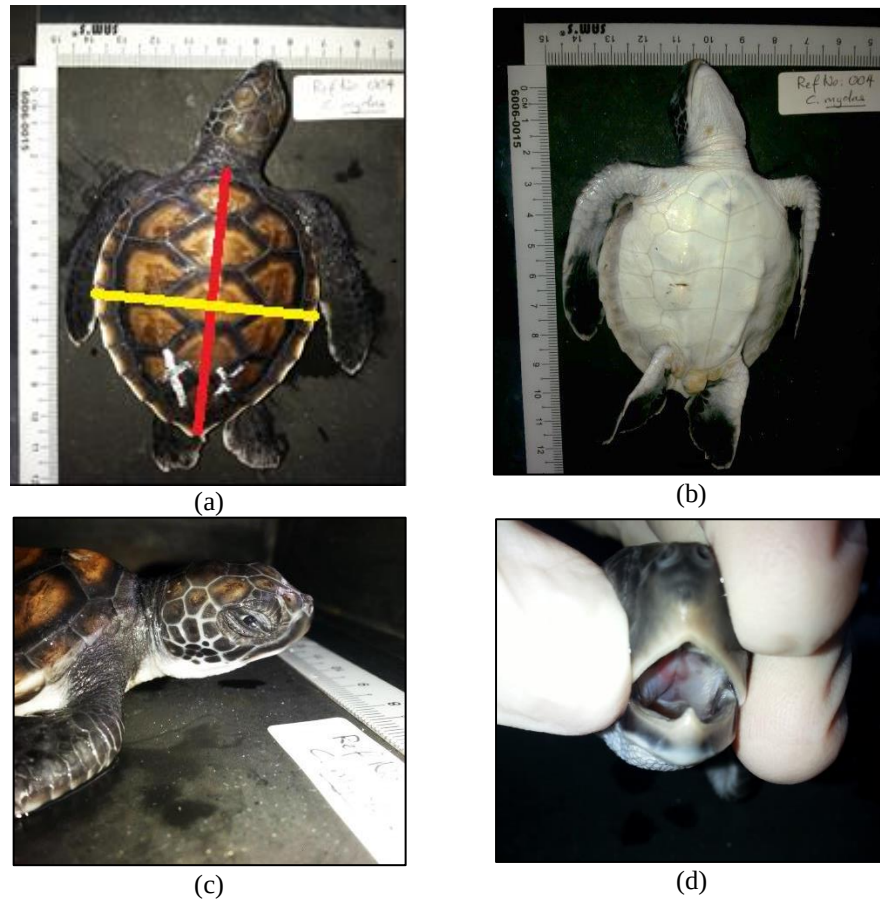


Figure 5.1: (a) measurement of carapace and dorsal view, (b) ventral view, (c) Eye and dorsal-ventral head view, (d) oral cavity view

Table 5.1: Number of hatchling and juvenile samples from batch 1 and 2

	Hatchling	Juveniles
1.Age (months)	1 to 2	3 to 12
2.Total samples obtained:	42	59
3.Number of samples obtained from Batch 1:	37	56
4.Number of samples obtained from Batch 2:	5	3

5.2.3 Culture of Bacteria and Fungi

All obtained samples were plated on Nutrient Agar, Tryptic Soy Agar, Blood Agar and Potato Dextrose Agar. To support organisms with an obligate salt requirement, all media contained 3 % Sodium Chloride (NaCl). The sample was plated using the 16 streak

dilution technique until a pure culture was obtained. Once a pure growth was obtained, the culture was processed for DNA extraction using Modified Cetyltrimethyl ammonium bromide (CTAB) protocol for bacteria following Doyle & Doyle (1987). For fungi, fungal DNA extraction was carried out following protocols of Cubero *et al.* (1999).

5.2.4 Molecular Identification of Bacteria and Fungi

The pair of primers that was used for the 16S rRNA gene, a DNA fragment of 1500 bp for the identification of bacteria was 27F (5' - AGA GTT TGA TCM TGG CTC AG-3') as forward primer and 1492R (5' -TAC GGY TAC CTT GTT ACG ACT T-3') as reverse primer following Frank *et al.* (2008). Amplification of the 16S rRNA gene used PCR protocol as suggested by Frank *et al.* (2008). For identification of fungi, the pair of primers that were used to amplify an approximately 700 bp ITS region was ITS1 primer (5'-TCC GTA GGT GAA CCT GCG G-3') as forward primer and ITS4 primer (5' - TCC TCC GCT TAT TGA TAT GC-3') as reverse primer, following Gardes & Bruns (1993). Each 25 µL PCR reaction contained 5× PCR buffer, 10mM of dNTP, 25mM of MgCl₂, 0.2 µM each of both primer per gene and also 5 u/µL of Taq DNA Polymerase. The thermal cycling profile used for amplification of 16S rRNA gene for bacteria is as shown in Figure 5.2 while the thermal cycling profile used for the amplification of ITS region of fungi is as shown in Figure 5.3 which was successful following protocol by Gardes & Bruns (1993). The reaction was carried out using PCR machine "Little Genius". The presence of PCR products was checked using a 1% agarose gel electrophoresis (AGE) in 1× TAE buffer at 90V for 1 hour. The pre-mixed ethidium bromide gel was then visualized using UV light and the gel photograph was captured using the Gel Doc system. For all bands obtained of

the correct size and clear, the PCR products were purified using Promega PCR Clean-Up System, followed by AGE in 1× TAE buffer at 110 V for 30 minutes. The gel was then visualized, and if clear bands were obtained, the purified PCR products were then sent to FirstBASE Sdn. Bhd. for DNA sequencing.

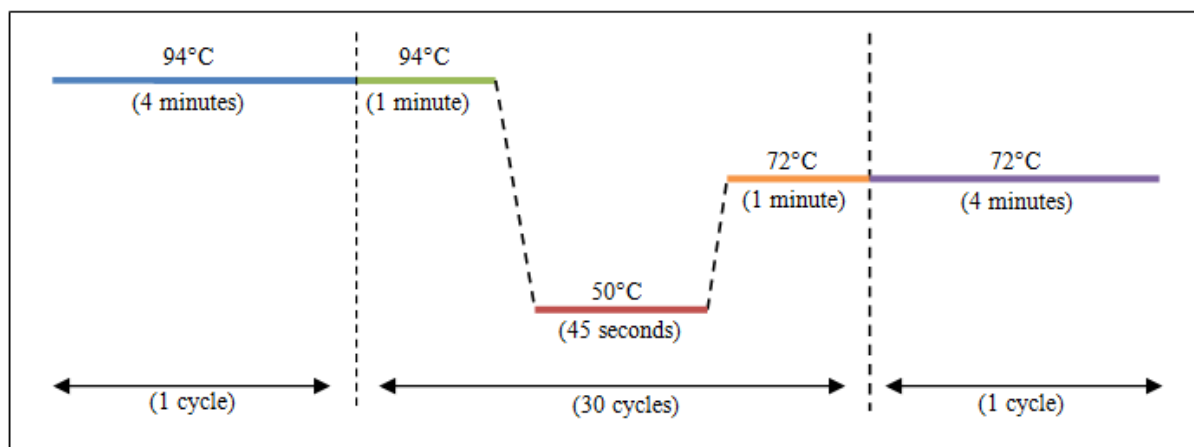


Figure 5.2: Thermal cycling profile for amplification of 16S rRNA gene of bacteria

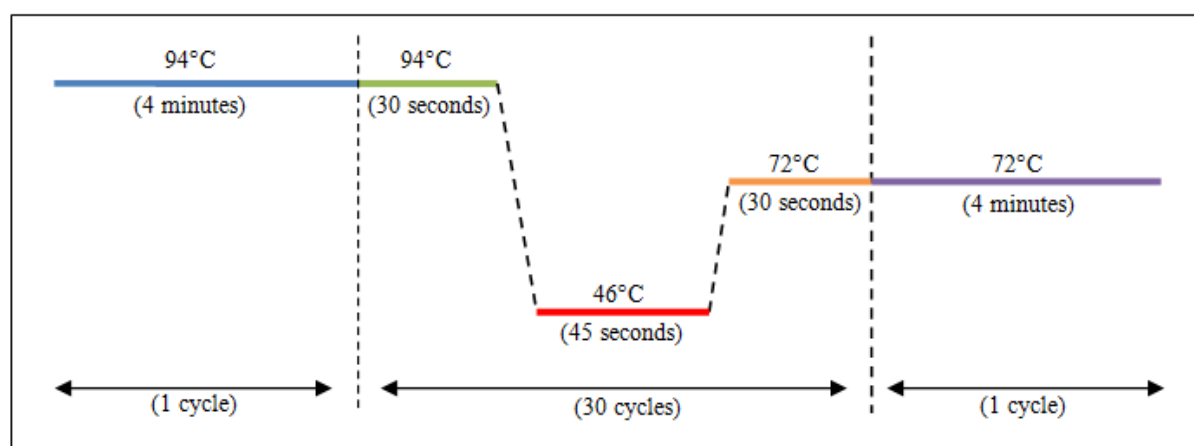


Figure 5.3: Thermal cycling profile for amplification of ITS region of fungi

5.3 Results

5.3.1 External Examination and Necropsy Procedure of Carcass

An external examination was conducted on the carcasses of hatchling and juvenile obtained in the headstart study followed by post mortem for internal examination. The general classification was done based on external observation of the samples and was further classified into age group of hatchling and juvenile (Table 5.2).

Table 5.2: Classification of carcass based on external observation.
Number in brackets () indicate number of samples that undergo post mortem examination

Classification based on external observation	Number of samples obtained	
	Hatchling	Juvenile
Eye infection	11 (3)	6 (2)
Severe bite marks	15 (5)	21 (8)
Red or Blue marks	4 (2)	3 (0)
Bloated plastron	6 (1)	16 (4)
Emaciated (sunken plastron)	6 (2)	13 (6)

5.3.2 Diseases Found on Sea Turtle Carcass

Upon the completion of the internal examination, the diseases found were further classified into two main categories namely infectious and non-infectious disease. The type of diseases found were confirmed after post mortem and after examining the affected parts of the body and the symptoms shown by the turtles before they died.

Diseases that were found to be infectious in this study are liver disease, ulcerative stomatitis, bronchopneumonia, obstructive rhinitis and the sudden hatchling death syndrome.

Meanwhile, under non-infectious disease, three different types of stressors were found to be the main cause of the diseases found in this study namely, metabolic

disturbance, genetic inheritance and trauma. The type of metabolic disturbance experienced by the turtles in this study was mainly nutrient deficiency which was associated with soft shell disease, eye lesion and emaciation while wrong diet consisted of faecal impaction and intestinal prolapse. Only congenital blindness and curved beak were observed as a condition caused by genetic inheritance. Lastly, the diseases that are caused by traumatic effect such as biting among turtles and physical abrasions are termed as ulcerative dermatitis and blue or red marks. The types of diseases found in this study are summarized in Figure 5.4.

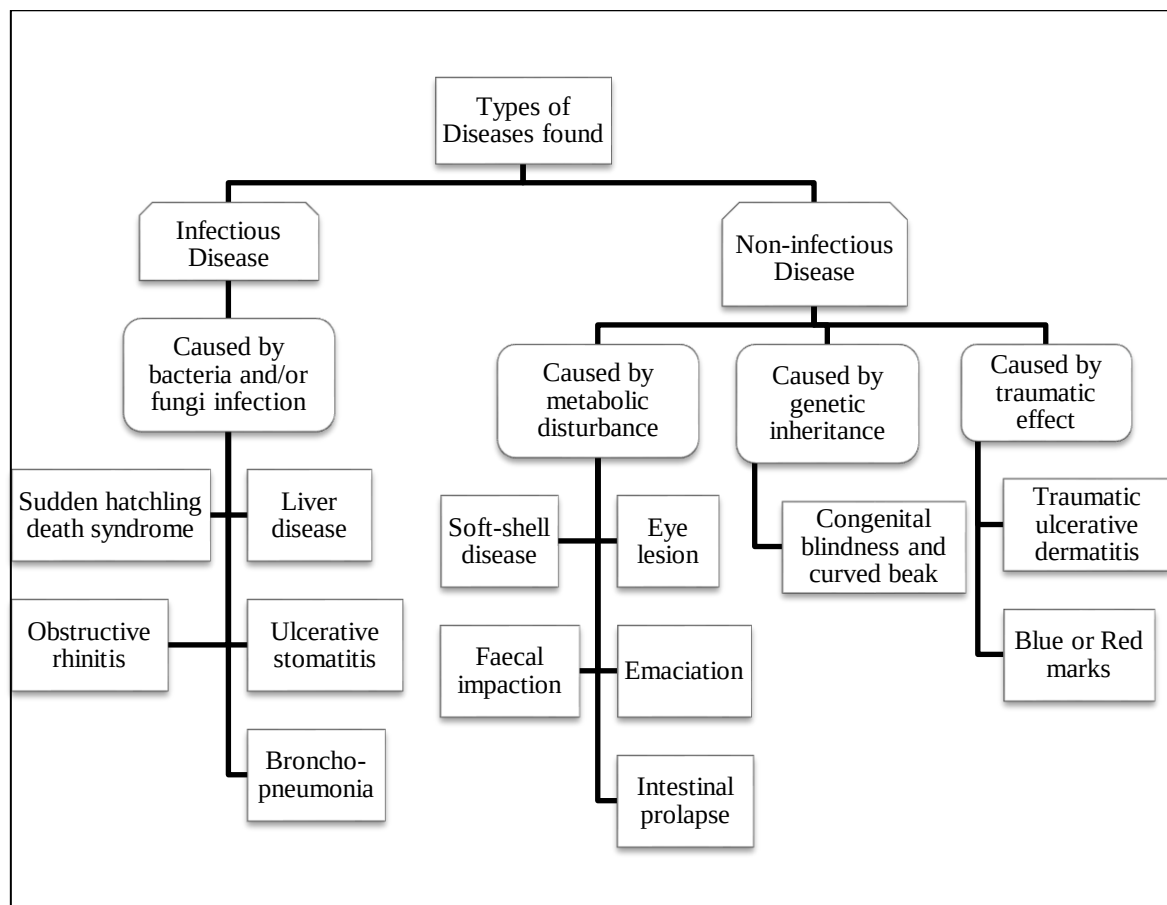


Figure 5.4: Flow chart detailing classification of diseases found in the study

5.3.3 Differences in Diseases Acquired by Batches

For Batch 2, only 8 out of 50 turtles suffered and died from diseases found in the study which was a small number compared to 93 turtles from Batch 1. The type of diseases that affected both batches was specified in Table 5.3. Infectious and non-infectious diseases were majorly found in Batch 1. From Table 5.3, it is important to note that no cases of soft-shell disease, eye lesion, emaciation and congenital blindness plus curved beak were found in Batch 2.

Table 5.3: Diseases found affecting sea turtles by batch. Batch 1(n=93), Batch 2(n=8)

Type	Disease caused by	Type	Number affected by disease	
			Batch 1	Batch 2
Non-infectious	Metabolic disturbance	Soft-shell disease	3	0
		Faecal impaction	18	4
		Intestinal prolapse	12	2
		Eye lesion	17	0
		Emaciation	39	0
	Genetic inheritance	Congenital blindness, curved beak	1	0
	Traumatic effect	Ulcerative dermatitis	86	7
		Blue/Red marks	33	1
Infectious	Bacteria / Fungi	Sudden hatchling death syndrome	68	3
		Liver disease	10	3
		Ulcerative stomatitis	4	1
		Obstructive rhinitis	4	1
		Bronchopneumonia	6	2

In this study, multiple types of disease could occur in one turtle carcass, thus making the total number of individual affected by the disease in Table 5.3 different from the total number of carcasses obtained in Table 5.1.

As for the infectious diseases, a necropsy procedure has to be carried out on a carcass to determine whether the carcass has symptoms of the disease internally to justify

the fact that it has contracted the disease. Therefore, it is important to note that not all the carcasses obtained from this study undergo necropsy, due to time and resource limitations. However, one of the infectious diseases that only require observation to determine was the sudden hatchling death syndrome. For this disease, all carcass involved was accounted for.

5.3.4 Identification of Pathogens using DNA Sequencing Approach

In this study, CTAB buffer protocol had successfully extracted DNA from both bacteria and fungi. The following subsections are results of the PCR and the DNA sequencing.

5.3.4.1 Amplification of Bacteria's 16S rRNA Gene using PCR

Amplification of the 16S rRNA gene was successful. At the very beginning of this study, the bands that were obtained were mostly multiple bands. However, after series of PCR optimization, single and clean bands of approximately 1500 bp could be observed in Lane 1 to 12 (Figure 5.5). In this study, if multiple bands were obtained, PCR of desired length (1000-1500 bp) was cut and subjected to PCR product purification procedure prior to DNA sequencing.

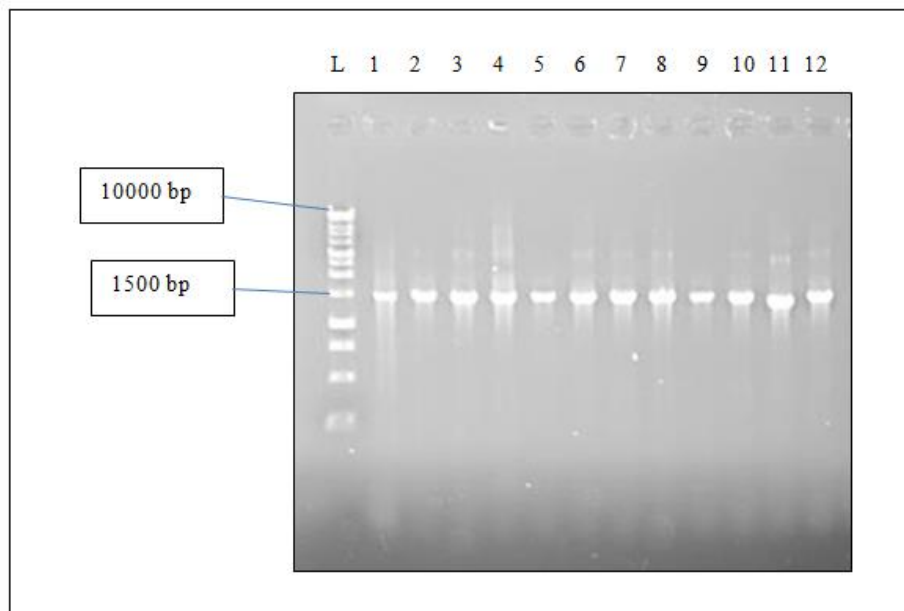


Figure 5.5: Gel picture showing PCR product of 16S rRNA gene of bacteria

Lane L = 1kb DNA ladder (Promega)

Lane 1-9 = PCR product of 16S rRNA of bacteria

5.3.4.2 Amplification of Fungi's ITS Region using PCR

Clear bands of approximately 650 bp were obtained in Lane 1 to 5 (Figure 5.6) after optimization steps were repeatedly carried out. The obtained PCR products were sent to DNA sequencing.

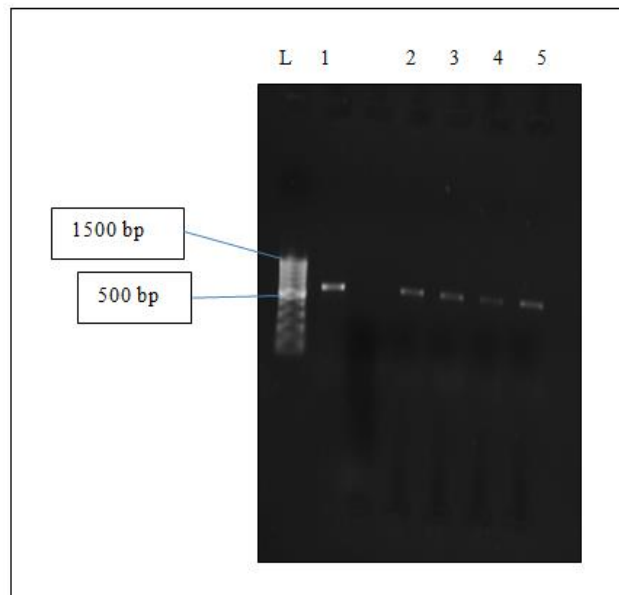


Figure 5.6: Gel picture showing PCR product of ITS gene of fungi

Lane L = 100 bp DNA ladder (Promega)

Lane 1-5 = PCR product of ITS gene of fungi

5.3.4.3 Identification of Disease causing Pathogens

Table 5.4 shows results of DNA sequencing of bacteria from the carcass of hatchlings using the 16S rRNA gene while Table 5.5 is the result of DNA sequencing of fungi that were obtained using ITS region. The results for both bacteria and fungi were compared to known causative pathogens found in the literature in Table 5.4.

Table 5.4: Sequencing result of bacteria that were found on hatchling's carcasses and the known causative bacteria. Highlighted bacteria/fungi are similar to known causative agents. **B**= bacteria and **F**= Fungi.

Expected Disease	Found	Known causative bacteria/fungi
Eye lesion	B: <i>Rahnella aquatilis</i> , <i>Bacillus</i> sp.	B: <i>Vibrio harveyi</i> (Ishimaru & Muroga, 1997)
Traumatic Ulcerative Dermatitis	B: <i>Streptococcus parauberis</i> , <i>Exiguobacterium indicum</i> , <i>Vibrio alginolyticus</i> , <i>Vibrio parahaemolyticus</i> , <i>Acinetobacter</i> sp., <i>Bacillus</i> sp. F: <i>Cladosporium sphaerospermum</i>	B: <i>Mycobacterium avium</i> (Greer <i>et al.</i> , 2003) F: <i>Fusarium scirpi</i> , <i>Penicillium</i> sp. (Glazebrook & Campbell, 1990)
Ulcerative stomatitis	B: <i>Paracoccus denitrificans</i> , <i>Morganella morganii</i> , <i>Proteus mirabilis</i>	B: <i>Proteus</i> sp., <i>Staphylococcus</i> sp. (Oros <i>et al.</i> , 2004)
Obstructive rhinitis	B: <i>Psychrobacter celer</i> , <i>Pseudomonas</i> sp., <i>Proteus mirabilis</i> , <i>Klebsiella pneumonia</i>	B: <i>Klebsiella pneumonia</i> (Aguirre <i>et al.</i> , 1994), <i>Pseudomonas</i> sp. (Oros <i>et al.</i> , 2004)
Broncho-pneumonia	B: <i>Citrobacter freundii</i>	B: <i>Arizona hinshairi</i> , <i>Flavobacterium</i> sp., (Glazebrook & Campbell, 1990) F: <i>Cladosporium</i> sp., <i>Sporotrichium</i> sp., <i>Paecilomyces</i> sp. (Jacobson <i>et al.</i> , 1979)
Obstruction of the gastro-intestinal tract (faecal impaction and intestinal prolapse)	B: <i>Shewanella upenei</i> , <i>Shewanella haliotis</i> , <i>Paracoccus</i> sp., <i>Morganella morganii</i> , <i>Pseudomonas pseudoalcaligenes</i> , <i>Citrobacter freundii</i> , <i>Shewanella algae</i> , <i>Vibrio harveyi</i>	B: <i>Bacillus</i> sp., <i>Corynebacterium</i> sp., <i>Citrobacter freundii</i> , <i>Morganella morganii</i> , <i>Proteus penneri</i> , <i>Proteus vulgaris</i> , <i>Proteus alcalifaciens</i> , <i>Vibrio alginolyticus</i> , <i>Acinetobacter Iwoffi</i> (Aguirre <i>et al.</i> , 1994)
Liver disease (hepatitis)	B: <i>Vibrio</i> sp. , <i>Citrobacter freundii</i> , <i>Shewanella algae</i> F: <i>Cladosporium</i> sp., <i>Penicillium steckii</i> , <i>Aspergillus flavus</i> , <i>Aspergillus terreus</i> , <i>Gibberella moniliformis</i> , <i>Aspergillus niger</i>	B: <i>Citrobacter</i> sp., <i>Escherichia coli</i> , <i>Proteus</i> sp., <i>Staphylococcus</i> sp., <i>Streptococcus</i> sp., <i>Vibrio alginolyticus</i> (Oros <i>et al.</i> , 2004)

Bacteria that are found to be similar to the known pathogens in the literature for infectious disease are *Proteus mirabilis* for ulcerative stomatitis, *Pseudomonas* sp. and *Klebsiella pneumonia* for obstructive rhinitis, and *Citrobacter freundii* for liver disease. For non-

infectious disease, only *Morganella morganii* and *Citrobacter freundii* were similarly identified to literature for obstruction of gastrointestinal tract.

In Table 5.5, four different taxa of fungi were identified namely *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp. and *Gibberella* sp. from liver, skin and heart samples.

Table 5.5: Sequencing result of fungi found on hatchling's carcasses

Specimen No.	Specimen	PCR Result	Sequencing Result	Homology %	Sequence length (bp)	Gram stain
39-009	Liver, fungi	+	<i>Cladosporium</i> sp.	99	526	-
41-009	Liver, fungi	+	<i>Penicillium steckii</i>	99	569	-
43-009	Liver, fungi	+	<i>Aspergillus flavus</i>	99	570	-
44-007	Liver, fungi	+	<i>Aspergillus terreus</i>	99	587	-
45-004	Liver, fungi	+	<i>Gibberella moniliformis</i>	99	519	-
46-007	Liver, fungi	+	<i>Aspergillus niger</i>	99	574	-
40-010	Skin, fungi	+	<i>Cladosporium sphaerospermum</i>	99	525	Positive
42-010	Heart, fungi	+	<i>Penicillium steckii</i>	99	567	Positive

5.4 Discussion

5.4.1 External Examination

A preliminary study by Zulkifli (2015) on 70 individual carcasses obtained during headstart program from Chapter 4 had suggested that disease is one of the factors that caused the death of the hatchlings. Based on physical examination alone, Zulkifli (2015) reported that 86 % had biting marks, 39 % had shrinking plastron, 33 % had blue or red marks, 16 % were bloated and 11 % had bacterial infection on eye. She reported that only small or medium characters (injuries or deformities) were found on the carcasses. Statistical analysis concluded that the hatchling's death during the headstart program was likely to occur by chance (not influenced by injuries or infection and the size of the character intensity). In contrast to that, for this study, 90 % of carcass was found to have bite marks, 45 % were found with shrinking plastron, 30 % were bloated, 16 % with blue or red marks and 9 % for bacterial infection on eye. Glazebrook & Campbell (1990) had similar findings in which ulcerative skin lesion due to biting were found to severely affect 88 % of hatchlings while 8.6 % of bacterial infection on eye were found to affect farmed turtles.

5.4.2 Non-infectious Disease caused by Trauma

According to Collin (2004), the non-infectious disease is defined as a disease that is not caused by microorganisms such as bacteria, viruses or fungi while disease was defined as a condition that stops the body from functioning in the usual way. Based on Mader (2006), the non-infectious diseases are classified into the causative factor namely traumatic effect, metabolic disturbance and congenital disease. For this study, the non-infectious are

classified into caused by traumatic effect, genetic inheritance and metabolic disturbance (Figure 5.4).

5.4.2.1 Traumatic Ulcerative Dermatitis

Ulcerative skin lesion caused by aggressive biting occurs in 80 % of the overall hatchlings reared in this project. Approximately 90 % of the affected hatchlings were categorized as severely affected. The hatchlings attacked each other regularly at soft areas such as the tips and trailing edges of the flippers, tails and necks. Ulcerative skin lesion (Figure 5.7) was caused by biting (Leong *et al.*, 1989). He stated that there is no evidence that aggressive biting contributed directly to the death of a hatchling but the inflicted wound could provide an opening for pathogenic microorganisms. Biting marks were observed on 90 % of the carcasses and this most likely was the major factor that caused the spread of bacteria or virus infection among the hatchlings. During the headstart project, to overcome the problem, the aggressive hatchlings were first isolated for 15 minutes and were carefully observed for a period of time. As soon as calm behaviour was displayed, they were then allowed to mix with the other hatchlings again. However, in many occasions, the isolation technique was not effective enough in controlling the aggressive behaviours. Therefore, slices of floating cucumber were introduced as a distraction technique to lessen the aggressive behaviour of turtle hatchlings. This floating cucumber slices had managed to control the problem.



Figure 5.7: Ulcerative dermatitis on the neck of a juvenile

5.4.2.2 Blue or Red Marks

The blue or red marks (Figure 5.8) that were observed on the carcasses as stated by Zulkifli (2015) were likely to be caused by (i) mishandling of the hatchlings by visitors to the facility or (ii) accidents as the young hatchlings crashes onto rocks or the sides of the tanks during episodes of aggressive biting among the hatchlings. Overall, 33 % carcasses showed blue or red marks on its plastron. For problem (i), the rearing facility had been improved by stepping up the safety measures around the rearing tanks by construction of higher and stronger fence. For problem (ii), the aggressive biting had been controlled using floating cucumbers.



Figure 5.8: Red or blue marks on plastron

5.4.3 Non-infectious disease caused by genetic inheritance

Conditions that are caused by genetic inheritance are usually observed at birth and these conditions are usually inherited or passed on from parents (Turton, 2004). Collin (2004) had defined congenital as existing at or before birth.

5.4.3.1 Congenital blindness and curved beak

One hatchling was found to have cross beak and congenital blindness (Figure 5.9). Both defects are congenital abnormality that causes the hatchling faced difficulty in feeding and locating food. The hatchling however did survive for 19 days, perhaps due to isolation in a smaller holding tank with a healthy hatchling as its guide.



Figure 5.9: Hatchling with congenital cross beak (arrow) and blindness (in white circle)

5.4.4 Non-infectious disease caused by metabolic disturbance

Metabolic disturbance is disruption in the chemical processes which are continually taking place in the body such as processes that convert food into energy (Collin, 2004).

According to Turton (2004), metabolic diseases are an upset in the normal functioning of the animal.

5.4.4.1 Eye Lesions

The observed eye lesion that occurred during the project was usually yellow deposits growing on the cornea adjacent to the eyelids. These lesions could have occurred as a result of nutritional deficiency or external trauma. Glazebrook & Campbell (1990) stated that the condition where the yellow deposit grows on the cornea indicates keratoconjunctivitis while the condition where the upper eyelid had been completely eroded was called ulcerative blepharitis. Eye infection (Figure 5.10) on the hatchlings was observed to be present during the first 4 months of the rearing and was most probably caused by combination of environmental factors and nutritional deficiency. Elkan & Zwart (1967) had mentioned that vitamin A deficiency could cause metaplastic changes in aquatic turtles. In order to overcome this problem, diet of the hatchlings was changed to fishes rich in vitamin A, like sardines. In addition, the water in the rearing tanks was changed every two days or whenever deemed necessary, following Kowarsky (1977).



Figure 5.10: Yellow eye deposit on hatchlings (arrow)

5.4.4.2 Soft-shell Disease

Carapace deformation or soft-shell disease (Figure 5.11) usually occurs after the hatchling or juvenile has recovered from certain kinds of illness such as emaciation syndrome (Leong *et al.*, 1989). This occurrence made the shell of the hatchlings relatively soft (it could be bent, manually) thus the hatchlings were retarded. Leong *et al.* (1989) also stated that this condition could possibly be the result of impaired calcium metabolism, as an outcome of the self-starvation problem. In this study, only 3 % of the hatchlings had soft shells. To overcome this problem, dietary requirements had been improvised by adding turtle pellet food as extra nutrition source.



Figure 5.11: Curvy and pliable shell of a juvenile turtle, an example of soft-shell disease

5.4.4.3 Emaciation

The self-starvation condition observed was actually emaciation which causes the shrinking of the hatchling's plastron (Figure 5.12). According to Leong *et al.* (1989), the symptoms of hatchlings experiencing emaciation include: (i) gradually losing weight, (ii) sunken plastron, (iii) wrinkled and lean-looking neck, and (iv) common advanced cases of anorexia. They also reported that the growth of hatchlings after recovery from emaciation is often stunted, therefore the animal retained a dwarfed physique and an unhealthy

appearance. Approximately 2 % of recovering cases were observed in this study, but they maintained soft and pliable shells for at least 2 months.

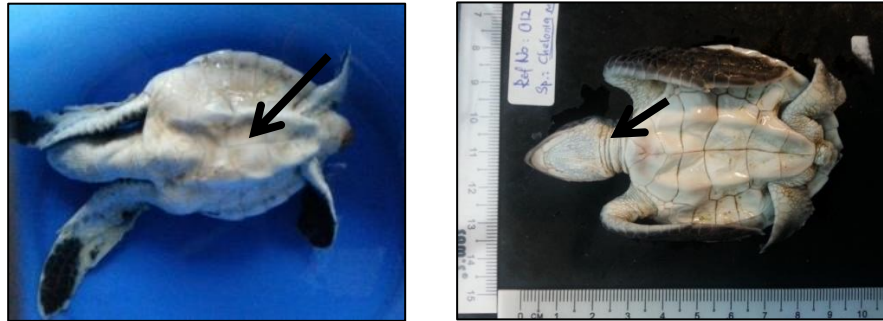


Figure 5.12: Hatchlings with wrinkled and lean looking neck

5.4.4.4 Obstruction of the Gastrointestinal Tract

This situation happened due to incorrect ingestion of a foreign material or incorrect diet. Some of the foreign materials that were found in the stomachs and intestines of the hatchlings were rocks and small pieces of plastics. The hatchlings with this disease were usually lethargic and anorectic for several days to weeks before dying, similar to reports by Glazebrook & Campbell (1990) who claimed that in extreme cases, the hatchlings could be floating motionlessly while they were continually attacked by other turtles which eventually could be a factor contributing to the death of the hatchlings.

Blocked cloaca due to faecal impaction (Figure 5.13a) happened due to obstruction of the gastrointestinal tract (Figure 5.13b) or due to the presence of foreign body or incorrect diet (Glazebrook & Campbell, 1990). During this study, the offending materials were removed using a pair of forceps to stop the swelling of the cloaca. Hatchling and juveniles have a tendency to eat anything due to its opportunistic feeding behaviour thus causing these problems to arise. The hatchlings that experienced this problem were

isolated, as they were lethargic and exposed to attacks by other hatchlings. The hatchlings that suffered this disease had bloated plastrons, perhaps due to faecal impaction.

Meanwhile, the jutted anus or intestinal prolapse disease (Figure 5.13c) was usually observed after the constipation problem was settled. Leong *et al.* (1989) had described the disease as protruding lower part of the intestine from the anus. Observation during this study showed that the disease did not pose any serious problem as long as isolation was carried out on the hatchlings and water in the tank was replaced on a daily basis.

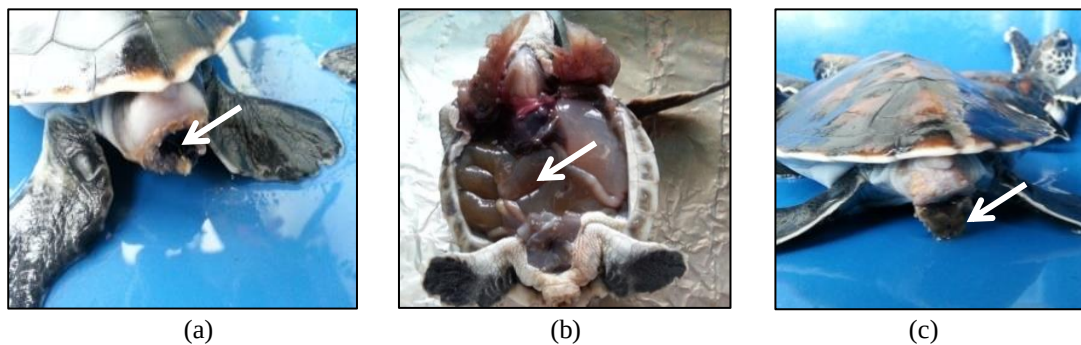


Figure 5.13: (a) Faecal impaction, (b) obstruction of the gastrointestinal tract (c) intestinal prolapse

5.4.5 Infectious diseases caused by bacteria and/or fungi infection

Infectious diseases are diseases caused by microorganisms such as bacteria, viruses or fungi that can be transmitted to others by direct means (Collin, 2004). Herbst & Jacobson (2003) mentioned that infectious disease was traditionally understood as a secondary process following a primary environmental stress like resource depletion. The causative agents of infectious disease that are found during the necropsy procedure were tested in this study and are discussed in the following sub chapter and simplified as in Figure 5.4.

5.4.5.1 Liver Disease

Figure 5.14 shows the presence of round pale coloured focal lesion on the liver of necropsied hatchlings. Approximately 30 % of the hatchlings necropsied possessed this lesion. Further observation of the lesions with aid of stereo microscope showed that the lesions were parasite-free. George (1997) reported that mycotic granulomas caused by systemic mycotic infection can be found in the liver and throughout the coelomic cavity. This disease was most likely caused by fungi after an episode of bacterial infection that has weakened the immune system of the hatchlings or could have also been caused by septicaemia. In addition, Glazebrook *et al.* (1993) reported that swollen hepatocyte in centro-lobular area of the liver was an indication of a septicaemic-toxaemic condition in a turtle. The possible agents causing the liver disease is fungi from various taxa, as shown in Table 5.4.

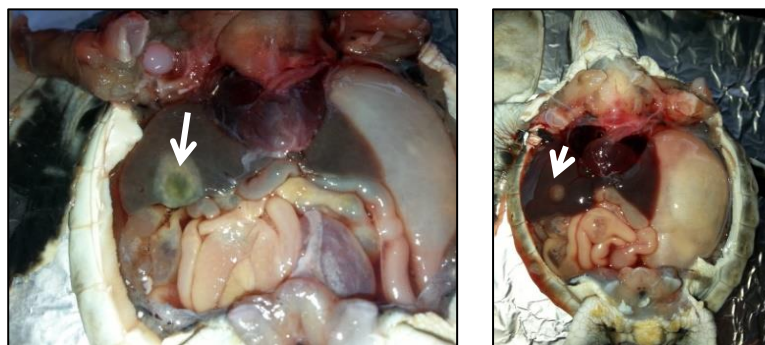


Figure 5.14: Spot on liver discovered during necropsy

5.4.5.2 Sudden Hatchling Death Syndrome

This syndrome was observed on 47.5 % of the hatchlings when they were less than 2 months old (batch 1). The episode started with one or two hatchlings that were found to be lethargic, bloated and floating passively on the water surface with their front flippers

hanging loosely downwards. These lethargic animals usually died overnight. The number of hatchlings infected and dying then started to increase by day, until they were 3 months old. A total of 57 turtles were lost in one month period. Similarly, Glazebrook *et al.* (1993) mentioned that the epizootic cases observed on most islands of their studies, began with a small number of individuals, increased to a peak at 5 to 6 week and by week 12, it had virtually disappeared. In addition, Haines *et al.* (1974) claimed that a condition called ‘early hatchling mortality’ related to green turtle hatchlings was thought to be an early manifestation of the grey-patch disease which was later attributed to a herpes virus infection. However, there was no observed sign of grey-patch disease on the hatchlings during this study. Necropsy findings of the hatchlings showed similar results reported by Leong *et al.* (1989) where they revealed feed-packed stomachs and faecal impaction in the lower bowels (Figure 5.15). It is possible that the disease occurred due to immunosuppression associated with stress of captivity which could later led to bacterial infections (Alfaro *et al.*, 2006). The disease discontinued after water in the holding tanks was changed twice daily (instead of once) and an antibacterial solution was administered in small quantity (200 ppm every 5 litre of water) to control the pathogens.

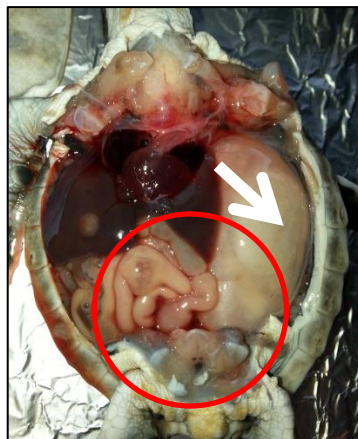


Figure 5.15: Feed packed stomach and fecal impaction discovered during necropsy

5.4.5.3 Ulcerative Stomatitis

According to Glazebrook & Campbell (1990), ulcerative stomatitis was the second most important bacterial disease after traumatic ulcerative dermatitis among farmed turtles. During this study, the disease was identified by the appearance of yellow, caseous material found in the oral cavity (usually on the dorsum of the tongue) of the hatchlings (Figure 5.16), aged between 4 to 9 weeks old. The accumulation of caseous material in the oral cavity left a distinct depression upon the oral mucose when subjected to debridement, leading to chronic weight loss. These features were common to turtles and tortoises with ulcerative stomatitis (Glazebrook *et al.*, 1993). Other than that, it was also noted that the lesions on the roof of the oral cavity were generally smaller and that the oral mucosa was noticeably inflamed in the immediate vicinity of the lesion. Glazebrook *et al.* (1993) also mentioned that mouth ulcers in green turtles were termed sub-acute because of the caseo-purulent material present and the zones of inflammation which surrounded them. To overcome the disease from spreading, hatchlings that were found to be infected were isolated in a separate holding tank, with approximately 200 ml (400 ppm) of TMSTM (contains alkyl dimethyl benzyl ammonium chloride 40 %) added into every 5 litre of water of each holding tank.

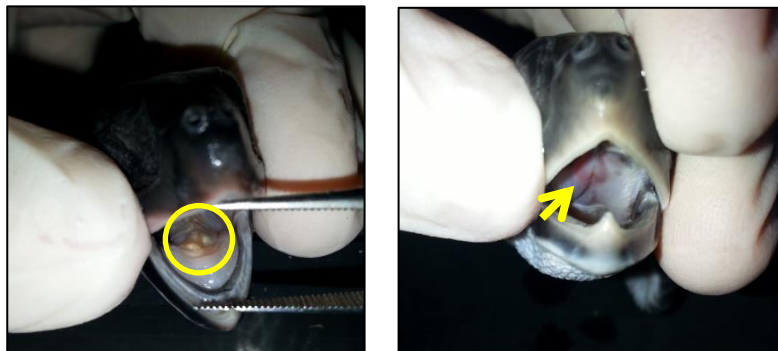


Figure 5.16: Yellow deposit on the tongue and ulcer found on the upper oral cavity of hatchling

5.4.5.4 Obstructive Rhinitis

Turtles that have this disease usually have a blocked nasal passage, causing difficulty in breathing through its nose (Glazebrook & Campbell, 1990). Yellow or white caseous material was found lodged in either one or both nasal passage thereby inhibiting normal respiration. Glazebrook & Campbell (1990) stated that this syndrome happened during the active expulsion of water by the hyoid apparatus on surfacing. The turtles that contracted this disease usually displayed open mouth breathing (Figure 5.17). According to Glazebrook *et al.* (1993), 70 % of cases were diagnosed during hatchling stage which was undoubtedly caused by the small diameter of their nasal passage. During this study, the hatchlings that were infected with obstructive rhinitis did not survive, despite being isolated and treated with TMS™ solution.



Figure 5.17: Juvenile displaying open mouth breathing

5.4.5.5 Bronchopneumonia

Turtles that are infected with this disease usually displayed a definite list (up to 40°) to one side when they are floating in water and laboured respiration (Figure 5.18). Glazebrook & Campbell (1990) reported that the disease affects turtles that are between 2

to 8 months old as a complication of ulcerative stomatitis and this usually happens to farmed turtles with the exception of a single case from Magnetic Island, Townsville, Australia. The severely affected hatchlings were also observed to be floating motionlessly on the surface of water, with occasional bursts of activity. Lung infection or bronchopneumonia was observed to have infected less than 5 % of the hatchlings during this study, in which only a small number of infected hatchlings had recovered or survived. Hatchlings that were able to overcome the disease were still tilted after 4 weeks of recovery. No special treatment was administered for this disease; the infected individuals were only singly isolated and not fed until the hatchlings showed desire to eat. The water of their holding tank has to be at only approximately 5 cm deep and changed at least 3 times daily.



Figure 5.18: Hatchling displaying slanted position due to bronchopneumonia

5.4.6 Differences in Diseases Acquired by Batches

The occurrence and type of disease was found to be more profound in Batch 1 as the management strategy was still not optimised to suit the Sarawak environment. Differences in handling techniques have definitely affected the survival and the disease occurrence rate between batches. The level of morbidity and mortality in Batch 2 was lower when

compared to Batch 1. The health level was apparently better in Batch 2 as reflected in its 82 % survival rate. However, the type of bacteria that were found in carcasses of Batch 2 that acquired infectious diseases was still the same as Batch 1 namely *Proteus mirabilis*, *Pseudomonas* sp., *Klebsiella pneumonia* and *Citrobacter freundii*.

5.4.7 Identification of Disease causing Pathogens

Various hemolytic and non-hemolytic, gram positive and gram negative bacteria were isolated from every lesion collected during this study. However, not all collected samples were successfully cultured. This situation is unavoidable because fastidious bacterial microorganisms may require special culture techniques for isolation. Moreover, there is limitation in terms of chemicals and facilities during this study.

A wide range of bacteria (Table 5.3), including *Acinetobacter* sp., *Citrobacter* sp., *Pseudomonas* sp. *Streptococcus* sp. *Shewanella* sp., *Bacillus* sp., *Morganella morganii*, *Paracoccus* sp. and *Vibrio* sp. were consistently isolated from all groups. These potentially pathogenic bacteria could act as opportunistic invaders of tissues damaged by trauma, and had been associated in farmed sea turtles with ulcerative shell disease, traumatic ulcerative dermatitis, ulcerative stomatitis, obstructive rhinitis, keratoconjunctivitis, septicemia, bronchopneumonia and osteomyelitis (Lauckner, 1985; Glazebrook & Campbell, 1990; Oros *et al.*, 2005). Oros *et al.* (2005) have considered pulmonary lesions as an important cause of mortality among stranded sea turtles in Canary Islands as the same bacteria were isolated by Glazebrook & Campbell (1990) from bronchopneumonia lesions. Similar bacteria such as *Bacillus* spp., *Citrobacter* spp.,

Proteus spp., *Pseudomonas* spp., *Streptococcus* spp. and *Vibrio alginolyticus* were present in this study.

The source of bacterial infection in this study was suspected to be from dermal, cloacal or oral routes. Jacobson (1992) stated that bacteraemia in fresh water turtles often originate from skin infections which in this case could have started from the aggressive biting behaviour of hatchlings and heavy exposure to faecal coliforms (Balazs *et al.*, 1993).

Cooper & Jackson (1981) have stated that *Citrobacter freundii* causes septicaemic cutaneous ulcerative disease in other turtle species, and was isolated from 50 % of the sea turtles sampled in this study. Leger (2001) has also mentioned that shell and skin disease as a common sequelae to trauma or injury which could become infected with bacteria such as *Vibrio alginolyticus*, *Pseudomonas* sp. and *Citrobacter* sp. It was also stated that these infections are often long standing and can progress to osteomyelitis and fatal septicaemia. *Vibrio* spp. have also been documented to cause endocarditis in a free-ranging leatherback turtle (Obendorf *et al.*, 1987), skin disease in captive loggerhead turtles (Wiles & Rand, 1987) and rhinitis, pneumonia, stomatitis and septicaemia in captive green turtles (Glazebrook *et al.*, 1993). However, Aguirre *et al.* (1994) have pointed out that *Vibrio* spp. are also common commensals in cloacal flora of green turtles with and without fibropapillomatosis from Hawaii. Saprophytic bacteria including *Bacillus* sp., *Micrococcus* sp. and *Proteus* sp. are considered part of the normal skin flora of sea turtles (Glazebrook & Campbell, 1990).

However, there are possibilities that the organisms cultured could have been natural microbiota of the sea water and not related to the infection. In this study, water used in holding tanks was pumped on daily basis from nearby sea without treatment. Thus, the

presence of bacteria from natural environment is unavoidable as George (1997) stated that the aspiration of sea water as one of the primary modes of bacterial entry into the respiratory system thus causing clinical disease.

Fungi obtained from necropsy procedures had been successfully cultured (Table 5.4). Fungi such as *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp., and *Gibberella moniliformis* were found from the liver of hatchlings with a focal lesion (Table 5.4). Similarly, Jacobson *et al.* (1986) had reported the isolation of a *Cladosporium* sp. isolated from the liver of a turtle suffering from lung, eye and trachea disease (LETD). Several fungi namely *Paecilomyces* sp., *Fusarium scirpi* and *Penicillium* sp. were isolated from pneumonic lesions of farmed green turtles by Glazebrook and Campbell (1990) while *Sporotrichium* sp., *Cladosporium* sp. and *Paecilomyces* sp. were isolated by Jacobson *et al.* (1979) from the same lesion but from captive reared green turtles.

According to George (1997), mycotic infections of wild and captive turtles primarily occur in the lung but mycotic granulomas could also be found in the liver and the coelemic cavity. *Paecilomyces* sp., *Sporotrichium* sp., *Cladosporium* sp. and *Scolecobasidium* sp. have been isolated from infected turtles and were incriminated as agents that are responsible for a variety of systemic mycotic infections (Jacobson *et al.*, 1979; Leong *et al.*, 1989; Glazebrook & Campbell, 1990). *Aspergillus* sp. has been found in lesions on flippers of loggerhead hatchlings (George, 1997) and was mentioned to have been caused by water quality.

In this study, only eight fungi taxa had been identified and this finding may underestimate the number of fungi species causing or associated with lesion as technical problems such as lack of chemical and instruments were faced during laboratory works.

No parasitic infection was detected in this study. Leger (2001) had mentioned that turtles from pelagic stage have little to no infection, suggesting these parasites are acquired once turtles recruit to near shore habitat. In this study, no parasite was found on carcasses suggesting that the water and food used to rear the hatchlings were clean and parasite-free.

Due to unavailability of virus detection facility, determination of virus associated with lesion could not be carried out during this project. It is possible that viral infections played roles in the mass death occurrence of hatchlings.

5.5 Conclusion

The diseases that were found in this study were revealed to be either caused by bacteria or fungi or caused by traumatic effect, genetic inheritance and metabolic disturbances. Bacteria or fungi that are found in the carcass of turtles or other possible cause of death such as ingestion of foreign materials have been properly determined. More often than not, mortality could have been caused by a combination of reasons that may have to be checked through other methods like haematology test so that we could discern the actual cause of mortality. Batch 1 suffered more diseases compared to Batch 2 suggesting better water management and handling technique to reduce the risk of disease among turtles reared in captivity.

CHAPTER 6

COMMUNITY-BASED GREEN TURTLE CONSERVATION AND THE DEVELOPMENT OF EDUCATIONAL TOOLS

6.1 Introduction

Sea turtles co-exist with humans, thus their influences on culture have been reflected in arts, crafts and music (Campbell, 2003). *Via* cultures, priorities could be set for conservation and development; for example in wildlife conservation, culture is used to explain particular sets of human-animal relationships. In the culture context, originally, subsistence economies that relies on extracting the basis of existence from the natural environment focuses on existing food needs rather than accumulating surplus (Nietschmann, 1973). However, such regimes have been devalued and destroyed by the existence of market economy (Escobar, 1992). However, view by Escobar (1992) had been redefined by Robert (1992) to individual producer's socio-biological survival under conditions of accumulation of capital. Therefore, the depletion of sea turtles (and other species) has often been blamed on the change from subsistence to market economy, further highlighting the importance of environmental education and awareness programs to the local communities and the public that shares the same culture and values to the environment.

Environmental education is an important tool for the conservation of species, since it enables the expansion of knowledge about animal ecology and promotes favorable attitudes that protect the environment and the conservation of natural resources (Reigada & Tozoni-Reis, 2004). The application of environmental education among children was

claimed to be effective in raising the awareness on environmental issues, since this is the key period for learning (Basile, 2000).

One of the exemplary conservation programs had been conducted by the late Dr. Archie Carr and his supporters namely the Caribbean Conservation Corporation (CCC) in 1959, where it is the world's oldest sea turtle research and protection organization renowned for its long term conservation project at Tortuguero, Costa Rica (Godfrey & Harrison, 2009). The sharp increase of green turtle nesting at Tortuguero in 1970 compared to the 1950s, had suggested the effectiveness of the conservation program.

According to Godfrey & Harrison (2009), CCC has helped built ethics of sea turtle conservation by building strong relationship with the Government of Costa Rica and the community of Tortuguero. CCC had developed a complex economic mechanism to ensure that the local people are able to participate and benefit from turtle tourism. In addition to that, legal strategies for safeguarding of the turtles and its nesting habitat from exploitation are practised in the area, Tortuguero National Park was established, regional turtle conservation agreement was made among neighbouring countries, a ban on turtle and egg harvest was implemented, and a development regulatory plan to control coastal construction and limit human encroachment along Tortuguero coastal strand was constructed. CCC has become the culture preserver, social issue resolver and a stable employer in Tortuguero whose presence has helped built the turtle conservation program (Godfrey & Harrison, 2009).

Ecotourism is an alternative form of tourism which has been defined as responsible travel to natural areas that conserves the environment and sustains the well-being of the local people (Page & Dowling, 2002). The concept of ecotourism basically means that the tourists provide income to the local residents to protect the resources they wish to view.

There have been good turtle ecotourism for example turtle walk which was conducted in the U.S. and served as an educational tool designed to gain public support for protecting nesting beaches (Johnson *et al.*, 1996). Other than that, Peskin (2002) mentioned that in Tortuguero, Costa Rica, the income earned by ecotourism can replace the money that was earned formerly *via* green turtle fishery. In Australia, ecotourism has been promoted to provide economic rationale for conserving species (Wilson & Tisdell, 2001). Guided turtle walks at Playa Grande, Costa Rica have generated income and awareness needed to justify for maintenance of nesting beach in an area of dense tourism activities (Campbell, 2002).

The existence of sea turtle in Sarawak has not been known to the locals when compared to other Sarawak's flagship animal such as orang utan, proboscis monkey and hornbills. Lack of awareness among the public have hindered successful sea turtle conservation program. Therefore, the aim for this study is to instil a sense of responsibility among the local community of Lundu and Sarawak, emphasizing on conservation of green turtle population that nests in Talang- Satang National Park, Sarawak.

This chapter describes the awareness programs that had been carried out in Lundu, Sarawak. Upon completion of the program, an educational toolkit specifically made for turtle conservation had been successfully developed and later evaluated during UNIMAS Innovation and Technology Exhibition 2016 (InTEX2016).

6.2 Materials and Methods

6.2.1 Development of Educational Toolkit

Adobe Premiere CS6 for Windows was used to develop the video while the information kiosk (IK) was developed using Adobe InDesign CS6 for Windows. Both softwares are

licensed to UNIMAS, under the care of Faculty of Applied and Creative Arts. The IK was implemented on a kiosk platform.

6.2.2 Turtle Release and Local Community Involvement

Table 6.1 shows the turtle release date, time, session and participants. The tagging was done on the turtles a day before they were released back to the sea. The time of release of the turtles were chosen based on the ebbing tide schedule. Turtles reared for 18 months were released during two occasions: (i) 3rd December 2015 and (ii) 4th May 2016. For the first activity, turtles were released in stages namely 10 individuals at approximately 10.30 am and 29 individuals at 9.00 pm. Morning activity is a part of post-conference activity for participants of the Regional Taxonomy and Ecology Conference 2015 (TEC 2015) while the night turtle releasing activity focused on the participation of local people. For 4th May 2016 activity, releasing ceremony received a better coverage by the media inclusive of TV program. This activity was conducted by Sarawak Museum as collaborator of this project. A total of 26 turtles were released by VIPs during 4th May 2016 releasing session.

Table 6.1: Details of turtle releasing ceremony and participants of the releasing ceremony

Session	Organizer	Date	Time	Number of turtles	Participants	Tagging date
1	UNIMAS	3/12/2015	1030	10	UNIMAS researchers, TEC participants, local people	2/12/2015 (By SFC)
			2100	29	Local people, UNIMAS researchers	
2	Sarawak Museum & Turtle Board	4/5/2016	1030	26	UNIMAS researchers, VIPs of Sarawak state, Sarawak Museum and Turtle Board staff, local people.	3/5/2016 (By SFC)

6.2.3 Turtle Awareness Campaign and Survey

The awareness campaigns were conducted after receiving endless requests from schools and various organisations. As it was the first local headstart project, it is unavoidable to attract public interest, thus the initiative to educate the locals about sea turtles and the dangers that they are facing begun. Officially, the awareness campaign was started on October 2014 and ends in November 2015. A total of 666 participants had joined in the awareness program (Table 6.2). Out of 666 awareness program participant, 259 of them had filled out a survey (Appendix 3) about their experience with sea turtles and their feedback on the awareness program carried out during this study.

Table 6.2: List of participants (in groups) involved in turtle awareness program

No.	Participating Group	Date	Time	Number of Participant
1	SJK Chung Hua No. 4, Kuching	4/10/2014	9.30 am	100
2	SJK (C) Chung Hua, Meludam	1/11/2014	4.00 pm	36
3	SMK St. Thomas, Kuching	7/12/2014	9.30 am	36
4	SMK Bandar Kuching No.1	14/3/2015	10.00 am	120
5	Persatuan Wanita Universiti Malaysia Sarawak (Group 1)	10/6/2015	10.00 am	30
6	Persatuan Wanita Universiti Malaysia Sarawak (Group 2)	11/6/2015	10.00 am	30
7	The Christian Church of God, Kuching	18/7/2015	4.30 pm	49
8	SMK Sungai Tapang	26/7/2015	9.00 am	40
9	SM Sains Kuching (Science Club)	12/9/2015	9.00 am	25
10	WS49-Aquatic Resource Science and Management Program , UNIMAS (2 nd year students)	11/9/2015	10.00 am	50
11	WS49-Aquatic Resource Science and Management Program, UNIMAS (2 nd year students)	11/9/2015	2.30 pm	50
12	WS49-Aquatic Resource Science and Management Program, UNIMAS (1 st year students)	31/10/2015	10.00 am	50
13	WS49-Aquatic Resource Science and Management Program, UNIMAS (1 st year students)	31/10/2015	2.00 pm	50

6.3 Results

6.3.1 Development of Educational Toolkit

In this study, an educational toolkit comprising three products related to green turtle conservation campaign had been successfully developed. As an evaluation process, the toolkit had been displayed during UNIMAS Innovation and Technology Exhibition 2016 (InTEX2016) on 23rd to 24th May 2016. The educational toolkit comprises of the following:

1. Manual on interaction between human and sea turtles. The 16 pages manual described step by step instructions for:
 - (i) Standard Operating Procedure (SOP) for human-turtle interaction, both in hatchery and at the beach (where turtles nests) (Chapter 4, Section 4.3.1).
 - (ii) Husbandry practice including egg and hatchling management techniques, water quality management, feeding process and daily care of hatchlings, and proper handling of sea turtle hatchlings (only applicable for facilities that are permitted to conduct headstart program) (Chapter 4, Section 4.3.1.2)
 - (iii) A standard survey, suitable for age 7 to 50 years old (and above). The questionnaire is bilingual (English and *Bahasa Melayu*), to check the effectiveness of the turtle awareness campaign (Appendix 3).
 - (iv) Sea turtle species recognition for the public where the basic identification key for 7 species of sea turtle is described along with some description of sea turtle's body parts (Appendix 2)

2. Video

The video summarizes the turtle conservation activity from June 2014 to November 2015 in the duration of 2 minutes. Footage includes a brief insight of the turtle awareness campaign's power point and video presentation, sea turtle feeding and nursing session (practical session), the turtle tagging process and the turtle releasing ceremony.

Brief outlook of the video as following:



Participants arrived at Pandan GoldCoast Holiday Villa to join the awareness program.



Hatchling coming of the nest inclusive of sea turtle hatchlings piping out of the nest.



An example of program conducted: power point presentation by the main researcher. This particular group involved the children of Persatuan Wanita Universiti Malaysia Sarawak (MASNITA).



Active participation: Participants eager to answer questions about sea turtles asked by the main researcher.



Turtle handling demonstration being carried out by main researcher before the nursing and feeding session.



Hands on experience: Kids participating in feeding session.



Observation of sea turtle's habit: turtle eating a slice of cucumber.



Hands on experience: sea turtle nursing session by future sea custodians.



Hands on experience: Using the right technique to return the turtle back into holding tank.



A snapshot of the victory moment. Turtles to be released were being carried in a basin (after tagging), ready to be released to the sea.



Sarawak Forestry Corporation (SFC) staff in action: Measuring, weighing and documenting (before tagging).



Sarawak Forestry Corporation (SFC) staff tagging sea turtles that are to be released.



Sea turtles swimming in the sea right after releasing program.



Words from main supervisor for the future generation.

3. Interactive information kiosk (IK)

This item is a joint venture project with researchers from Faculty of Applied and Creative Arts (FACA) of Universiti Malaysia Sarawak (UNIMAS) namely Miss Aslina Mohd Jainal, Mr. Anuar Ayob, AP Dr. Zulkalnain Zainal Abidin, Mr. Hasrunnaim Hassan and Mr. Mohammad Suffian Abdul Kadir.

The information kiosk comprises of touch screen facility that is mainly operated with the help of a control unit and a touch screen sensitive LCD monitor. It is usable in a touch screen gadget thus making this tool easily handled by small children. This e-

book styled kiosk is a form of interactive learning. This kiosk offers information on green sea turtle biology where relevant pictures and videos are included so that the learning experience will be fun. The contents of the kiosk are: green sea turtle physical characteristics and other facts, life cycle (Figure 6.1), habits of green sea turtle in terms of locomotion, feeding and resting position, threats faced by sea turtles, the nesting site of green sea turtles, its conservation status and conservation efforts that are being carried out in Sarawak.

The aim of this information kiosk is to provide the public useful information to help them understand the ecology and biology of green turtles. Information kiosk has advantage as a valuable component of today's information age. It is a new type of technology which is more appealing to the new generation thus enabling knowledge dissemination in a more effective way. The information kiosk was designed to be user friendly therefore it is a compatible tool even for a person who does not have any computer use experience. Information kiosk is a new innovation for learning, preferable by younger generation.

Brief outlook of the information kiosk (IK) as following:

The front page and all other pages in the information kiosk have click-able buttons that is indicated by the arrow in Figure 6.1. All subsequent round buttons on the right and left lower corner of the pages are buttons which could either lead to a new page or return to the previous page while the orange and green buttons seen on all pages of the kiosk are click able buttons which could lead to a specific page.

Meanwhile, on the third page, the small round orange buttons at the right upper corner of the page could lead to different pictures of turtles that were captured for introduction of the information kiosk. Near the orange buttons are instructions notifying users to click button to view pictures. In the life cycle page in Figure 6.1, videos were also made available to users of the information kiosk. An instruction to click coloured pictures of sea turtles to view images and videos of sea turtle's life cycle is indicated on the page.

The final pages of the information kiosk shows the link to a webpage of Sarawak's other turtle conservation program for example the 'Adopt-a-Turtle' program conducted by Sarawak Forestry Corporation so that it will be easier for future sea custodians to participate in other awareness or voluntary programs to save the sea turtles. The last page shows the symbol of collaborators that enabled the project to happen while the last page contains the list of UNIMAS researchers that developed the information kiosk and video.

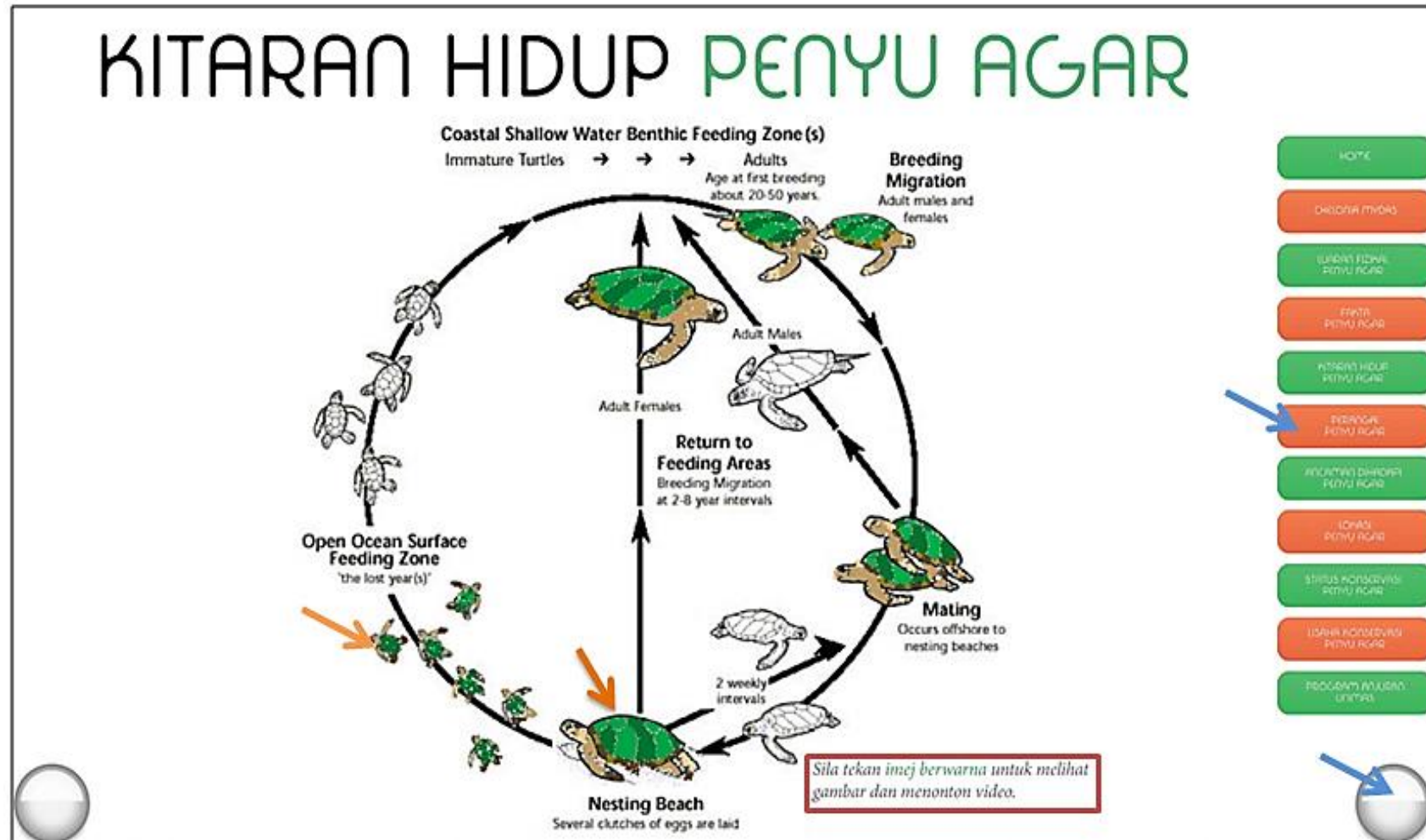


Figure 6.1: The life cycle page of information kiosk consists of buttons that could lead to pictures, videos and to other contents of the information kiosk. The blue arrows point to click-able buttons that could lead users to other pages or to a specific page while the orange arrows point to coloured images that could lead users of information kiosk to pictures and videos of sea turtles. The instructions in the red box will help users use the information kiosk.

6.3.2 Turtle Release and Local Community Involvement

The project was graced by the media and was reported in a few newspaper and online articles (Utusan Borneo, The Sunday Post and Sin Chew Daily) (Appendix 4) especially during the releasing ceremony of the turtles. During the releasing ceremony, the locals came during both day and night release to help release the turtles back to the sea.

After the releasing ceremony (post-release), the locals even made an effort to inform the researcher about the turtle's whereabouts around the area (especially when they are seen during post-release). Local people continuously made efforts to roam along the Pandan Beach, Lundu to check for any turtle stranded on beaches, up to the time when this thesis is being prepared. The summary of biometry of the released turtles is in Table 6.3. The complete information of released turtles with their tag number is in Appendix 5.

Table 6.3: Details of turtles released during this project

Session	Number of turtles	Minimum			Maximum		
		CCL (cm)	CCW (cm)	Weight (kg)	CCL (cm)	CCW (cm)	Weight (kg)
1	39	33.5	29.2	4.1	42	41	9.4
2	26	29	27	2.7	41	38	8.1

6.3.3 Turtle Awareness Campaign and Survey

In relation to the turtle awareness campaign, it is important to know the rate of effectiveness of the awareness campaign and to measure the acceptance of the community towards such programs for future conservation planning. Table 6.4 shows the result of the surveys conducted on different age groups and on civilians of variable scientific knowledge and background. In total, there were 259 participants for the survey.

Table 6.4: Result of the turtle awareness program [Level of Science knowledge= 1 (not so knowledgeable) - 5 (very knowledgeable)]
 Masnita= Persatuan Wanita Universiti Malaysia Sarawak; SMK= Sekolah Menengah Kebangsaan; SM= Sekolah Menengah; WS49= Aquatic Resource Science and Management Program

Institution/Club	Participant age group	Number of participant	Level of Science knowledge (1-5)	Interested in sea turtles before program	Was really listening to /understood the talk	Tasted turtle egg before	Still want to eat turtle egg after program	Will attend another conservation program (%)
Masnita	7-12	30	1	12	25	3	0	100
Masnita	7-12	1	1	1	1	0	0	1
	13-17	13	2	7	9	5	0	12
	>50	1	2	1	1	1	0	0
The Christian Church of God, Kuching	18-25	44	3	6	42	12	0	42
	>50	5	2	3	4	3	1	4
SMK Sungai Tapang (Biology Club)	13-17	40	3	7	37	8	0	39
SM Sains Kuching (Science Club)	13-17	25	3	7	24	4	0	25
WS49, UNIMAS (2 nd year students)	18-25	48	5	12	47	8	0	46
	26-50	2	5	2	2	0	0	2
WS49, UNIMAS (1 st year students)	18-25	48	4	11	47	9	0	47
	26-50	2	4	1	1	1	0	2

Based on Table 6.4, the age group of the participants had been divided into five age groups namely 7 to 12, 13 to 17, 18 to 25, 26 to 50 and above 50 years old. This is in accordance to the usual living status and education level in Malaysia where the 7 to 12 is in primary education, 13 to 17 is in secondary education, 18 to 25 in tertiary education or working, 26 to 50 is usually a fully mature adult phase (working life) while for the age group of over 50 is considered a veteran with a comfortable life. A group of people from different backgrounds were randomly chosen to balance the overall survey after the awareness program.

From the survey, it was found that 70 of the participants were readily interested in sea turtles even before the program. Efforts have been made by these sea custodians to learn about sea turtles in which 41.4% of them travelled to other states such as Sabah, Perak, Kedah and Terengganu while 24.3 % went to places like aquaria (*ex-situ* conservation spot) and the other 34.3 % turned to documentaries on television for learning.

A rank of scientific knowledge was given to the groups in which 1 was given to civilians with infancy level of science knowledge while 5 was given to individuals with science education at a higher tertiary level. There were apparent differences between the highest and lowest group in terms of knowledge gained from the program. The feedback obtained from the group that ranked 5 was more positive in terms of participation and knowledge while the groups that are lower than that requires more help to understand the facts presented to them.

Turtle eggs are local delicacies to Sarawak. Therefore, it is not surprising that individuals from all age groups in this survey have tasted them before the program with the majority (53.7 %) being in age group of 18 to 25. However, after the program, the willingness expressed by the participants to stop eating turtle eggs were commendable as

only 1 more person from the age group of above 50 out of the initial 54 people was unwilling to give up on turtle eggs. Finally, the survey shows that the support received from the 259 participants was highly positive as 96.5 % of the total participants were willing to attend another conservation program in the future with 97.7 % giving good feedback regarding the activities carried out during the awareness program.

6.4 Discussion

This subsection is divided into educational toolkit development, community and public involvement and turtle awareness campaign and survey section.

6.4.1 Educational Toolkit Development

It is not possible to continuously educate the public without public awareness program carried out in tandem, unless (i) the program is being recorded and shown to the public repeatedly or (ii) using another method such as information kiosk (or E-book), video and manual. According to Clark (1991), combination of printed materials, audio-visual presentations and face-to-face interaction is probably the best way to start an education program. Hudson (1988) has evaluated techniques that can be used to deliver conservation message and he reported that video has been a beneficial tool to environmental educator, as the equipment needed for video making is relatively cheap and easy to use. Moreover, video is a catchy tool that could attract the children's attention.

Mayer (2001) supported that video is one of the most effective ways to disseminate knowledge by mentioning that well-designed multimedia instructional messages (could be in interactive information kiosk with incorporated videos) could promote active cognitive

processing in students, even when learners seem to be behaviourally inactive. In addition, Dede (1987) stated that any visual media delivery system that is capable of supporting learner's interactivity while at the same time facilitating inter-connectivity of images and symbols has the potential to become an extremely powerful educational tool.

During InTEX2016, the judges had given constructive criticisms as follows: (i) Information Kiosk: The judges had suggested that the information kiosk should be made bilingual (in English and *Bahasa Melayu*) in order to reach a wider and global audience. (ii) Video: additional video which focuses on green sea turtle (biology, ecology) conservation should be made. This video should come in *Bahasa Melayu* with English subtitles.

Constructive comments were also received from the visitors of InTEX2016, as follows: Visitors to the booth on 23rd of May commented on the information kiosk technical problems, and suggested a few solutions, which were later improved by the team. The new version was displayed on the 24th of May. In addition to that, visitors had suggested that materials for the education kit (videos, information kiosk software) should be made into a form that is ready to be purchased during the expo. Other suggestion was to produce videos in cartoon version of green sea turtle similar to 'Finding Nemo' so that it could be more appealing to younger kids (pre-school kids).

In the near future, changes and upgrading will be carried out to offer a better learning experience on green sea turtle biology, ecology and conservation. There are plans to upgrade the information kiosk with addition of complete 3D (three dimensional) hologram to make it more surreal. Other plan is to make interactive sea turtle games to be incorporated into the information kiosk.

6.4.2 Turtle Release and Local Community Involvement

This project received enormous support from the local community of Lundu, as well as from the public. During the 18 months of the headstart program, there were frequent visits either by local people or visitors (tourists). On many occasions, civilians visit the facility to see the turtles and donated money to help with the turtle's food cost. Majority of people in Lundu are fishermen, who would drop by the facility just to observe the progress of the project or to donate fish and seagrasses found at sea for the turtles on a daily basis. Occasionally, when the fish stock for turtle feed became low, the fishes could be directly bought from these fishermen.

In addition, the local fishermen were seen to make extra efforts to locate sick or injured turtles around the area which was later sent to the facility before being released back to the sea when they were ready to do so. Sometime in October and December of 2014, the locals had brought 2 turtles with injuries to the headstart facility to be cared for before releasing them to the wild. Moreover, since the public awareness campaign started, the local fishermen constantly went to check on their nets to ensure that no turtles get entangled and drown. Unfortunately, the nets with Turtle Excluder Device (TED) has not been used in the Lundu area, therefore drowning of turtles caught in fisherman's nets are unavoidable. However, the turtle drowning incident seemed to be lessened with cooperation of the local fisherman.

The public awareness project soon became popular, attracting requests for schools and organisations to visit and learn about turtles at the headstart facility. Soon after, school and university students became the main participants of the public awareness program. These visits have led to beach cleaning activities which were parts of turtle conservation

effort around Lundu, Sarawak. The high number of participants (locals of Sarawak) is the evidence of the public's interest in sea turtle conservation program.

Other agencies that are involved and has contributed to the success of the headstart and awareness program are Pandan GoldCoast Holiday Villa as the financial supporter and provider of rearing facility, Sarawak Museum as advisor of the project, Forest Department Sarawak for granting the permit for the project, Sarawak Forestry Corporation for tagging turtles before release and advices, Universiti Malaysia Sarawak for 'Dana Pelajar PhD' and the Ministry of Higher Education for MyPhD scholarship.

This project is the first community-based green turtle conservation in Sarawak. It involved educational initiatives, involvement of local people as well as the public and government agencies. In terms of public involvement in conservation activities, community based conservation (CBC) for sea turtle had been carried out worldwide. In Ostional, Costa Rica, a community development association is responsible for all aspects of an egg collection conservation program where Campbell (1998) had described details of the project with addition of community willingness to undertake extra conservation effort.

Meanwhile, Nichols *et al.* (2000) had pointed out a CBC effort in Baja, California where community approach is needed in an isolated region with limited enforcement. His study had shown that local values are more often than not oversimplified by outsiders. Nichols *et al.* (2000) had reported that although research and conservation efforts were initiated by outsiders in Bahia Magdalena, an organization has been formed to promote sea turtle recovery in the region. Moreover, the fishermen in the organization were reported to exclude those who hunt for turtles from participating in research activities of sea turtle.

Another example of a famous CBC is 'Project TAMAR' which covers a large portion of the Brazilian coast. Detailed description of the project had been described by

Macrovaldi & Macrovaldi (1999) where it was mentioned that TAMAR's approach is usually for environmental education and provision of alternative economic activities. Lima (2001) had identified TAMAR's activities of assisting local communities in developing alternative economic activities including making artificial reefs, a community vegetable garden and embroidery and lace making in addition to their extensive environmental education activities at a meeting between TAMAR and a community association.

6.4.3 Turtle Awareness Campaign and Survey

The survey carried out during the public awareness program asked the participants about their exposure to sea turtles. 72.8 % of the participants mentioned that they have never seen a live sea turtle before. Approximately 30 % of them were not aware of the existence of sea turtles in Sarawak. When the public is not aware of the situation, it is hard to cultivate the sense of urgency to protect the animal. To date, Sarawak promotes wild animals such as proboscis monkey, orang-utan and hornbill as flagship animals. Although turtles nest at Talang-Satang National Park on yearly basis, the public are not aware of the situation, let alone the conservation programs that were being carried out on turtles of Sarawak.

As mentioned by Toronto Zoo (2012), the opportunity to view native and exotic animals in simulations of their natural habitats will catch the attention of the general public and set the platform for them to learn about the biology of the organism as well as the challenges that they are facing in their natural ecosystems. It is generally agreed that through educational initiatives, the public will become inspired to do what they can to preserve the nature thus highlighting the importance of the headstart facility as the platform for conservation education. According to Swingle *et al.* (1990), the primary

conservation role of zoological parks and aquarias has been one of public education in which it acts as centers for information transfer; they function as the main outlets for results of basic biological research to reach the public in an understandable form.

Apart from that, it is also not surprising that many of the conservation program participants mentioned Sabah as a place where they have seen turtles. According to Palaniappan (2001), the Sipadan Island (Sabah) supports a large density of green turtles, second only to the Turtle Islands Park. Joseph & Chong (2014) had mentioned that the juvenile green turtles in Sipadan Island consist of mixed stocks from Sabah Turtle Islands, Philippine Turtle Islands, Australia, Micronesia, Papua New Guinea and Indonesia. Sabah is a neighbour state to Sarawak, with good promotion on activities related to sea turtles, therefore the public are willing to travel to Sabah just to see the turtles. It is timely for Sarawak to step up promotion about the existence of turtles in the state and related activities on turtle conservation so that the locals could play a more active role in sea turtle conservation for the state of Sarawak.

Human play a major and invasive part to environment where human activities and behaviour, regardless of their feeding habits lead to issues such as climate change, biodiversity loss, pollution and others (Chan, 2004, 2006; Kotchen & Reiling, 2000). According to Chan (2006), a long history of extensive egg exploitation has been named as the major cause of population decline in Sarawak where continued egg harvest for many decades has led to failure in protection of sufficient numbers of marine turtle eggs required for population maintenance. Although regulations regarding the ban on ownership and selling of any parts or components related to sea turtles exists in Sarawak (Sarawak Wild Life Protection Ordinance 1998), the enforcement of the law is a continuously challenging task, perhaps due to the lack of manpower and the vast area of beaches of Sarawak.

From observation, traditional custom has led to a long history of turtle egg trading especially at the border of Sarawak (Serikin) and at Sematan (both areas are closest to Lundu). In the survey, 54 participants had confessed to have eaten or tasted turtle eggs before the program. After the program, when asked, only a single respondent from the above 50 age group would still want to consume turtle eggs. This is similar to the situation faced by Abd. Mutalib *et al.* (2013) that mentioned about reluctance of the older generation in Setiu, Terengganu in stopping turtle egg consumption when compared to the younger generation due to habit, norm and culture. Moreover, the older generation are aware of the declining numbers of sea turtles but had believed that since turtle eggs are produced in large numbers, consuming them would not have any effect on the sea turtle population (Abd, Mutalib *et al.*, 2013). This ‘belief’ would have to be rectified before they are passed down to the next generation.

Hence, the public education and awareness plays an important role in the issue of endangered species conservation. Awareness programs to promote behaviour change, which is of great importance for the success in long term conservation programs (Jacobson, 1987). Furthermore, UNEP (2007) stated that nearly 30 % of the global population comprises of young people who will be the decision makers of the future and their involvement has been internationally recognised as critical to sustainable development.

UNEP (2007) suggested that awareness campaigns are often the most successful when they are targeted at specific groups, because information can be tailored to the activities, needs and challenges of the group. During this project, two sets of talks (power point presentations) and sessions were prepared for the participants. The first set of presentation was prepared for children aged 7 to 12 years old while the second set of presentation was prepared for age 13 and above. Public education regarding sea turtle

issues may have wider spill-over effect as a successful conservation of sea turtle (in terms of habitat or others) may benefit other less charismatic species.

To strengthen public understanding on the issue of sea turtle egg consumption, there is a need to emphasize the dangers of eating turtle eggs in the future awareness programs. Joseph *et al.* (2014) stated that the level of lead detected in turtle eggs collected in Malaysia exceeded the permissible limits of the Malaysian Food Regulation 1985, whereby five heavy metals (Mn, Cu, Zn, Cd and Pb) were detected at high level in the eggs. Therefore, the public needs to be informed about the biomagnification and bioaccumulation concepts, where the environmental contaminants such as heavy metals could be dangerous to human (Joseph *et al.*, 2014). These dangers need to be made available to the public from a young age to discourage continuation of the sea turtle egg consumption based on habit or norm. It is believed that early education instilled through school systems could help curb this matter.

6.5 Conclusion

Eighteen months sea turtle headstarting project carried out in Lundu Sarawak had open up opportunity for small scale yet systematic awareness campaigns to educate the public on the importance of turtle conservation. Voluntary involvement of local people in this project promotes further understanding on the human-sea turtle relationships. This pioneer project had boosted the sea-custodian sprits among local people and the public, which is important for survival of this endangered species. A video, manual on human-turtle interaction and information kiosk had been produced as a tool for future environmental conservation and awareness program. Incorporation of awareness education in an early school education stage could be the key to conservation success. Future work should involve an in depth

documentation of sea turtle biology and ecology including life cycle, and sea turtle at its natural foraging and breeding habitat.

CHAPTER 7

GENERAL DISCUSSION

Conservation of green turtle in Sarawak has started since the 1950s with the establishment of a rookery and it was considered successful after the rapid decline in female nesters in 1949 which was reflected by the constant equilibrium of turtle landing starting in 1980s (Chapter 3). However, there is still a debate on the level of success of this program as the number of nesting females became unclear due to over harvest effect of eggs in Sarawak from 1927 to 1950s and due to the data not being readily available. The data from Chapter 3 will clearly show the declining and upward trend of Sarawak's green turtle population by describing all egg collected, incubated and hatched on Sarawak's Turtle Islands' (STIs) hatcheries from year 1980 to 2016.

Another problem that has been proposed by Jensen *et al.* (2016) was lack of male produced in Sarawak waters which exacerbated it by causing an increase in unfertilized eggs which was found in the hatcheries of Sarawak. This study (Chapter 3) however estimated the number of female nesters in Sarawak to be increasing based on data from 2010 to 2014 which is a good sign for Sarawak waters now, but may not end on a good note if the hatchery practice is not changed. The effect of the unfertilized egg now will only be reflected on the population size in 30 years' time. Therefore, for the good sake of future population, it would be an ideal move to make the change now. The remigration interval of nesting females was found to be every 3 to 4 years with internesting frequency of 3. The calculated data of remigration interval and internesting frequency should be compared to internal and more detailed data by Sarawak Forestry Corporation. If the data is not tally, it shows that there is lack of food or increasing threat to the mature nesters

which deters them from going back to their natal nesting ground in the estimated time. If so, a more suitable conservation management practice should be applied on the route from the turtle's nesting to foraging ground.

The growth rate of the turtles in the Sarawak Management Unit is still unknown. It is believed that if the growth rate of the green turtles is made known, a much clearer picture of Sarawak's turtle population could be painted thus the hatchery management of STIs could be improved. This is because the number of turtles that are growing in Sarawak rookery could be predicted and the cause of unfertilized egg could be determined thus enabling stabilization of the male:female ratio through proper hatchery management. To get an estimate of green turtle's growth, mark recapture technique would be the best method. However, lack of funds, manpower and expertise for that has diminished the chance to do so. Therefore, to collect growth and biometry data from green turtles, a headstart project was carried out in Lundu, Sarawak (Chapter 4). The main aim of the headstart project was to gain information on survival, growth rate and general behaviour of green turtles during their 'lost years' while working on improving the turtle landings in Lundu. This project has successfully established an SOP and a growth function related to Sarawak green turtle population. The SOP includes a new and tested behavioural management procedure for small scale headstart project that is suitable even for public use.

This is the first study using headstart approach in Sarawak and it recorded 82 % survival, relatively good growth (average 4,780 g after 18 months) suitable to be released to the wild. Burke (2015) stated that headstarting is one of the ways of turtle conservation effort whereas Pritchard (1981) mentioned that headstarted turtles have a greater chance of becoming part of a breeding population than hatchlings that were not as they are released at size and age that is not prone to predation. Because of the practicality of the SOP, it is

believed that the procedures described in the SOP could be easily replicated for future headstart programs. Other than that, an age-at-length table that was based on captive turtle data and secondary data (for first time mature female size) had been postulated. From that data, it is clear that all that is needed for a perfect growth function, which could be used for the Sarawak rookery is mark and recapture data comprising of body size of sub-adult, adult and nesting mature female turtles with properly documented time intervals. The age-at-length data and the growth function data have to be used with caution, as the data used to produce only based on captive turtles that are known to grow faster in comparison to wild turtles due to food availability (Wabnitz & Pauly, 2008).

While carrying out the headstart project, different types of diseases were seen affecting the turtles which merit further detailing and identification to help prevent future occurrence of these diseases. Therefore, the carcasses of the hatchlings and juveniles were brought back to the lab. Detailed examination through post mortem procedures has been carried out (Chapter 5). A combination of symptoms observed before the turtles died, external physical examination and necropsy procedures have enabled the identification of different types of infectious and non-infectious diseases that were affecting the turtles during the headstart program which is similar but less variable to those reported by Leong *et al.* (1989) and Glazebrook & Campbell (1990). Molecular work using 16S rRNA and ITS gene had pointed out the possible causative agent as bacteria and/or fungi of the infectious diseases which were found to be the same for some when compared to available data from literature. Other than that, a further differentiation between batches in the study has shown a more variable type and occurrence of diseases in Batch 1. This suggests that proper handling and management of green turtles are important during headstart. In this chapter, preventive steps were highlighted so that future headstart project could be carried

out in a more systematic and safe manner. It is very important to note the type, symptoms and time of diseases that occurs during headstart so that the turtles released could be ensured of good health and would not spread dangerous pathogens in their natural habitat.

From historical perspective and through the headstart project, a better understanding of the current status of Sarawak's turtle conservation had been achieved. Lack of awareness and involvement of the public in Sarawak's sea turtle conservation had limited the success of the effort. To instil a sense of responsibility about the charismatic megafauna onto the public, sea turtle awareness programs were conducted at the headstart facility (Chapter 6). Following a positive response from the locals of Sarawak, an educational toolkit for sea turtle conservation had been produced to allow continuous conservation effort and support for green sea turtle conservation in Sarawak despite the halting of the headstart project. This study highlights community-based green turtle conservation in Sarawak where many civilians are involved and exposed to sea turtle conservation. The survey that was conducted at the end of every program for 259 people had shown that there were 96.5 % of people who were willing to join the program again and to stop eating turtle eggs after the program. The study has also found out that some Sarawakians were not aware of green turtle's existence in Sarawak. Therefore, the data and tool developed in this study should be used in schools for classroom education in conservation or as part of co-curricular activity. The limitation of this study is time and discontinuation of the program after the headstart program had been completed. It is hoped that the population of green turtle in Sarawak waters will continue to exist and thrive through public education.

CHAPTER 8

CONCLUSION AND RECOMMENDATIONS

8.1 Conclusion

Sea turtles are highly migratory and long living animals with a complex life history. The management action of these megafauna requires an in depth study of its historical and recent data. This study estimated that the current female nesters of Sarawak to be re-migrating back to their natal beach every three to four years and to nest thrice in that particular visit. Over the course of 36 years, the nesting trend of Sarawak's green turtle population have shown a fluctuating trend that is predicted to improve in the upcoming years providing a balanced sex ratio is produced in the hatchery and the percentage of hatching success improves continuously. Historical and recent data showed that the number of eggs incubated is at maximum stage and that all conservation effort is boosting the population of Sarawak's green turtle population. However, the current nesting population is a result of the past 30 year's effort to conserve the animal. Therefore, the currently estimated number of 3,088 will only be achieved if proper hatchery and egg management practice is carried out properly.

The growth of post hatchling turtle in their first 18 months of life using headstart program has been successful. Growth data obtained had successfully developed the current growth function. The growth function will be used as a guideline in estimating maturity rate of green turtles of Sarawak as the estimated age-at-length is readily available now and this will further aid the conservation effort of this species. The headstart project with reference to problem of insufficient male in Sarawak green turtle population had introduced approximately 30 male green turtle back to the sea. Moreover, the headstart

project has produced an SOP with disease and behavioural management tactics and recommendations for a small scale headstart program.

Rearing turtle in captivity had its challenges especially in disease and behavioural management. The type of diseases and the possible causative agent for infectious diseases had been determined. For every type of non-infectious or infectious disease observed and identified, the symptoms observed on the morbid hatchlings, together with the steps taken to control the diseases from spreading were documented. Other than that, a necropsy procedure was carried out on the carcasses to confirm the suspected infectious disease. This study highlights the importance of both behavioural management and cleanliness factor in any headstart project to prevent disease outbreak. It was evident from the different survival rate, disease variation and severity of the disease as Batch 1 was more inflicted compared to Batch 2.

Furthermore, after the commencement of sea turtle awareness program, the locals of Sarawak has provided this research with a positive response in words and action by displaying a community-based conservation in collaboration with private companies and government bodies. The encouraging response has led to the development of a manual, an information kiosk with infusion of informational videos that could be used in E-book style and an introductory video for a lasting conservation effort. This tool should be introduced in schools as a part of science or conservation subject. The awareness program was an eye opener to the current situation for sea turtle conservation in Sarawak as many participants had confessed to have eaten turtle eggs (all age groups involved) and claimed that they were unaware of Sarawak's sea turtle population. The awareness program should be carried out regularly and consistently to maintain the future of green sea turtle population in Sarawak.

8.2 Recommendations

In future, a few factors should be considered for Sarawak's green turtle rookery including obtaining mark recapture data on a yearly basis, changing the hatchery management practice, investigating the sex ratio of hatchlings produced along with the nest incubation factor. In addition, the fertility of eggs produced in Sarawak hatchery needs to be checked on a yearly basis so that the current fertility rate could be monitored. Moreover, the data that is available should be published and used to maintain and improve the current condition. A study on climate change should be conducted on coastal areas of Sarawak and all of Sarawak's Turtle Islands to measure the temperature change inflicted by global warming besides measuring the rise in level of sea water and its temperature. These data will be useful in predicting the future trend so that a proper countermeasure could be prepared beforehand.

A thorough stock analysis should be done on the rookery of Sarawak and the suspected foraging ground of adult and immature turtles. A mark and recapture should be performed at these places on a yearly basis as this will enable collection of a more reliable age-at-length and maturity-at-age data. A genetic mixed stock analysis and haematology analysis should be performed on the turtles obtained during mark recapture so the origin and route of the turtles could be determined alongside blood profile that could provide us with the health status of green turtles in Sarawak. A survey on the health condition and parasites on green turtles should also be performed during mark recapture.

Furthermore, a permanent public friendly and reachable awareness program centres need to be established and maintained on the mainland of Sarawak to promote awareness on sea turtle and other marine animals among locals. The threats in which all the marine

animals have faced due to pollution and development by human should be emphasized in this centre. The centre could also promote other awareness programs such as recycling or saving the mangroves and would act as an interactive and information hub for the public.

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APPENDICES

Appendix 1:

Paired T-test Result for weight of batch 1 and 2

Paired Samples Statistics

	Mean	N	Std. Deviation	Std. Error Mean
Pair 1 LG1	2.5109	31	.63218	.11354
LG2	2.6512	31	.63545	.11413

Paired Samples Correlations

	N	Correlation	Sig.
Pair 1 LG1 & LG2	31	.997	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
					Mean	Std. Deviation			
Pair 1	LG1 - LG2	-.14033	.04794	.00861	-.15791	-.12274	-16.298	30	.000

Paired sample t-test result for SCL of batch 1 and 2

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	LGSC1	2.0795	31	.19199	.03448
	LGSC2	2.1593	31	.22865	.04107

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	LGSC1 & LGSC2	31	.990	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
					Mean	Std. Deviation			
Pair 1	LGSC1 - LGSC2	-.07983	.04675	.00840	-.09698	-.06268	-9.507	30	.000

Paired sample t-test for survival of Batch 1 and 2

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	LGS1	1.5457	31	.21442	.03851
	LGS2	1.6293	31	.02123	.00381

Paired Samples Correlations

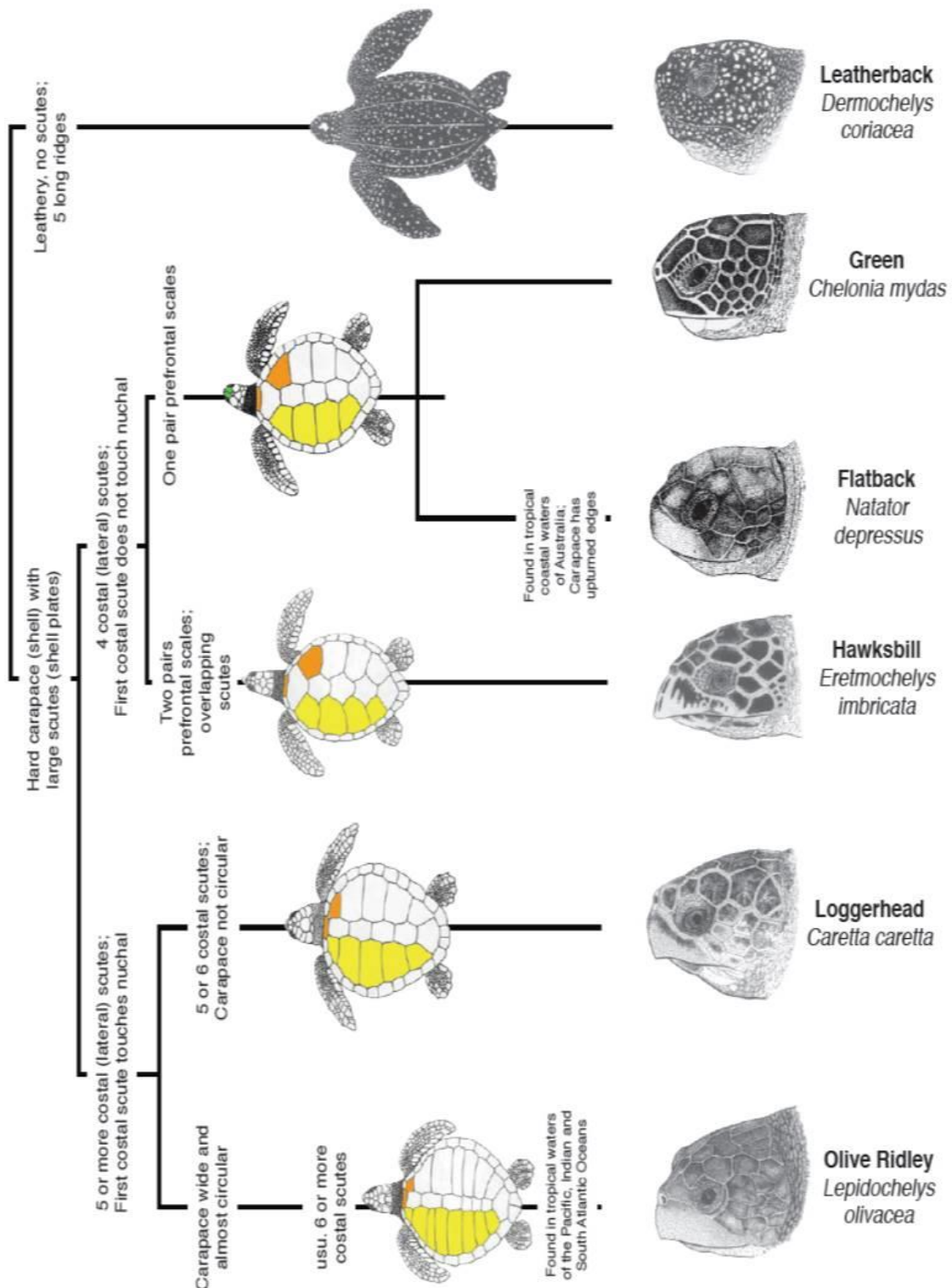
		N	Correlation	Sig.
Pair 1	LGS1 & LGS2	31	.930	.000

Paired Samples Test

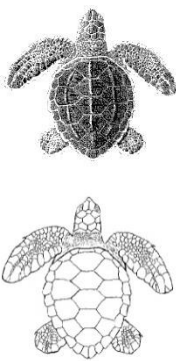
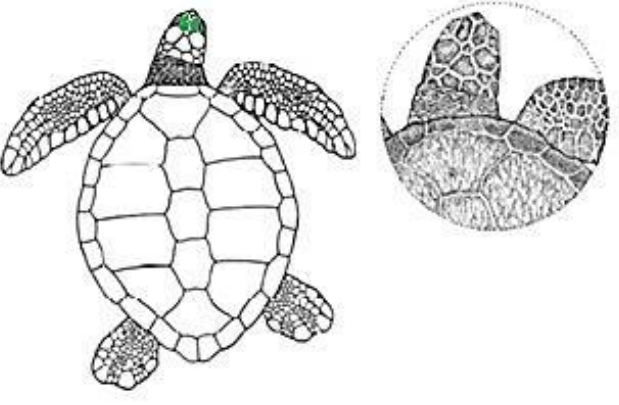
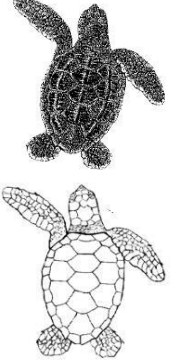
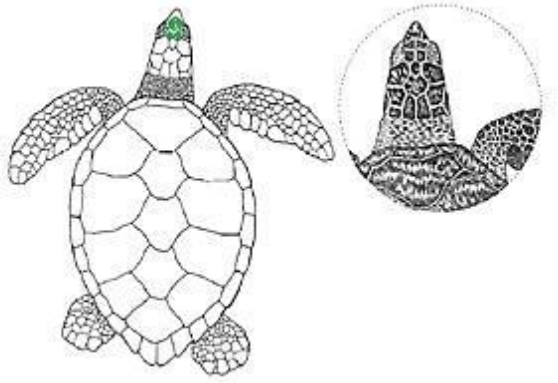
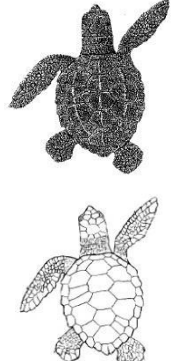
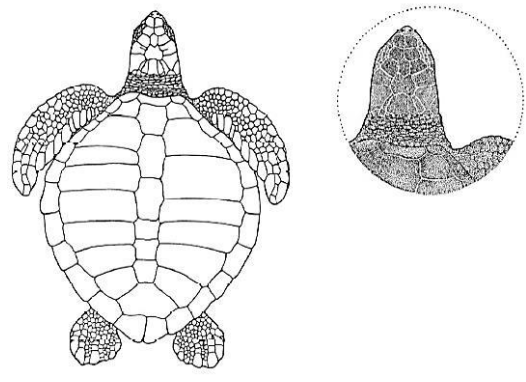
		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
					Mean	Std. Deviation			
Pair 1	LGS1 - LGS2	-.08362	.19484	.03499	-.15509	-.01215	-2.390	30	.023

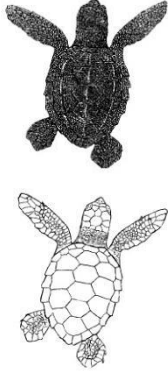
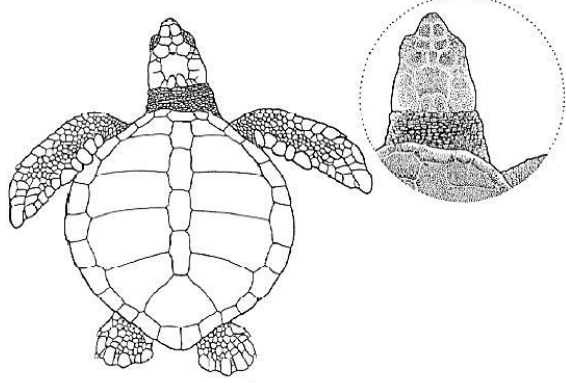
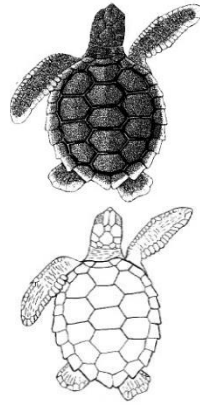
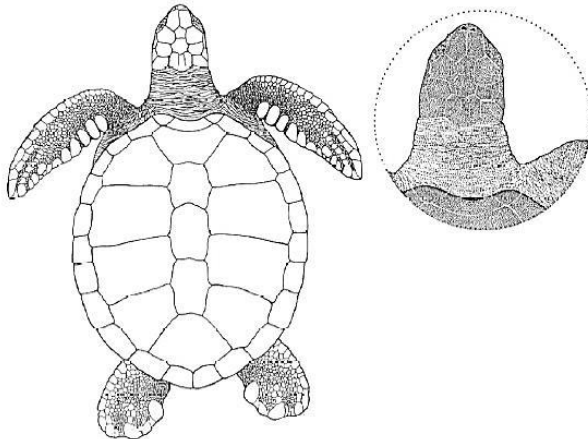
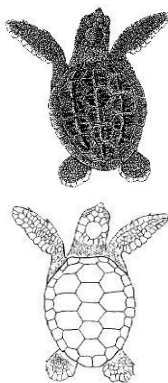
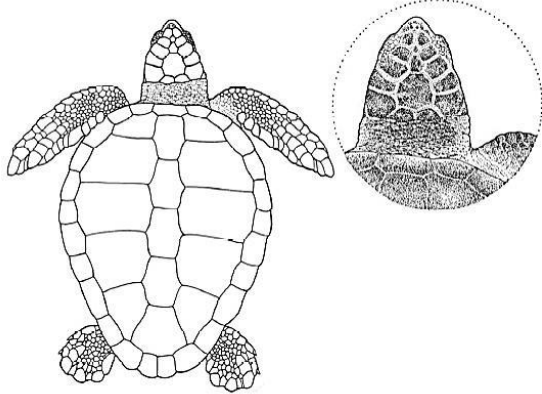
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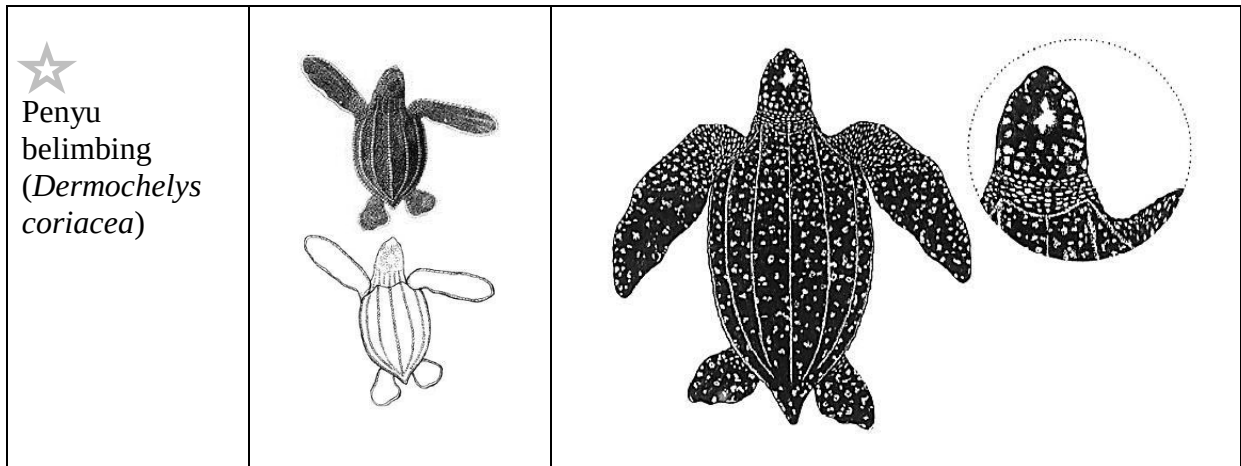
Panduan Bergambar untuk Mengenal pasti Penyu



Rajah 1: Panduan bergambar untuk mengenali penyu Indo-Pacific. Kuning menunjukkan 'costal scute', hijau menunjukkan sisik 'prefrontal' dan jingga menunjukkan kedudukan 'costal scute' yang pertama kepada 'nuchal scute'. Diadaptasi dari Speirs & Richter (2009).

Nama	Anak Penyu	Penyu Dewasa
☆ Penyu agar (<i>Chelonia mydas</i>)		
☆ Penyu karah/sisik (<i>Eretmochelys imbricata</i>)		
☆ Penyu lipas (<i>Lepidochelys olivacea</i>)		

Penyu ‘Kemp’s Ridley’ (<i>Lepidochelys kempii</i>)		
Nama	Anak Penyu	Penyu Dewasa
Penyu ‘Flatback’ (<i>Natator depressus</i>)		
Penyu ‘loggerhead’ (<i>Caretta caretta</i>)		

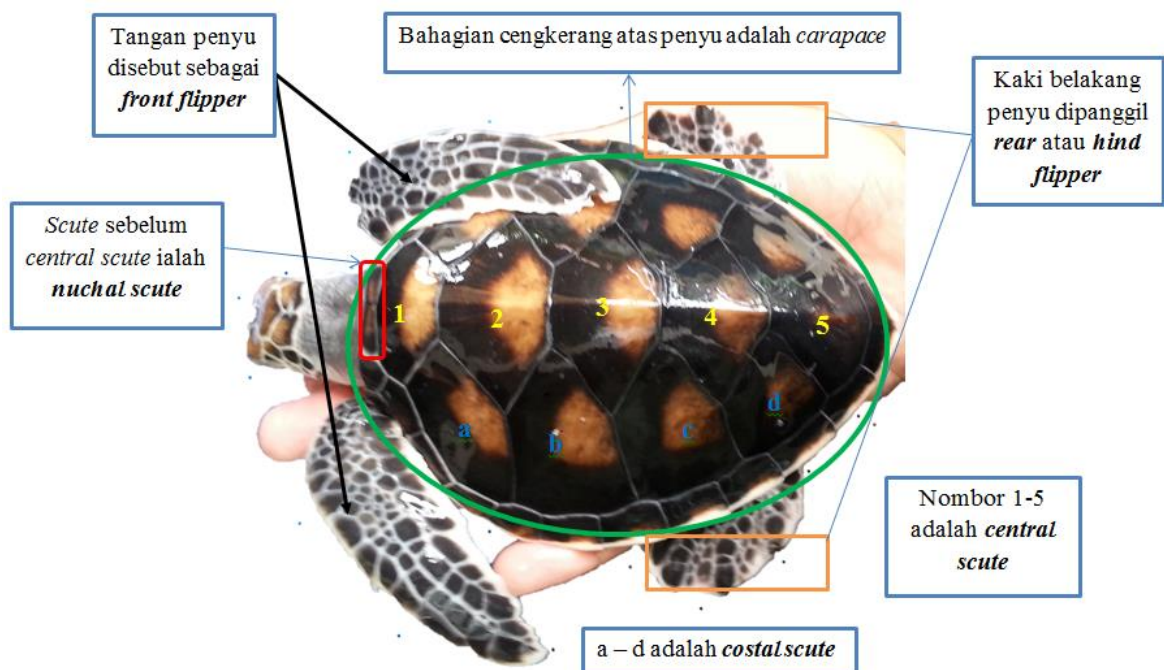


☆ Penyu yang selalu ditemui di Malaysia.

* Gambar diadaptasi dari Pritchard & Mortimer (1999).

Mengenali bahagian badan penyu:

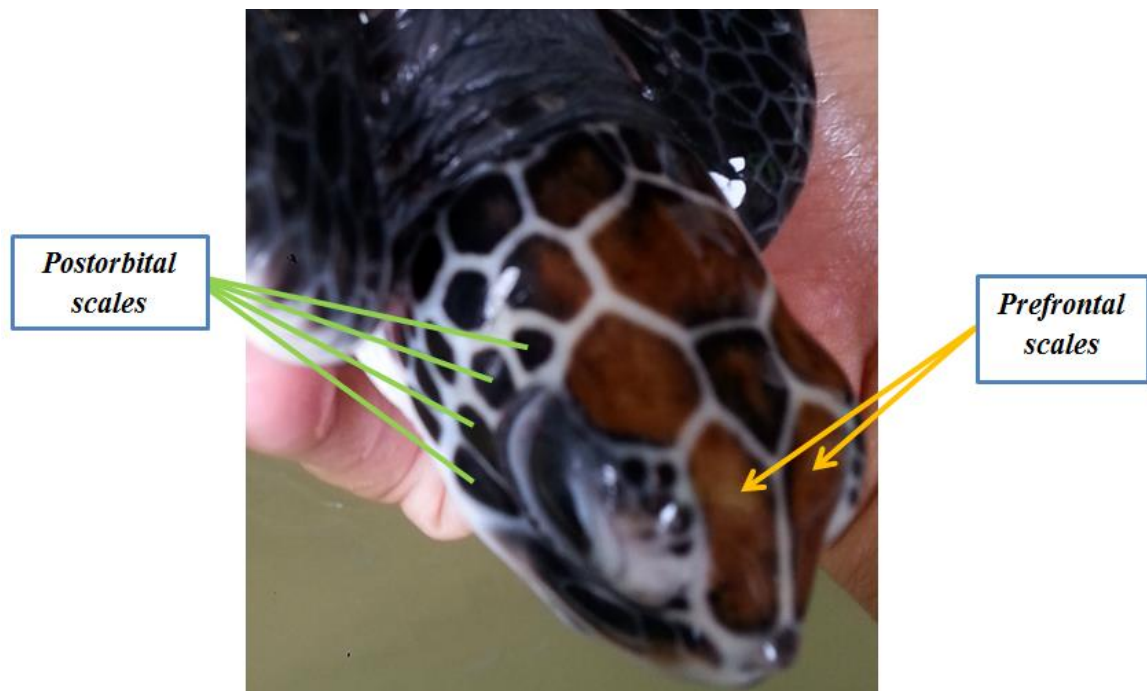
(a) Bahagian atas (*dorsal view*) badan penyu



(b) Bagian bawah (*ventral view*) badan penyu



(c) Muka penyu



Rujukan:

- Speirs, M. & Richter, V, (2009). Standard Operating Procedure No: 12.5 Semi-permanent marking of marine turtles using flipper and PIT tags. Department of Environment and Conservation Nature Conservation Service.
- Pritchard, P.C.H. & Mortimer, J.A. (1999). Taxonomy, External Morphology, and Species Identification. Pages 21-38 in Eckert, K.L., Bjorndal, K.A., Abreu-Grobois, F.A. and Donnelly, M. (eds.) Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4, Pennsylvania.

Appendix 3

Survey for Turtle Awareness Program

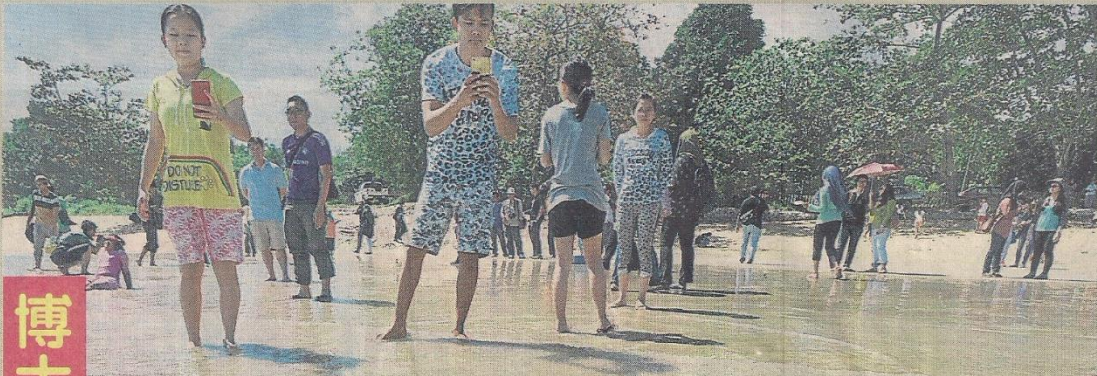
Please tick (√) wherever applicable / Tolong tandakan (√) di tempat yang berkenaan:

1. Age group/ Kumpulan umur:
7-12(), 13-17(), 18- 25 (), 26-50 (), >50 ()
2. Have you ever seen a sea turtle before? / Pernahkah anda lihat penyu sebelum ini?
Yes / Ya () Where / Di mana? : _____
No / Tidak ()
3. Did you understand the information on the sea turtles given during this visit? / Adakah anda faham informasi tentang penyu yang disampaikan sebentar tadi?
Video: Yes / Ya () No / Tidak ()
Talk / Ceramah: Yes / Ya () No / Tidak ()
Practical or demonstration / Amali atau demonstrasi: Yes / Ya () No / Tidak ()
4. Have you eaten turtle eggs before? / Pernahkah anda makan telur penyu?
Yes / Ya () Will you eat it again after today? / Adakah anda masih akan makan telur penyu selepas ini? _____
No / Tidak ()
5. What is the age of the turtles in the tank? (estimate) / Berapakah umur penyu di dalam tangki?

6. Are plastics floating in the sea dangerous for turtles? / Adakah plastik terapung di laut berbahaya kepada penyu?
Yes / Ya () No / Tidak ()
8. Will turtles drown when caught in a net? / Adakah penyu akan lemas bila terperangkap di dalam jaring?
Yes / Ya () No / Tidak ()
9. What is your opinion about this programme? / Apa pendapat anda tentang program ini?
Boring / Sangat membosankan ()
Exciting / Teruja ()
Useless and such a waste of time / Sangat tidak berfaedah dan membazir masa sahaja ()
10. Will you come for another conservation program? / Adakah anda akan datang lagi untuk program konservasi seperti ini?
Yes / Ya () No / Tidak ()

THANK YOU / TERIMA KASIH.

Appendix 4:



博士生領養研究

海
龟慢慢适应海
水的温度后，渐
渐游远，重回大
自然。

39隻海龜回歸大海

■ 報道 田蘇恩

（古晋4日讯）39只平均重达5至6公斤的海龟，昨日在三马丹班丹海滩被释放到大海。这群海龟慢慢向前爬行，适应海水的温度后，渐渐游远，重新拥抱大自然！

一批来自砂拉越大学的学生在活动策划人兼攻读水产学科博士学位的诺哈迪妮卡玛利亚率领下，将这群海龟放到班丹海滩，让它们回到属于它们的大自然环境。

诺哈迪妮卡玛利亚受访时指出，她于去年6月份获得许可证，从大达浪达浪岛领养了200只稚龟。她在伦乐一个家庭式度假屋内，将这群稚龟分别饲养在两个池塘，并定时供应稳定的新鲜海水。

昨日的放生活动也引起当地人及游客的注意，纷纷上前围观。

■ 稚龟首6个月易患病

诺哈迪妮饲养这群海龟已有一段

宜现阶段释放。

她指出，基本上，平均每1千只海龟中，只有一只得以生存。它们一直都处于危险边缘，因为海龟在成功孵化后首6个月很容易患上疾病，而且它们是自然捕食，容易被人类的各种活动影响而死亡。

她解释，海龟在20至30多岁期间才开始孵蛋，而根据她的博士论文，她将达到两项目标，那就是收集足够的数据和资料来进行海龟的精确年龄与大小的

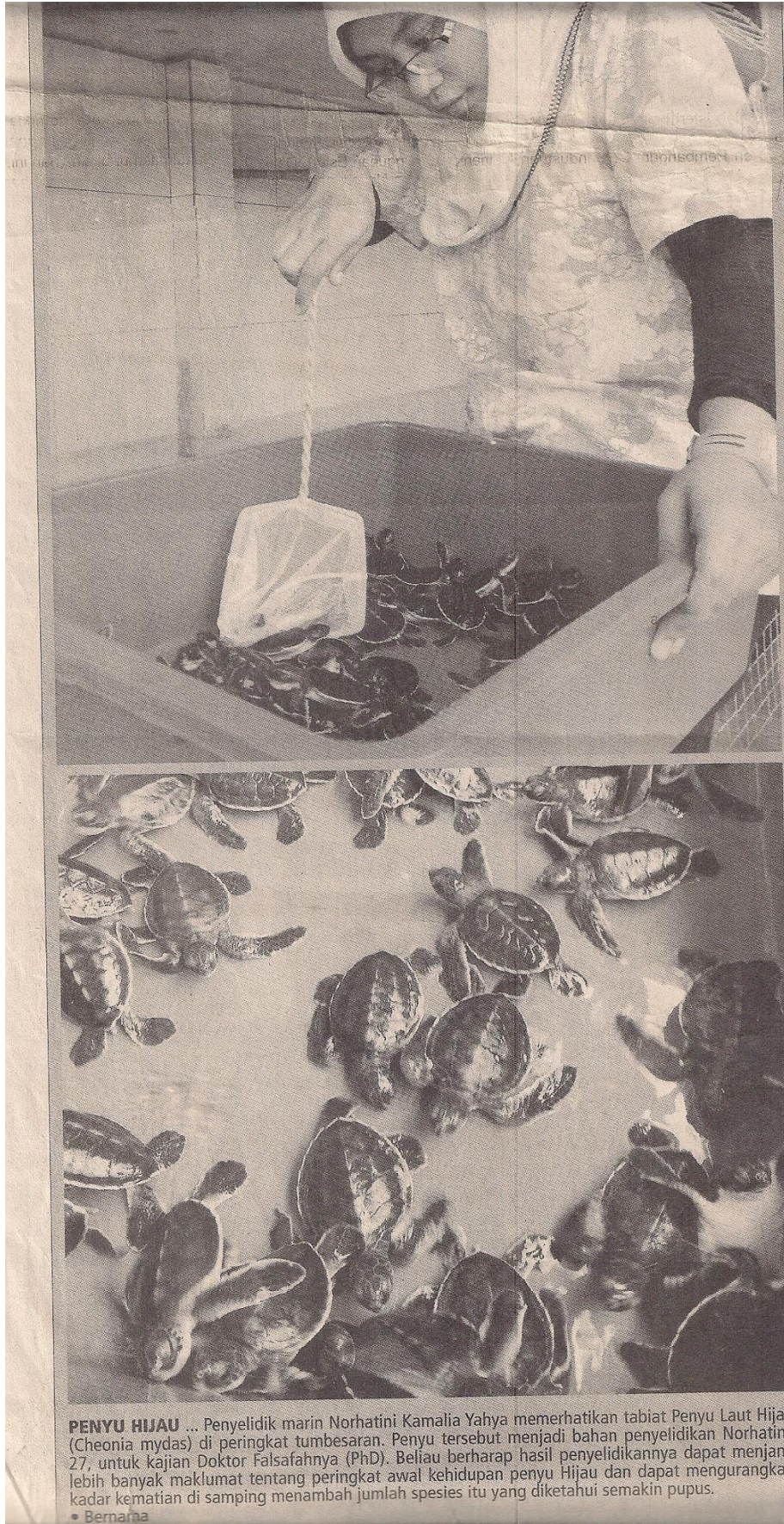
成长图，以及研究相关疾病。

“目前，在海洋动物的数据中存有间隙，可说已失去追踪轨道。如果拥有良好和完整的图表，我们就可透过一只海龟的大小，来计算或预测它的年龄。无论如何，研究野生海龟的经费非常昂贵。”

她也说，当稚龟在成长过程中（长出四肢之前到达重量190公斤），无法追踪它们，因为它们仍处于皮肤脱落的阶段，很难嵌入监督的标签。



海，重新拥抱大自然。
← 砂大生将39只平均重达5



Appendix 5:

Tagged by :Alfanso, Christopher, Nelson & Phay Swee Ann

Tagging method :Inconel Ear Tag

Location :Gold Coast Resort, Pandan

Date :3rd May 2016

ID	Tag no.(Left)	Tag no. (Right)	CCW (CM)	CCL (CM)	Weight (kg)	Sex	Species
01/16	27776	27777	37	40	6.9	F	CM
02/16	27778	27779	38	39	6.3	F	CM
03/16	27780	27781	30	34	3.1	M	CM
04/16	27785	27783	32	36	4.6	F	CM
05/16	27786	27788	37.5	40	8.1	F	CM
06/16	27782	27789	35	37	5.9	M	CM
07/16	27790	27791	37	41	7.1	M	CM
08/16	27794	27793	38	41	7.2	F	CM
09/16	27795	27796	32	36	5.5	F	CM
10/16	27797	27798	33	35	6.1	M	CM
11/16	27799	27775	31	33	4.1	M	CM
12/16	27774	27773	31	34	4	M	CM
13/16	27772	27771	27	29	2.7	M	CM
14/16	27770	27769	28	31	4.3	M	CM
15/16	27768	27767	37	40	7.9	M	CM
16/16	27766	27765	33	37	5.5	M	CM
17/16	27764	27763	36	38	6.4	M	CM
18/16	27762	27761	33	36	5.1	M	CM
19/16	27760	27759	31	34	4.5	F	CM
20/16	27754	27753	33	37	6.3	M	CM
21/16	27756	27755	34	40	5	F	CM
22/16	27754	27753	32	34	4.7	M	CM
23/16	27752	27751	35	40	8.1	M	CM
24/16	27750	27749	37	41	7.3	F	CM
25/16	27748	27747	34	37	5.9	M	CM
26/16	27746	27745	35	37	5.5	M	CM

Tagged by: Tonny, Christopher, Nelson & Phay Swee Ann

Tagging method: Inconel Ear Tag

Location: Pandan GoldCoast Holiday Villa, Date: 2nd December 2015

ID	Tag No. (Left)	Tag No. (Right)	CCL (cm)	CCW (cm)	Weight (kg)	Species
1	21525	21576	37	32	5.4	CM
2	21577	21578	37.5	35.2	5.8	CM
3	21579	21580	38.2	35.4	6.9	CM
4	21581	21582	37	33.2	5.6	CM
5	21583	21584	35	32.2	5.3	CM
6	21585	21586	34.5	32.9	5.1	CM
7	21587	21588	35	32.5	5.4	CM
8	21589	21590	38	35	6.3	CM
9	21592	21591	36	32.2	5.5	CM
10	21595	21593	38	34.5	6.5	CM
11	21597	21596	38	34.5	6.1	CM
12	21599	21598	37	32.8	5.6	CM
13	21526	21600	34	31.2	4.6	CM
14	21528	21527	36.5	33	5.7	CM
15	21530	21529	34	31	4.5	CM
16	21532	21531	38	33	5.9	CM
17	21534	21533	35	30.3	4.7	CM
18	21536	21535	35	30.6	4.9	CM
19	21538	21537	34	30.2	4.6	CM
20	21540	21539	36	32	5.9	CM
21	21542	21541	38	35	6.2	CM
22	21544	21543	37	33	6.1	CM
23	21546	21545	35	32.1	5.2	CM
24	21548	21547	35	34	5	CM
25	21550	21549	37	34.5	6.1	CM
26	21552	21553	37	33.1	5.6	CM
27	21555	21554	33.5	30	4.6	CM
28	21558	21557	37	33	6	CM
29	21560	21559	37	34.5	6.6	CM
30	21562	21561	37	32.5	6.4	CM
31	21564	21563	34	30.3	4.8	CM
32	21566	21565	42	37	8.5	CM
33	21569	21568	42	41	9.4	CM
34	21571	21570	35	34	5.3	CM
35	21573	21572	40	36	7	CM
36	21575	21574	42	38	7.8	CM
37	15402	15401	38	35	6.9	CM
38	15404	15403	34	29.2	4.1	CM
39	15406	15405	40	38	7.3	CM

Appendix 6:

List of Publication

Hassan, R., Yahya, N.K., Ong, L.M., Kheng, L.K., Abidin, Z.Z., Ayob, A. & Jainal, A.M.
(2017). Public Awareness Program and Development of Education Toolkit for
Green Sea Turtle Conservation in Sarawak, MALAYSIA. *International Journal
of Environmental and Science Education*, 12(3), 463-474. doi:
10.12973/ijese.2016.1241p



Giving them a fighting chance

PhD student releases green sea turtles into the ocean after few months of protection

story by
YU JI

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KUCHING: Aquatic science PhD student Nurhartini Kamalia Yahya is facing the end of her time with around 100 green sea turtles. In June last year, the Penang-born scientist obtained a permit to collect 200 hatchlings from the Talang Besar island. She reared them at a family-run resort at Lundu, from two aquariums (when they were small) into two ponds, with a constant supply of fresh seawater pumped in.

The sea creatures have been a hit. Holiday makers were attracted to them; kids from nearby villages fascinated.

It was with a heavy heart last Thursday when Kamalia, who was joined by resort workers, villagers and even the local police, released the first batch of 39 green sea turtles into the ocean.

“I think I am sad. But it’s for the best. Honestly I did not like to see them confined to a small space. It hurt me,” Kamalia told reporters at Pandan Beach.

“I got to keep the animals up to a certain size, now that they are less affected by predation, it’s time to release them into the wild. Their survival rates would have increased.”

Roughly, only one in 1,000 sea turtles survive into maturity. They are endangered because they are naturally preyed upon, prone to diseases for the first six months of their lives, and affected by human activity.

“Sea turtles do not start laying eggs until

well into their 20s or 30s,” Kamalia said, who began her university life studying their DNA.

For her PhD thesis, she has two goals: To collect enough data for an accurate age-size growth chart and to study diseases.

Currently there is a gap in the sea animal’s data, which Kamalia called the “lost years”. “With a good chart, when you see one turtle, you check its size, and you’ll be able to estimate its age.”

Sea turtles in the wild are expensive to study.

When they are growing (before ending up as big as four-feet long, weighing up to 190kg) it is almost impossible to track them. This is because they are still shedding skin, making it hard to embed simple tags and global position sensors.

For the duration of Kamalia’s research, she has been living at the resort. A typical day would involved about three to four hours of labour – weighing the turtles, feeding time, cleaning the ponds, operating the pumps – and the rest of the day to crunch the numbers.

She had pre-selected the larger ones to release first. They were mostly between 5kg and 6kg. “In January, we’ll release more. Some still in the pond are only about 2kg.”

In the end, the release of the 39 turtles took just minutes. From the pond, they were placed into blue plastic trays (some by giggling children), carried to the beach and laid on the sand.

Kamalia told everyone to face the turtles “towards the sun, because they’ll follow it”.

The green sea turtles crawled towards the crashing waves. And then they were gone, submerged beneath the sea. For Kamalia, she would always have the turtles present in her charts.



- 1 A turtle crawls on Pandan Beach.
- 2 A toddler looking at a turtle that is making its way into the ocean.
- 3 A turtle crawls on Pandan Beach toward the sea after being released.
- 4 Kamalia releasing a 5kg turtle.

— Photos:
ZULAZHAR
SHEBLEE / The
Star