Project Title: GREEN TURTLES OF MALAYSIA

Principal Investigator(s):	**Dr. Joan Whittier**	**Mr. Kamarruddin Ibrahim**
Position/Title:	Senior Lecturer	Senior Researcher
Organisation/Affiliation:	University of Queensland Dept .of Anatomical Sciences St. Lucia, Qld 4072 Australia	Marine Fishery Resources Development & Management Dept., Turtle and Marine Ecosystems Centre (TUMEC), Rantau Abang, Terengganu, Malaysia

RESEARCH SITES:

In 2004, research was conducted at two main locations in Terengganu on the eastern coast of Peninsular Malaysia:

Ma'Daerah Beach on the mainland and Mak Kepit Beach on Pulau Redang. Ma'Daerah Beach is a 1.8km stretch of mainland beach with relatively coarse yellow/orange sand, in a more remote portion of the coast. Pulau Redang, an island approximately 15 km from the mainland, has a series of small isolated beaches, including Mak Kepit. These island beaches have fine, light coloured sands and are well shaded by natural vegetation, resulting in a cooler incubation temperature throughout the nesting season.

Local Management Status of the Research Site(s) (e.g. National Park, RAMSAR Site, World Heritage Site, IBA etc.):

The Terengganu state government has recently gazetted the Ma'Dearah Turtle Sanctuary. This ensures the protection and conservation of surrounding land and nearshore waters. Mak Kepit is a state government gazetted turtle sanctuary and Pulau Redang lies within a National Marine Park. This protects both the nesting beach and coastal waters extending for 2 nautical miles from the shore.

Scientific names of primary species being studied (if appropriate): ***Chelonia mydas***

Key Research Objectives:

1) **Gain a greater understanding of the biology of green turtle eggs in Peninsular Malaysia.**
2) **Gain a better understanding of the incubation process in the artificial hatchery and compare this to natural in situ nest environments.**
3) **To understand the effects of sand temperature and nest position on the hatching success, health and fitness and sex ratios of hatchlings produced.**
4) **To formulate specific management recommendations to aid in the conservation of the green turtle.**
5) **To conduct sampling experiments involved with testing hormonal interactions between adults nesting females, eggs and hatchlings, and the effects of marine environmental pollutants on the reproductive processes in the turtles.**
6) **Together with staff of the Malaysian Department of Fisheries, we help to determine the status of the nesting population of green turtle in Peninsular Malaysia.**

BACKGROUND AND OBJECTIVES:

The worldwide populations of marine turtles are declining rapidly. All the extant seven species of marine turtles have been classified in the Red Data Book of the IUCN as endangered or threatened, and they are included on Appendix I of the Convention on International Trade in Endangered Species (CITES). These trends are also applicable for populations of marine turtles in Malaysia (Siow & Moll, 1982; Leh, 1989; Chan, 1990; Limpus, 1991; Kamarruddin, 1994).

The green sea turtle (*Chelonia mydas*) has a worldwide distribution in tropical and subtropical regions. It represents one of four marine turtle species in Malaysia. It is the most abundant, and its nesting populations are distributed unevenly all over the Peninsula, Sabah and Sarawak. High breeding concentrations are found in Malaysia, with an estimated 1000+ nests per year on each of the Sabah Turtle Islands, Pulau Redang (nearshore Peninsula) and Sarawak Turtle Islands. However, there have been large declines in nesting at each of these areas over the past 50 years. In addition, the population of the green turtle in Peninsular Malaysia has significantly declined over the last few decades. For instance, the Terengganu green turtle population has been depleted by at least 60% since the 1950s (Limpus, 1993; Kamarruddin, 1994). This population is at risk of extinction if appropriate remedial actions are not taken immediately.

A diversity of factors has been implicated in the decline of sea turtles. Turtles are long-lived, have low reproductive success and high juvenile mortality. Particularly important is human-influenced mortality of adults. The major threats to Malaysian marine turtles include a continuous over-exploitation of eggs for human consumption, incidental capture of turtles in fishing gear, habitat loss and environmental degradation, direct human disturbances and pollution. The threatening processes continue and the problems seem unresolved although various conservation efforts such as legislation, operation of beach hatcheries and establishment of sanctuaries have been attempted.

One of the contributing factors leading to insufficient marine turtle conservation in Peninsular Malaysia has been the incomplete knowledge of the turtles' biology and ecology in this area. This lack of knowledge has hindered efforts of developing effective conservation measures. Previous research in the 1960s and 1970s had focused on tagging leatherback turtles, with research and monitoring of other marine turtles beginning in the late 1980s. This followed a national workshop on Sea Turtle and Conservation and Management in December 1987, and the establishment of the Intensification of Research in Priority Areas Strategic Panel by the Malaysian government in the same year. At present, little is known of the biology and ecology of the green turtles in this region.

There is a long cultural history of human consumption of marine turtle eggs in Malaysia. Unless turtle eggs are actively protected there is typically little or no hatchling production from Malaysian turtle rookeries. In the context of hatchling production as a tool in the conservation of green turtles in Malaysia, the technique of replanting eggs in central beach hatcheries was initiated in the 1950s (Leh, 1994). It is still being practiced in Malaysia at the present. The technique, however, has been observed to produce relatively low hatching success in comparison with that obtained from natural incubation (Eckert and Eckert, 1990; Kamarruddin and Thalathiah, 1994). This is thought to be related to movement-induced mortality (Limpus et al., 1979; Parmenter, 1980) and/or the alteration of beach temperature (Miller and Limpus, 1983). In addition, artificial incubation can have a profound effect on the sex ratio of hatchlings (Mrosovsky, 1982; Dutton et al., 1985; Limpus et al., 1985). The fact that physical environment, particularly temperature, affects the survival and sex ratios of embryos and hatchlings reinforces why understanding the biology and ecology of eggs is important for management of turtle conservation.

This study is designed to provide information and scientific knowledge of the green turtle. The scope includes the gathering of information on the status of nesting populations as well as the biology and ecology of eggs, hatchlings and adults. There is very little information concerning the incubation ecology and natural sex ratio of green sea turtles in Malaysia. The pivotal temperatures at which sex is determined during incubation are now known for both Malaysian island and mainland nesting populations of this species (K.Ibrahim, C. Limpus and J. Whittier, unpublished data). We suspect that the past and present practices of hatchling production in hatcheries are likely to produce skewed sex ratios of hatchlings of green turtles. The extent that hatchery practice has and does influence adult sex ratios in populations needs to be considered. Ideally hatchery practice should be modeled on natural incubation environments to simulate the production of natural sex ratios in populations.

The findings of this study are used to the Department of Fisheries in Malaysia in order to help improve the existing management and conservation practices. These include improving egg and hatchling management as well as protection of turtles and habitats. It forms a basis for the establishment of a national and international cooperative effort. The contribution to scientific knowledge will in turn help to maintain the long-term sustainability of the green turtle population. Consequently, it will contribute significantly in generating socio-economic benefits to the local communities and revenue to the government, primarily through turtle-based tourism. The findings will also highlight Malaysia as a model country in turtle conservation in the region.

Our primary objective is to determine the status of the green turtle population and to assist in its conservation. We will describe the biology and ecology of green sea turtles at two locations in Peninsular Malaysia. We expect that our work will update knowledge of the nesting population and the conservation status of the green turtle in Peninsular Malaysia. Problem areas, weaknesses and issues related to conservation should be identified. We expect to gain an understanding of some important biological and ecological factors that affect successful production of green turtles in the region. We will gather important scientific information on egg and incubation biology of the green turtle. We will also evaluate management of hatchling production in the study area. From these studies specific recommendations will be made to help improve existing conservation practices. We will also seek to identify future needs for scientific study of marine turtles in the region.

Specific Objectives

The primary research objective is to determine the population status and conservation needs of the green sea turtle in Peninsular Malaysia. We plan to study the nesting biology and reproductive and population ecology of this species. This project further aims to systematically identify all factors that may affect egg incubation and hatchling quality and to use this information to produce a set of structural and procedural parameters to maximise the production of high quality hatchlings with near natural sex ratios in hatcheries. The findings will be used to formulate recommendations for the future conservation management of the species.

We will seek to gain a greater understanding of the nesting ecology of the green turtle in Peninsular Malaysia. Basic data on the size and condition of nesting females, as well as the number of eggs laid per clutch through an eight week period of the nesting season will be collected. We will also focus on the biology of green turtle eggs in Peninsular Malaysia. Since turtles are under threat by egg collection, egg and hatchling management are critical to the conservation strategy for the species. Of particular importance is that we gain a better understanding of the incubation process in the artificial hatchery environment. To effectively protect the turtles, data on the influence of the hatchery environment on hatching success and quality is needed. Also information about the sex ratio of hatchery-produced offspring is needed. These data will be compared to similar data collected from natural, undisturbed nests, and to previously conducted laboratory incubation studies.

Work recently being conducted by Kamarruddin Ibrahim in Malaysia determined the pivotal temperature of green turtles from the nesting beaches at Chendor (another mainland hatchery) and Pulau Redang is 29.5C. When this study began in 1999, our monitoring of nest temperatures at all unshaded hatcheries indicated that most hatchery temperatures exceed the pivotal temperatures by 2 to 4 degrees C. This would indicate a highly female skewed sex ratio. The higher temperature hatcheries (33C) also produce more hatchlings with abnormal scale morphology. Hatchlings produced at these temperatures are also less vigorous and would be more susceptible to predation during their post-hatching dispersal phase. At Chendor in 2000, the hatchery was shaded 70% and nest temperatures were lowered to 31C. More hatchlings were produced, with fewer scale abnormalities and with greater vigor, in comparison to other hatcheries and to 1999 data from Chendor. In 2001 and 2002, studies at Ma'Daerah, found that shading of the hatchery lowered the temperature closer to pivotal temperature, producing a sex ratio of 70:30 male:female and increasing the vigour of emerging hatchlings to exceed that of natural nest hatchlings (Van de Merwe et al., 2005a). Also, in 2001 and 2002, a more in-depth investigation into nest temperatures revealed that metabolic heating of the eggs was an important factor in determining nest temperatures and hence sex ratios. Another study in 2001 found that increasing the nest depth in hatcheries did not significantly decrease the nest temperatures although the quality of hatchlings was different with clutches relocated to 50cm depth producing hatchlings with greater size and vigour compared to clutches relocated at 75cm (Van de Merwe et al., 2005b). An experiment on nest density in 2002 also indicated that running speed and mass:SCL ratios were decreased when the hatchery nests were placed closer together. These results all highlight the need to further investigate different hatchery designs that can significantly lower nest temperatures to maximise the quality and sex ratios of emerging hatchlings.

Specific objectives in 2004 were to investigate the effects of retaining hatchlings in nest nets after they have emerged and further investigate sand temperature profiles within the hatcheries. These particular pieces of information will further our understanding into the effects of current management practices and bring us closer to determining a set of design and procedural parameters for optimal hatchery management in terms of producing large numbers of high quality hatchlings at near natural sex ratios.

DATA COLLECTION AND RESULTS:

Animal Ethics and Wildlife Permits

We currently hold an Animal Experimental Ethics Approval Certificate from the Animal Welfare Office of the University of Queensland, ANAT/265/04/MDF through 6/2005. Malaysian Wildlife Permits for this project on turtles are held by Kamarruddin Ibrahim.

Beach patrols

Early in the turtle-nesting season, beach patrols were conducted along each beach on foot by “beach teams”, lead by local rangers employed by the Fisheries Department of Malaysia. Beach patrols commenced at 2100 hours and continued until dawn. Each team carried a dim torch and a kit bag containing necessary tagging, data collecting and recording gear.

Nests were located by sighting beach tracks left by nesting females. Once nesting females were located, they were monitored until egg laying had commenced or until the turtle left the beach (when egg laying did not occur). At this stage, the base camp was notified by radio and reinforcements, including volunteers and a trained research staff, were sent to the nesting site to assist in data collection. At the end of egg laying or when returning to the beach, all nesting females were tagged on both front flippers using Inconel tags. Turtle measurements and other relevant data were collected as recommended by Pritchard *et al*. (1983) and the Queensland Turtle Research Project. This included recording existing tags or tag scars, or new tags applied, the time and date of emergence, an index of body size (curved carapace length or CCL), and any damage to the carapace or flippers. Volunteers also assisted field staff and rangers in a newly developed technique for measuring the weight of nesting females. Data also was collected on nest location and beach zone, as well as whether the nest was located on open beach, in grassy areas or under beachside vegetation. Nest location was determined by measurement to nearest beach marker posts (located every 25 m).

Nightly Nest, Egg and Hatchling Data Gathering and Processing

The base camp, which was situated adjacent to the hatchery, was manned by volunteers and staff between nightfall and daybreak each night. The team of volunteers was divided into two, with half working from 2000 to 0130 and the other half working from 0130 to 0700 (or until finished). The processing of eggs, nests and hatchlings differed slightly at the two sites. At Ma'Daerah Beach, all eggs were relocated to hatchery sites where they are protected, a necessity on the mainland beaches. At Pulau Redang a natural incubation study was conducted, with only eggs laid below the high water mark or those near the creek relocated. When relocated, egg statistics were collected, including the number of eggs, plus a measurement of egg diameter and mass for a sample of ten eggs from each clutch. After approximately 6-8 weeks of incubation, a mesh frame to briefly retain hatchlings was placed around nests ready to hatch. When hatchlings emerged, they were counted and a sample of 10 were weighed and measured, tested for running ability, scale counted to identify morphological abnormalities and tested for their ability to orient towards the ocean.

Artificial Incubation Study (at Ma'Daerah)

At the Ma'Daerah Turtle Sanctuary, the hatcheries are enclosed areas of exposed beach located above the high tide watermark. The main production hatchery, which can house more than 300 nests over the nesting season, was approx. 6 x 15m. It was fenced with netlon, covered with 70% black shade cloth and padlocked for security.
Throughout the 8-week study, a sample of 10-20 hatchlings was taken from 55 emerging nest, which were weighed and measured, scale counted and tested for running speed, flip speed and ability to orient towards the ocean. These tests were repeated with hatchlings from *in situ* nests at Mak Kepit and a comparison was made to determine if the quality of hatchery hatchlings is consistent with naturally incubated hatchlings.

In our study into the effects of retaining hatchlings in hatchery nests after they emerge, 25 hatchlings were collected from each of 10 nests over teams 2, 3 and 4. Immediately following emergence, 10 hatchlings were subjected to a running test and had their mass and straight carapace length measured. Subsequently, 5 hatchlings were subjected to the same tests at intervals 1 hour, 3 hours and 6 hours after emergence. We analysed the data to determine if there were any decreases in running speed and/or mass:length ratio over this time period.

Team 3 planted temperature data loggers in the sand in the hatchery at depths of 50cm, 60cm, 85cm and 95cm to determine sand temperature profiles in the hatchery and to investigate what depth is best to relocate nests to maintain high hatch success and natural sex ratios. These data loggers were set to record the temperature every hour and were left in the sand for 5 days. Temperature data was then downloaded and mean temperatures determined for each nest depth.

The emergence and hatch success of hatchery nests and *in situ* nests was determined by excavating the nests after hatchlings had emerged and examining the contents to determine the number of hatched, unhatched, and undeveloped eggs, assessed according to Limpus *et al.* (1985) and Miller (1985).

Partly due to our efforts, egg poaching at the hatcheries has been more closely monitored by hatchery staff. Unfortunately at Ma'Daerah one Malaysian staff member was let go in 2001 due to poaching activities. We hope to demonstrate to hatchery staff in future that it is in their own best interests to protect the turtle eggs as sustainable resource for tourism, rather than as a non-sustainable commodity.

Natural Incubation Study (at Mak Kepit)

At Pulau Redang approximately 44 clutches of eggs were studied. All of these clutches were incubated naturally. The emergence success and quality of hatchlings from naturally incubated nests at Mak Kepit is investigated to allow a comparison between artificially incubated hatchery nests and the natural situation. Early in this study, the *in situ* management was producing nests of a superior quality in terms of their emergence success and production of higher quality hatchlings. However, in 2001 and 2002, the emergence success of hatcheries reached that of *in situ* nests and the quality of hatchery produced hatchlings surpassed the natural nests. This provides evidence of natural variations between years and sites, as well as supporting the evidence found in 2001 that shallower nests produce higher quality hatchlings, as natural nests are significantly deeper than hatchery nests. Future monitoring of Mak Kepit and other *in situ* island situations is required to gain a better understanding of natural variation in hatching success and performance.

RESULTS

Are hatcheries producing hatchlings?

The results of the nest diggings and surveys completed with the aid of the Earthwatch volunteers at Ma'Daerah and Mak Kepit between 2000 and 2002 indicate that the success of hatchery production is high (Table 1). Hatchery figures compare favourably with the *in situ* site and also with long term average emergence rates for natural nests at Mon Repos, Queensland ranging from 80-90%.

Table 1. Emergence success of hatchlings from nests surveyed in Peninsular Malaysia, 2000-2004.

Site	2000		2001		2002		2004	
	Emergence Success*	Nests	Emergence Success*	Nests	Emergence Success*	Nests	Emergence Success*	Nests
Ma'Daerah (Hatchery)	75.0 ± 2.9	54	74.9 ± 4.6	35	90.5 ± 4.6	10	81.6 + 17	69
Mak Kepit (in situ)	79.8 ± 1.7	224	89.0 ± 1.2	247	86.7 ± 7.7	21	84.1 + 19	26

* emergence success in % ± SE

Are hatcheries too hot or cold, and what are the sex ratios of the hatchlings produced?

The sand temperatures (at nest depth) measured at the 50-70% shaded hatcheries in 2000 ranged from 31 to 33 degrees - a high range compared to natural nests in pristine areas that ranged from 27 to 31 degrees. With a pivotal temperature of 29.5^{0}C determined by Kamarruddin Ibrahim for this population of green turtles, these high sand temperatures would suggests that nearly all hatchlings produced were females and that further shading methodology needs to be tried in the future to bring nest temperatures down to a more natural range.

In 2001 we used temperature data loggers that could be placed in the middle of incubating clutches and began to experiment with alternative hatchery shading (70% and 100%) and nest depths (50cm and 75cm) to see if these variables affected nest temperatures (Van de Merwe et al., 2005a,b). A 70:30 male:female sex ratio was produced and all nest temperatures during this study ranged between 28C and 30C, including those under 70% shading that had been used in previous years when sand temperatures were 31C to 33C. This experiment further determined that increasing the depth (from 50cm to 75cm) or degree of shading (from 70% to 100%) had very little influence on lowering nest temperatures. Furthermore, the effect of metabolic heating of incubating eggs was found to be more influential on the mean nest temperatures than either nest depth or hatchery shading. This prompted us to further investigate the different temperature microenvironments within and around clutches in 2002. In a number of cases where the majority of eggs incubated the full course of incubation, we found that the data logger placed in the centre of the clutch recorded larger increases in temperature over the final third of incubation, when metabolic heating is coming into effect (Figure 1).

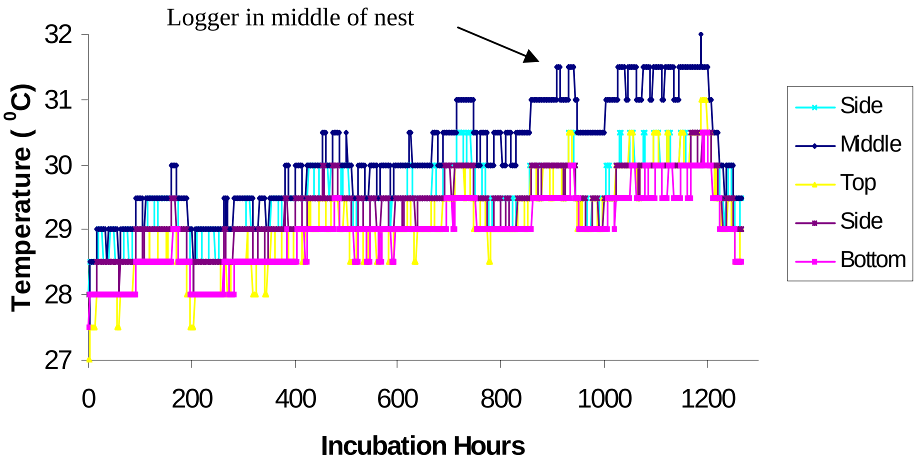

Figure 1. Example of the temperature recorded by the five data loggers placed at different locations in each clutch. Note the middle data logger recorded higher temperatures over the final third of incubation.

In 2004 we found that sand temperatures in the hatchery at Ma'Daerah actually increased with depth (Figure 2). This indicates that there maybe other thermal factors influencing nest temperatures and that relocating closer to the surface and the effect of the shade cloth may be optimal for reducing nest temperatures and maintaining natural sex ratios.

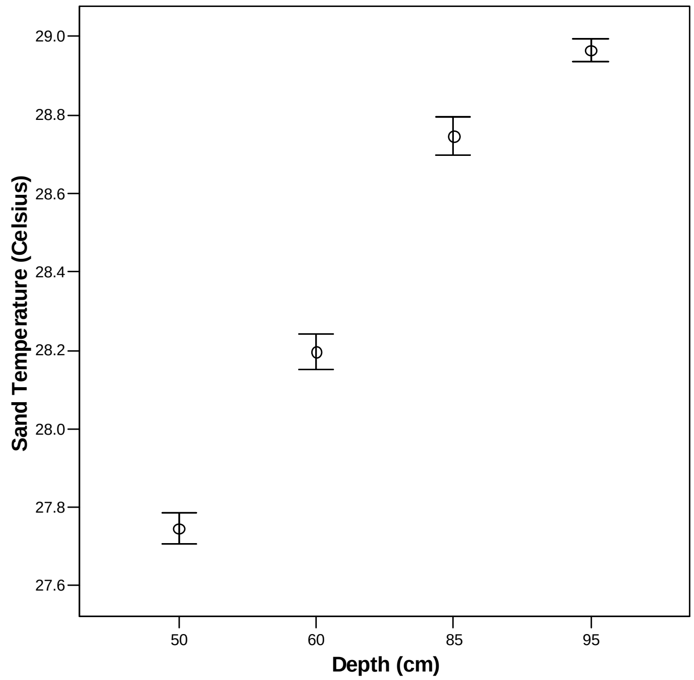

Figure 2. Mean nest sand temperatures at depth of 50cm, 60cm, 85cm and 95cm in the Ma'Daerah hatchery, Terengganu, Malaysia 2004.

Are hatcheries producing high quality hatchlings?

In 1999 and 2000, when hatchery sand temperatures were 31C to 33C, hatchlings from mainland hatcheries were found to have slower running and swimming speed, more scale abnormalities and smaller mass:length ratios than hatchlings emerging from the *in situ* nests of the island site, Mak Kepit. However, in 2001 and 2002, when nest temperatures were lowered to 28C to 30C, the running speed and mass:length ratios were found to be higher in hatchlings from the hatchery sites. It was also determined in 2001 that hatchlings emerging from shallower nests (50cm deep) had a higher mass:length ratio, indicating higher quality in terms of energy reserves for the offshore dispersal stage following emergence. In 2004, similar trends were observed with hatchlings from the Ma'Daerah hatchery being significantly faster at running (Figure 3) and having a significantly larger mass:length ratio (Figure 4) than hatchlings from the undisturbed *in situ* sites of Mak Kepit.

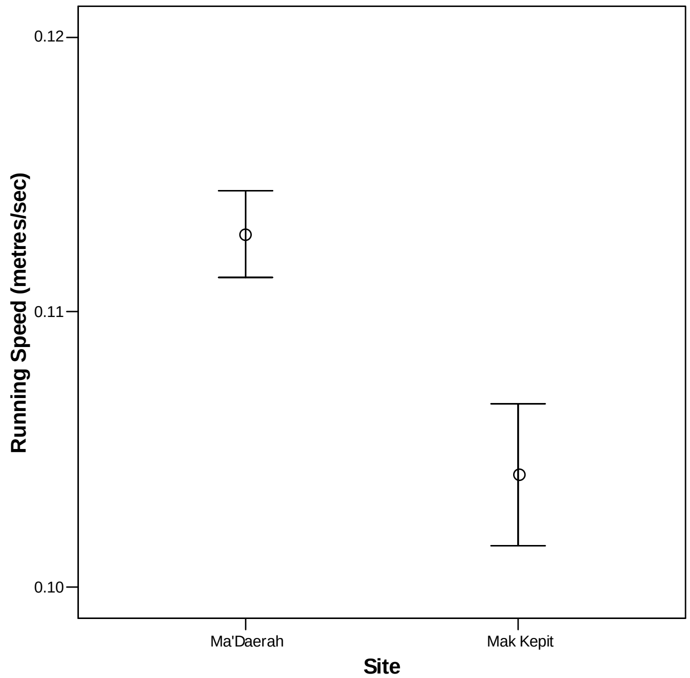

Figure 3. Running speed (ms^{-1} $\underline{+}$SE) of *Chelonia mydas* hatchlings emerging from nests at Ma'Daerah hatchery and Mak Kepit *in situ* site, Terengganu, Malaysia, 2004. Running speed of hatchlings from Ma'Daerah (0.113 ms^{-1} + 0.002) is significantly faster than hatchlings from Mak Kepit (0.104 ms^{-1} + 0.002) (ANOVA: df = 1, F = 9.208, P = 0.003).

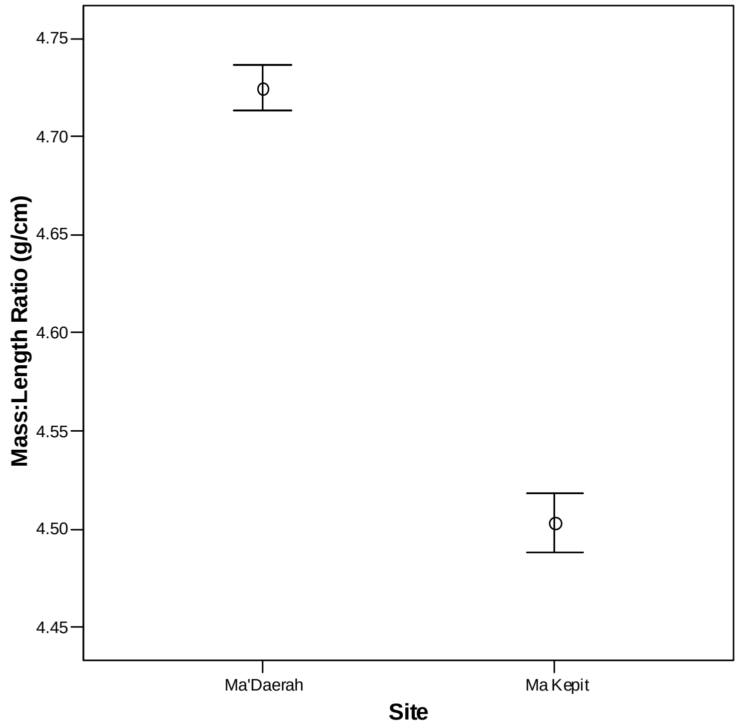

Figure 4. Mass:SCL ratio (gcm^{-1} $\underline{+}$SE) of *Chelonia mydas* hatchlings emerging from nests at Ma'Daerah hatchery and Mak Kepit *in situ* site, Terengganu, Malaysia, 2004. Hatchlings from Ma'Daerah (4.725 gcm^{-1} $\underline{+}$ 0.012) have a higher mass:length ratio than hatchlings from Mak Kepit (4.503 gcm^{-1} $\underline{+}$ 0.015) (ANOVA: df = 1, F = 135.033, $P < 0.0005$).

In contrast to these results, we found that hatchlings from the Ma'Daerah hatchery took more than 2 times longer to flip themselves back to the upright position (Figure 5).

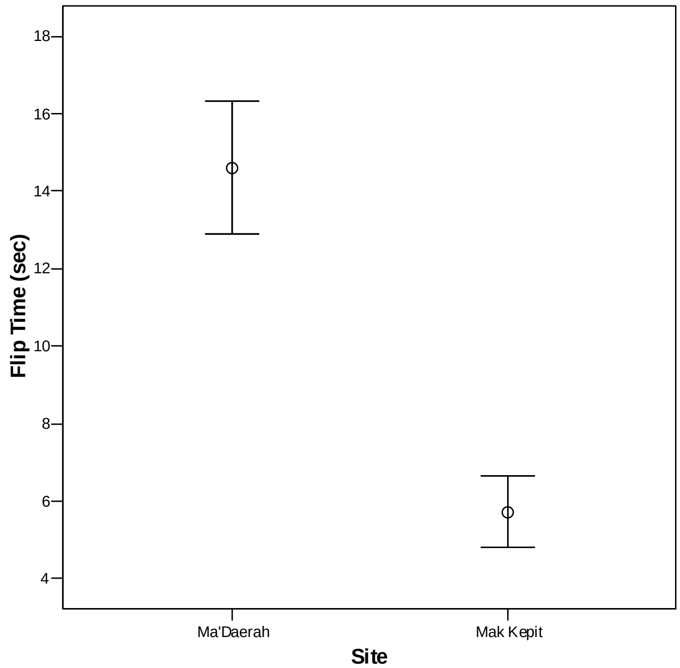

Figure 5. Flip time (sec. ±SE) of *Chelonia mydas* hatchlings emerging from nests at Ma'Daerah hatchery and Mak Kepit *in situ* site, Terengganu, Malaysia, 2004. Hatchlings from Ma'Daerah (14.6 secs ± 1.4) take significantly longer to flip back over than hatchlings from Mak Kepit (5.7 secs ± 2.0) (ANOVA: df = 1, F = 13.23, P <0.0005).

How does retention of hatchlings in hatchery nests affect hatchling quality?

One of the decisions faced by Malaysian hatchery managers is how often to check the hatchery to release any emerged hatchlings. Sea turtle hatchlings emerge in a state energetic frenzy and with a finite supply of energy in the residual yolk sac to fuel off shore dispersal. In Peninsular Malaysian hatcheries, emerging hatchlings are restrained from running directly to the ocean by nest nets and rely on hatchery staff for their release. We found that after retaining hatchlings for 1, 3 and 6 hours running speed of hatchlings decreased significantly over each time period and hatchlings tested 6 hours after emergence ran 50% slower than hatchlings tested when they first emerged (figure 6; Van de Merwe et al. 2005c). Mass:length ratio did not decrease after the first hour but significantly decreased after 3 hours and 6 hours of restraint (figure 7).

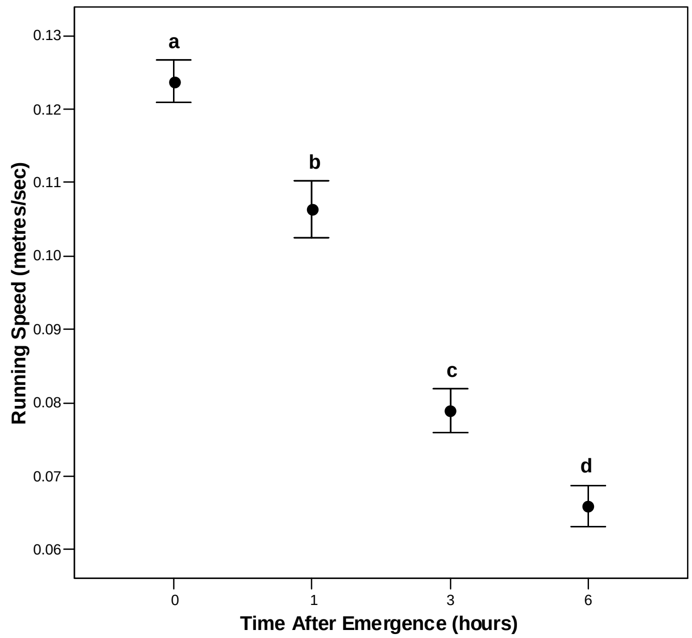

Figure 6. Mean running speed (ms^{-1} $\underline{+}$SE) for *Chelonia mydas* hatchlings tested at time 0, 1, 3 and 6 hours after emergence from nests in the Ma'Daerah Hatchery, Malaysia, 2004. a, b, c and d are significantly different (P<0.05).

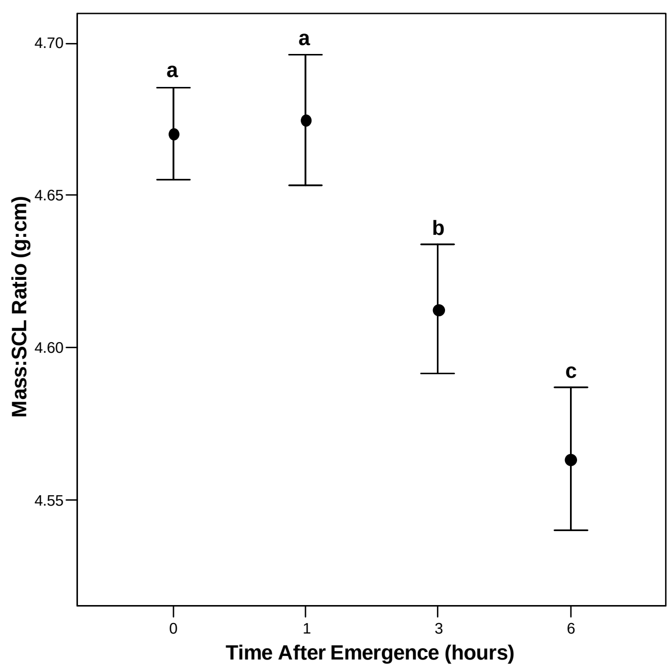

Figure 7. Mean mass:Straight carapace length (SCL) ratio ($\underline{+}$SE) of *Chelonia mydas* hatchlings measured at time 0, 1, 3 and 6 hours after emergence from nests in the Ma'Daerah Hatchery, Malaysia, 2004. a, b and c are significantly different (P<0.05).

SIGNIFICANCE AND BENEFITS OF THE RESEARCH

Nest Temperature

The nest temperature profiles for all relocated clutches show a consistent pattern over the course of incubation. The temperature remains relatively constant over the first third of incubation followed by a gradual increase peaking towards the end and then falling again just before the hatchlings emerge. This trend is not observed in temperature data collected from nearby sand at nest depth, which shows no general increase in temperature over the course of the incubation period. This indicates that the increase in nest temperature observed over the latter stages of incubation is not due to external factors (e.g. air temperature, rainfall) affecting the sand temperature in the hatcheries and is presumably due to metabolic heating of the clutch. This is further supported by the findings in 2002 that the data logger in the middle of the clutch recorded a greater increase in temperature over this final third of incubation. On the peripheries of the clutch (top, bottom and sides) there was not as a distinct increase in temperature as these areas are further from the heat source of the developing eggs.

Previous research has found metabolic heating to increase nest temperatures by 3-6^{0}C over the course of incubation. As each embryo develops, more tissue is synthesized and the embryo generates heat as it maintains tissues and continues growth. In this study, metabolic heating was estimated by the number of eggs in each nest that hatched successfully as each egg that incubates the full term to produce a hatchling would contribute to the metabolic heating of the nest. Nests illustrate evidence that this is a reasonable estimation with low hatchling numbers having much smaller increases in nest temperature over the course of incubation compared to nests with high hatchling numbers.

The provision of 50-70% shade beginning in 2000 was not initially adequate to balance the sex ratio of offspring, with temperatures still exceeding 31C, indicating nearly 100% female production. However, in 2001 hatcheries under 70% and 100% shade cloth reduced nest temperatures to between 28C and 30C, which produced a 70:30 sex ratio (determined histologically). Nest temperatures in 2002 under 70% shade cloth were also around pivotal temperatures, which also indicates a more balanced sex ratio being produced in the past two years. In a period of global warming, nest temperatures need to be continually monitored and hatchery designs need to be further investigated to reduce nest temperatures further in the future. These results highlight the problems of regional warming and the effect of the total loss of natural beachside vegetation in some areas. The continuation of green turtle production along some of these mainland beaches now may only be possible in a hatchery, as exposed beach sand areas are most likely lethal because they are too hot. Other means need to be explored for cooling down the beaches, and it would be ideal if these could be linked to programs that would encourage *in situ* nest incubation, even on the mainland.

The temperature profile of the hatchery sand determined in 2004 indicated that sand temperatures increased with depth. This information can be used by hatchery managers to determine the best nest depths for optimal sex ratios. To produce more male hatchlings, clutches should be relocated closer to the surface where sand temperatures are cooler.

What other factors might be important to help manage hatcheries better?

Our studies in previous years also found that there were significant differences in nest depths and in incubation times between hatcheries and the natural incubation site. Nest depths at all hatcheries were significantly shallower than those in natural nests at Mak Kepit. This result and the difference in sand colour and natural vegetation shading may account for the lower nest temperatures observed at Mak Kepit. Related to this, incubation times were significantly longer at Mak Kepit. Nest depth will also influence the time between hatching and emerging, as hatchlings would have further to climb from deeper nests. As hatchlings hatch with a finite energy supply for the 3-5 days of offshore dispersal, hatchlings emerging with larger yolk reserves may be able to maintain high-energy swimming for a longer period. Evidence of larger energy reserves has been observed in the larger mass:length ratios observed in hatchlings from the 50cm nests compared to hatchlings from 75cm nests in 2001. It may therefore be advantageous to relocate eggs at shallower depths to maximise the mass:length ratios and hence the energy available for the offshore dispersal.

The density of nests in the hatchery has also been found to influence the quality of hatchlings, with higher densities producing slower running hatchlings with lower mass:length ratios and hence less available energy for offshore dispersal. This could be due to reduced ability for gases to exchange between the eggs and the surrounding sand and needs to be considered in the management of all hatcheries around the world. Other factors such as oxygen and water in nests and their effects on hatchling fitness during digging also need to be studied. The observations on nest depths also indicated that some hatchery nests are much too shallow, especially at Chendor, and that hatchery staff members need to be educated about the importance of digging nests to a recommended depth. Although this problem was identified in 1999 no changes in nest depths were implemented in 2000. However, our results in 2001 demonstrate the importance of appropriate nest depths.

The reduced running speed and mass:length ratios observed in hatchlings retained in the nests after emergence also has direct hatchery management implications. A decreased running speed would increase the exposure of hatchlings to near shore predators and may indicate a reduction in activity and energetic status. Decreased mass:length ratio over the 6 hours indicated increased absorption of the residual yolk sac, which could compromise the duration of offshore dispersal. Therefore, we recommended that hatcheries be checked at least every thirty minutes to release all emerged hatchlings immediately and maximise the speed and duration of hatchling dispersal.

The results of these hatchery design and operation studies have contributed significantly to our primary objectives of learning more about the biology of incubation and hatchlings of Green Sea Turtles in Peninsular Malaysia. These results have important and direct implications for improving hatchling production at hatcheries. The results of these studies will be directly applied to improve hatchery management in 2005 and beyond. They also have indicated an essential area for further study that may lead to long-term improvement of sea turtle conservation both in this region and at other locations around the world.

We have also documented that the production of hatchlings from mainland hatcheries is high, comparable in numbers to what is found in natural nests on offshore islands and at other locations in the South Pacific region. This indicates that shading of the hatcheries has been successful to the extent that hatchling mortality has been significantly reduced in comparison with previous years. We have also shown that hatchling vigor improves with shading to the point where we are now observing hatchlings from hatcheries to be superior to *in situ* hatchlings in terms of running speed and mass:length ratios (an estimate of energy reserves).

The results of the hatchling vigour experiments are the first reported that suggest a difference in fitness of hatchery produced hatchlings from that of naturally produced hatchlings. This raises a larger question: How should one judge short-term success in hatchery programs? Most hatcheries report productivity as a measure of the ability of eggs to produce hatchlings or as the number of hatchlings released. There have been more recent attempts to manage incubation temperatures to provide optimal sex ratios of hatchlings. Our data suggest that good hatchery management should also include some measure of hatchling performance or fitness. We would also suggest, ultimately, that some measure of hatchling health be monitored as well. We have found abnormalities in scale counts of hatchlings are correlated with their low vigor in running and swimming performance measures. This suggests that simple, on-beach approaches to hatchery and hatchling monitoring may be possible.

We plan several future avenues of work that has resulted from this past year's study. More controlled incubation experiments could be done to test effects of different incubation temperatures and regimes on hatchling fitness. It is as important to determine if there is a "pivotal" temperature or temperature range above or below which the hatchlings are less fit. Research of this nature will either lead to further advances in hatchery management or, if not successful, may provide a stronger case for protection of more eggs on the beaches in natural nests.

REFERENCES

Chan, E.H. (1990). The status and conservation of sea turtles in Malaysia. Paper presented at the Symposium on the State of Nature Conservation in Malaysia, 24-26 August 1990.

Dutton, P.H., Whitemore, C. P. and Mrosovsky, N. (1985). Masculinisation of leatherback turtles *Dermochelys coriacea* hatchlings from eggs incubated in styrofoam boxes. Biological Conservation 31, 249-264.

Eckert, K.L. and Eckert, S. A. (1990). Embryo mortality and hatch success in in situ and translocated leatherback sea turtle *Dermochelys coriacea* eggs. Biological Conservation 31, 249-264.

Kamarruddin, I. (1994). The status of marine turtle conservation in Peninsular Malaysia. pp. 87-103 In: Proceedings of the First ASEAN Symposium-Workshop on Marine Turtle Conservation (Nacu et al., eds.). Manila, Philippines, 1993.

Leh, C.M.U. (1989). The green turtle, *Chelonia mydas* (L.) in Sarawak: is there a future? Proceedings 12th Annual Seminar of the Malaysian Society of Marine Sciences, pp. 219-225.

Leh, C.M. U. (1994). Hatch rates of green turtle eggs in Sarawak. Hydrobiologia 285, 171-175.

Limpus, C. (1991). Recommendations for the conservation and tourism usage of the Sarawak green turtle population. Queensland Department of Environment and Heritage, Brisbane, Queensland, Australia, 45 pp.

Limpus, C. (1993). Recommendations for conservation of marine turtles in Peninsular Malaysia. report to Department of Fisheries, Ministry of Agriculture, Malaysia. Queensland Department of Environment and Heritage, Brisbane, Queensland, Australia, 60pp.

Limpus, C.J., Baker, V. and Miller, J.D. (1979). Movement induced mortality of loggerhead eggs. Herpetologica 35, 335-338.

Limpus, C.J., Reed, P.C. and Miller, J.D. (1985). Temperature dependent sex determination in Queensland sea turtles: Intraspecific variation in *Caretta caretta*. In Biology of Australian Frogs and Reptiles (Grigg, G., Shine, R. and Ehman, H., eds.). Royal Zoological Society of New South Wales, Sydney, Australia.

Miller, J.D. and Limpus, C.J. (1983). A method for reducing movement-induced mortality in turtle eggs. Marine Turtle newsletter 26, 10-11.

Mrosovsky, N. (1982). Sex ratio bias in hatchling sea turtles from artificially incubated eggs. Biological Conservation 23, 309-314.

Parmenter, C. J. (1980). Incubation of eggs of the green sea turtle, *Chleonia mydas,* in the Torres Strait, Australia: the effect of movement on hatchability. Australian Wildlife Research 7, 487-491.

Siow, K.T. and Moll, E.O. (1982). Status and conservation of estuarine and sea turtles in West Malaysian waters. pp. 339-348 In: Biology and Conservation of Sea Turtles, Proceedings World Conference on Sea Turtle Conservation (Bjorndal, K.A., ed.) Smithsonian Institution Press, Washington, D.C.

Van de Merwe, J. K. Ibrahim and J. Whittier. 2005. Effects of hatchery shading and nest depth on the development and quality of *Chelonia mydas* hatchlings: Implications for hatchery management in Peninsular Malaysia. Australian Journal of Zoology, In Press.

Van de Merwe, J, K. Ibrahim and J. Whittier. 2005. Effects of nest depth, shading, and metabolic heating on nest temperatures in sea turtle hatcheries. Chelonian Conservation Biology, In review.

Van de Merwe, J., Ibrahim, K. and Whittier, J. 2004. The effects of delaying the release of *Chelonia mydas* hatchlings emerging from hatchery nests in Malaysia. Poster presentation at the 25th Symposium on Sea Turtle Biology and Conservation, Savannah, Georgia, U.S.A.

PUBLICATIONS AND OUTCOMES

Scientific presentations:

Van de Merwe, J. K. Ibrahim and J. Whittier. 2005a. Effects of hatchery shading and nest depth on the development and quality of *Chelonia mydas* hatchlings: Implications for hatchery management in Peninsular Malaysia. Australian Journal of Zoology, In Press.

Van de Merwe, J, K. Ibrahim and J. Whittier. 2005b. Effects of nest depth, shading, and metabolic heating on nest temperatures in sea turtle hatcheries. Chelonian Conservation Biology, In Press.

Van de Merwe, J., Ibrahim, K. and Whittier, J. 2005c. The effects of delaying the release of *Chelonia mydas* hatchlings emerging from hatchery nests in Malaysia. Poster presentation at the 25th Symposium on Sea Turtle Biology and Conservation, Savannah, Georgia, U.S.A., January, 2005.

Whittier, J. 2002. Hatchery management in peninsular Malaysia: a progress report. Earthwatch Australia Conference, Melbourne, Australia.

Schauble, C., Hamann, M., Ibrahim, K., Whittier, J., Kassim, A.R. 2002. Hatchery management in peninsula Malaysia: 3 years of data on nest and hatchling attributes. 22nd Symposium on Sea Turtle Biology and Conservation, Miami, Florida, USA.

Ibrahim, K., Kassim, A.R., Schauble, C., and Hamann, M. 2002. Making the most of hatchling production in peninsular Malaysia: an urgent need to increase egg protection in marine parks., Miami, Florida, USA.

Hamann, M. 2002. Hatchery Management of Green Turtles in Malaysia. Earthwatch Lecture, Royal Geographical Society, London, UK.

Whittier, J. 2001. Hatchery monitoring in Malaysia. Earthwatch Australia poster presentation, Melbourne, Australia.

Van de Merwe, J. 2002. The effects of nest temperature and nest depth on the morphology, performance and blood physiology of *Chelonia mydas* hatchlings in Peninsula Malaysia. Honours Thesis, University of Queensland, Australia.

Mark Hamann, Ibrahim, K. Schauble, C., Kassim A. R., Whittier, J. 2001. An Integrated Approach to Hatchery Management: Nest and Hatchling Attributes. 21st Symposium on Sea Turtle Biology and Conservation, Philadelphia, Pennsylvania, USA.

Chloe Schauble, Ibrahim, K., Hamann, M. and Whittier, J. 2001. Hatchery monitoring: A role for integrated measures of hatchling quality. 21st Symposium on Sea Turtle Biology and Conservation, Philadelphia, Pennsylvania, USA.

Whittier, J., Ibrahim, K. Hamann, M., and Jessop, T. 2000. Looking after Malaysian Sea turtles: Hatcheries or Habitats. Earthwatch Millenium Fellows Conference, Oxford, UK. Invited speaker, sponsored by Millenium Commission.

Whittier, J., Hamann, M., and Ibrahim, K. 2000. Green turtles of Malaysia. Poster presentation to Earthwatch Millenium Fellows Conference, Oxford, UK.

Hamann, M. Ibrahim,K. and Whittier, J. 2000. Measuring the success of sea turtle hatcheries in Malaysia: using emergence success, sex ratios and hatchling performance as indicators. 20th Symposium on Sea Turtle Biology and Conservation, Orlando, Florida, USA.