Asian Journal of Conservation Biology, July 2014. Vol. 3 No. 1, pp. 83–87 AJCB: SC0013
ISSN 2278-7666 ©*TCRP* 2014

Heavy metal compositions in Green turtle (*Chelonia mydas*) eggs from nesting beaches in Peninsular Malaysia

Juanita Joseph[1], Siti Norazliyana Ali[1] & Hing Lee Siang[2]

[1]*Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia;*
[2]*School of Marine Science and Environment, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia.*

(Accepted June 15, 2014)

This study investigates the concentration of heavy metals in fresh and un-hatched green turtle eggs from two major nesting beaches in (Ma' Daerah, Terengganu and Cherating, Pahang) Peninsular Malaysia. All five heavy metals (Mn, Cu, Zn, Cd and Pb) were detected in the eggs. Statistical analysis shows no significant difference of heavy metals concentration between the yolk of rotten and fresh eggs. Therefore, the yolk of rotten eggs can be used as heavy metals indicator for future study without sacrificing fresh eggs. The level of lead detected exceeded the permissible limits of the Malaysian Food Regulation 1985. The presence of toxic contaminants in sea turtle eggs can be attributed to the chemical pollutants present in their feeding and breeding sites. Sea turtles have long lifespan and can bio-accumulate contaminants from their food, sediment and water. The presence of toxic metals in green turtle eggs may cause health problem for those who likes to consume turtle eggs. This finding is important to increase public awareness on the danger of eating sea turtle eggs as well as to reduce egg collection for human consumption in Malaysia.

The green turtle, *Chelonia mydas* is listed as endangered species in IUCN Red List of threatened species and under the Appendix I of CITES. This species is threatened by all factors that threaten other sea turtles, including exploitation for eggs, meat, curio trade, loss of nesting and foraging habitat, incidental capture in fishing gears and marine pollution. In Peninsular Malaysia, one of the major reasons that contributed to the decline of green turtle nesting is the excessive collection of eggs for local consumption. Until recently, except for the leatherback turtle eggs, commercial sale of sea turtle eggs are still allowed in Peninsular Malaysia. Only Sabah and Sarawak had the total protection for these endangered marine animals. Differences in legislation to protect sea turtles by each state in Malaysia had accorded different protection to these animals.

In Peninsular Malaysia, the States Fisheries Department will issue license for egg collectors. Eggs collected must then be sold back to the Fisheries Department and incubated in the hatchery. However, the presence of many illegal egg collectors and sometimes lack of funding from the government to buy the eggs for incubation had resulted to high number of eggs being sold in the local market. In recent years, the protection of green turtle eggs in Peninsular Malaysia had increased where all the major nesting beaches were gazetted as turtle sanctuary. Yet, because of the high demand for sea turtle eggs in Peninsular Malaysia and no existing legislation to ban the selling of eggs, smuggling of turtle eggs had increased. WWF-Malaysia (2009) reported that most of the eggs sold in Pasar Payang, Kuala Terengganu were smuggled illegally from Sabah and the Philippines. Due to lack of enforcement and legislation, harvesting of turtle eggs had contributed as one of the major factor to the decline of sea turtle nesting in Peninsular Malaysia.

Previous studies had reported that sea turtle eggs are affected from toxic contaminants (Sakai et al., 1995, Godley et al., 1999 and Lam et al., 2006). Heavy metals measured in the egg yolks of sea turtles could beused to predict body burden in nesting females (Sakai et al., 1995 and Sakai et al., 2000). A study on the level of trace elements in green turtle eggs collected from Hong Kong shows that concentrations of Selenium (Se), Lead (Pb) and Nickel (Ni) in the eggs were generally higher (Lam et al., 2006). The fact that sea turtles have a long lifespan and high mobility increase their chances of being exposed to environmental toxicants. In today's modern world, most of the industrial, agricultural, aquacultures and others human activities waste were easily discharged into the sea without having proper treatments. Most of the waste contains toxic metals compositions that will accumulate in the marine environments or habitat of sea turtles. Off-shore mineral and oil explorations as well as the discharge of waste from oil tankers further complicate the marine pollution problems. Long range transport of pollutants is also a threat to aquatic species as well as humans (Serfoh-Armah et al., 1999). Besides from polluted environments, feeding behavior can also influence the accumulation of heavy metals in sea turtles. The diets of green turtle are mainly consists of marine algae and seagrasses, which are known to have the ability to accumulate heavy metals in their cells or tissues (Valchos et al., 1998; Khristoforva and Kozhenkova, 2002. These heavy metals can also be transferred from the mother to their eggs. Essential metals such as Fe, Mn, Zn and Cu are used in the development of the embryo within the eggs. As for toxic metals such as Cd, Pb and Hg, limited amounts will be transferred from the mother to the eggs. Non – essential and essential metals can act as toxicants at elevated concentrations in organisms (Devkota and Schmidt, 1999; Kobayashi and Okamura, 2004). The study of heavy metal composition in green turtle eggs in Peninsular Malaysia is potentially important for both conservation of sea turtles and for public health awareness. Information obtained in this study may stop the consumption and collection of sea turtle eggs in Peninsular Malaysia.

*Corresponding Author's E-mail: *juanita@umt.edu.my*

MATERIALS AND METHODS

Sample Collection

Egg samples were obtained from Ma' Daerah, Terengganu and Cherating, Pahang in 2008. Both sites have been gazette as turtle sanctuaries. All turtle nests at these areas were transferred and incubated in protected hatcheries. The sanctuaries are managed by the State Department of Fisheries. Both sanctuaries are the main nesting sites for green turtle in Terengganu and Pahang

A total of 16 fresh eggs from four different individuals were used in this study. Six eggs were collected from Cherating and the remaining eggs were collected from Ma'Daerah. All fresh eggs were taken immediately after the egg laying process. For the rotten eggs, a total of 18 samples from four different nests were collected randomly from the hatchery in Ma'Daerah and Cherating. Rotten eggs were eggs incubated in the hatchery but failed to hatch after more than 45 days. Both fresh and rotten eggs collected were preserved in a freezer (-20°C) until further analyses.

Sample preparations

Prior to metal analyses, samples were thawed at ambient room temperature and washed with distilled water. Calliper and portable scale were used to measure the size and weight of the eggs. Each of the eggs was separated to three parts which are the egg yolk, eggshell and albumen. The wet weight of egg yolk, eggshell and albumen were taken before drying it in the oven. Samples were dried at 80°C until it reached a constant weight. Mortar and pestle were used to grind the samples to a smaller component.

Heavy Metal Analyses

Digestion of samples were done following the EPA Acid Digestion Method (3050 B) with few modification. All digested samples were kept in the refrigerator before being analyzed using ELAN ® 9000 ICP-MS. In this study, five trace elements Mn, Cu, Z, Cd and Pb were analyzed. All concentrations are based on dry weight basis.

Data Analysis

Statitical analyses Anova Univariate and Mutivariate (SPSS ver. 11) were used to compare heavy metals concentration between the eggs compartments, fresh and rotten eggs, and the sampling site.

RESULTS AND DISCUSSION

Essential and non-essential elements in eggs

Results from this study showed high concentration of essential elements such as Zn and Cu. These metals were detected in all eggs compartments. Concentration of Zn, Cu and Mn were higher in egg shell, albumen and egg yolk of fresh eggs collected from Cherating compared to those that collected from Ma'Daerah (Figure 1). Zn was detected as the highest element in the egg yolks of fresh eggs. The concentration detected was 84.61 µg g^{-1} (Cherating) and 66.54 µg g^{-1} (Ma'Daerah). This result is an agreement with other studies. According to (Lam et al., 2006) the concentration of Zn in the egg yolk of green turtle eggs collected from Hong Kong was significantly higher than those of the albumen and egg shell. In this study, higher concentration of Cu and Mn were detected in the egg shell compared to albumen and egg yolk (Figure 1). Study on the concentration of heavy metals from green turtle eggs in Turkey shows a high concentration of Cu in egg shells compared with other eggs compartments (Celik et al., 2005). According to Storelli and Marcotrigiano (2003), sea turtle's egg shell contained highest levels of Cu and Mn. Stoneburner et al., 1980 suggested that the concentration levels of these essential metals were necessary for successful embryonic development. Zn is an important element in the development of yolks, whereas Cu and Mn is an important element in the development of egg shell.

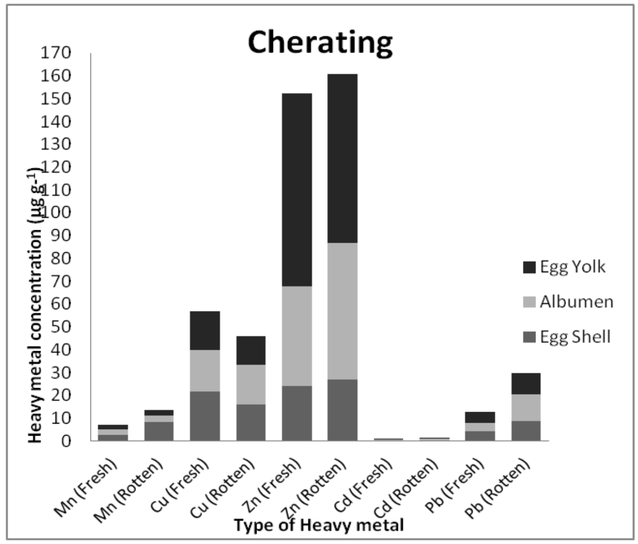

Figure 1. Comparison of heavy metals concentration in egg shell, albumen and egg yolk of fresh and rotten eggs collected from Ma'Daerah and Cherating (µg g^{-1} dry weight).

Non essential elements such as Cd and Pb were detected in all samples in this study. Concentration of Cd was higher in the egg shell and egg yolk of fresh eggs collected from Cherating compared to those collected from Ma'Daerah. The concentration of Cd detected in egg yolk of fresh eggs from Cherating was 0.34 µg g^{-1} while 0.23 µg g^{-1} from Ma'Daerah. For rotten eggs, the concentration of Cd was higher in albumen for samples from Ma'Daerah and Cherating which is 0.42 µg g^{-1} and 0.60 µg g^{-1} respectively. Pb concentration are higher in all part of fresh eggs collected from Ma'Daerah with the range from 5.06 µg g^{-1} to 6.21 µg g^{-1} compared to Cherating (3.56 to 4.78 µg g^{-1}). As for rotten eggs, higher concentration of Pb detected in egg yolk and albumen collected from Cherating compared with Ma'Daerah. Concentration of Pb detected in egg yolk and albumen of rotten eggs from Cherating were 9.37 µg g^{-1} and 11.43 µg g^{-1} respectively. While for Ma'Daerah, concentration detected was 5.72 µg g^{-1} (egg yolk) and 9.56 µg g^{-1} (albumen). Overall Cd and Pb concentration were highly detected in rotten eggs compartments compared to fresh eggs compartments (Figure 1). Statistical analyses (ANOVA – univariates) shows significance difference ($p < 0.05$) in concentration of heavy metals between egg shell, albumen and egg yolk of fresh eggs collected from Ma'Daerah and Cherating Turtle Sanctuary. Differences in heavy metals between individual turtles may be due to their behavior which is highly mobile where the distance between breeding and feeding grounds can be hundreds to thousands kilometres (Plotkin, 2003). It is possible that the contaminants found in the tissues of marine turtles can be attributed to chemical pollutants present in both their feeding range and breeding sites (Lam et al., 2004). Feeding behavior can also influence the accumulation of heavy metals in sea turtles. The main diets of green sea turtle are marine algae and seagrasses. Marine algae and seagrasses are known to have ability to accumulate heavy metals in their cells or tissues (Valchos et al., 1998; Khristoforva and Kozhenkova 2002). Celik et al. (2005) found high concentrations of Cd, Pb and Fe in the sea grass, *Posidonia oceanica* around the nesting environment of green turtles in Turkey. Since the adult green turtles are herbivorous and feed mainly on seagrass, they might be affected from heavy metal contamination (Celik et al., 2005). Benthic macroalgae can concentrate metal ions from seawater and thus, macrophytic algae, especially green and brown seaweed have been widely used as indicators of trace metal pollution (Sivalingam and Ismail, 1981; Ho, 1990).

Heavy metals are not sequestered equally in eggs (Burger, 1993). Maternal transfer of energy, nutrients and trace elements during egg production could be especially high in leatherback turtle (*Dermochelys coriacea*) because they have the highest reproductive output among reptiles (Miller, 1997). Leatherback turtle has a bigger eggs size and can accumulate and eliminate high concentration of heavy metals compared to green turtles. Study by Aguirre (1994) reported that the concentration of Cd in kidney of green turtle (39.4 µg g^{-1}) was higher compared to the concentration detected in liver (9.30 µg g^{-1}) and muscle (0.062 µg g^{-1}). Therefore, maternal transfer is likely to depend on the species accumulation and elimination capability, the level of contamination and the nature of the element considered.

Statistical analyses (ANOVA – univariates) shows that there was no significance difference ($p > 0.05$) in concentration of heavy metals between egg shell, albumen and egg yolk of rotten eggs collected from Ma'Daerah and Cherating Turtle Sanctuary. Generally, Cd is highly toxic to wildlife and potentially causing mutation. It is associated with increased mortality, growth reduction and reproduction (Eisler, 1985). At the biochemical level, Cd can negatively affect DNA, RNA, and ribosome synthesis (Gerhard et al., 1998), as well as deactivate several enzyme systems (Doi et al., 1993). At the organism level, Cd is known to be tetratogenic and embryotoxic (Sundermann et al., 1995). In addition chronic Cd exposure results in lower fecundity and decreased overall reproductive success in marine organisms (Singhal et al., 1985). Birds and mammals suffer effects from Pb poisoning such as damage to the nervous system, kidneys, liver, sterility, growth inhibition, developmental retardation, and detrimental effects in blood. Pb poisoning in higher organisms such as green turtles primarily affects hematologic and neurologic processes (Eisler, 1998).

Potential adverse effects of green turtle eggs consumption to humans

In this study, toxic contaminants such as Cd and Pb were detected in green turtle eggs. Figure 2 shows the concentration of heavy metals in albumen and egg yolk of fresh sea turtle eggs found in this study. Table 1 shows the comparison of Cd and Pb concentration detected in this study with the permissible value from the Malaysian Food Regulation (1985). The concentration of Pb in green turtle eggs exceeds the permissible limits of Pb, however the Cd concentration was below the concentration of permissible limits. Even though Cd concentration is low, Cd has the ability to accumulate in human's body. The presence of Pb and Cd in green turtle eggs might cause and adverse effects to human health due to the eggs consumptions.

Table 1. Guidelines of heavy metals (mg kg^{-1}) for food safety set by Malaysian Food Regulation (1985).

Element	Malaysian Food Regulation (1985) (mg kg^{-1}) (wet weight)	Metal level of green turtle eggs (this study) (µg g^{-1}) (dry weight)
Cu	**30.00**	25.74
Zn	**100.00**	106.23
Cd	**1.00**	0.61
Pb	**2.00**	9.46

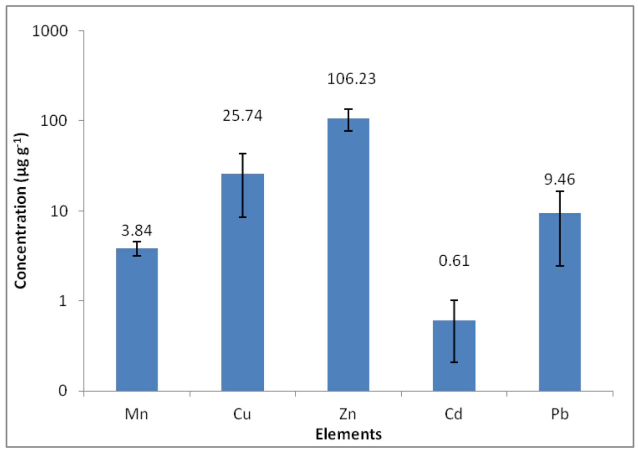

Figure 2. Overall concentration of heavy metals in fresh sea turtle eggs (albumen + egg yolk).

Environmental contaminants such as heavy metals may be transferred to humans upon consumption of contaminated seafood. Heavy metals persist and bioaccumulate in marine ecosystems and this compound reach especially high concentrations in long-lived organisms such as sea turtles. Pb toxicity can caused adverse behavioral, physiological and biochemical effects on humans. Neurological impacts of Pb include hyperactivity, poor attention span and low IQ especially in children. Besides, Pb can also disrupt the enzymes activity. It's inhibited the haemoglobin synthesis and caused insufficient transport of oxygen and anemia. Anemia caused children fatigue easily and are less able to learn well (Bradl, 2005; Wright and Welbourn, 2002; Landis and Yu, 2003). Exposure of Cd in humans' body system can contribute to renal dysfunction, increased risk of osteoporosis, possible link to type – 2 diabetes, renal and prostate carcinogen (Jarup et al., 1998; Goyer et al., 2004; Kazantzis, 2004).

CONCLUSION

The concentrations of heavy metals (Mn, Cu, Zn, Cd and Pb) were detected in all fresh and rotten eggs compartments. Most of the elements detected were found accumulated in the yolk compared to albumen and egg shell. The highest element concentration detected was Zn and the lowest concentration of element detected was Cd. Concentration of Zn and Cu was high due to their character as an essential element for the development of eggs. Statistical analyses (ANOVA – multivariate) shows that there was no significance different ($p > 0.05$) in heavy metal concentration between egg yolk of fresh and rotten eggs. However, there was a significance different ($p < 0.05$) in heavy metal concentration between albumen and egg shell of fresh and rotten eggs. Thus, egg yolk of rotten eggs is suitable for heavy metals indicator compared to shell and albumen. In future study, instead of sacrificing fresh eggs, rotten eggs can be used as heavy metal indicator in green turtle. The presence of non – essential elements such as Cd, and Pb in green turtle eggs can pose dangerous affect to human health. As such, the public should be informed about this and to stop the consumption of sea turtle eggs. When collection and consumption of sea turtle eggs had stopped, indirectly this will help the conservation of sea turtles in Malaysia.

ACKNOWLEDGEMENTS

We gratefully thank the Department of Fisheries Terengganu for providing us with sea turtle eggs for the heavy metal analysis. This research was funded by the SEATRU-INOS Turtle Trust Fund and the School of Marine Science and Environment, Universiti Malaysia Terengganu.

REFERENCES

Aguirre, A., Ballazs, G., Zimmerman, B. and Galey, F.D. 1994. Organic contaminants and trace metals in the tissues of green turtles (*Chelonia mydas*) affected with fibropapillomas in the Hawaiian Islands. Mar. Pollut. Bull. 28:109 – 114.

Bradl, H. 2005. Heavy Metals in the Environment: Origin, Interaction and Remediation. Elsevier/ Academic Press, London.

Burger, J. 1993. Metals in avian feathers: Bioindicators of environmental pollution. Rev. Environ. Toxicol. 5:203 – 311.

Celik, A., Kaska, Y., Bag, H., Aureggi, M., Semiz, G., Kartal, A.A. and Elci, L. 2005. Heavy metal monitoring around the nesting environment of green sea turtles in Turkey. Water, Air and Soil Polluton 169:67 – 79.

Devkota, B., Schmidt, G.H. 1999. Effects of heavy metals during the embryonic development of acridid grasshoppers (*Insecta Caelifera*), Arch, Environ. Contam. Toxicol 36:405 – 414.

Doi, R., Chowdhury, P., Nishikawa, M. and Rayford, P.L. 1993. Tissue distribution of Cd-109 after tracheal and gastric administrations in rats. Bull. Environ. Contam. Toxicol. 51:619 – 624.

Eisler, R. 1985. Cadmium hazards to fish, wildlife, and invertebrates : A synaptic review. U.S Fish and Wildlife Secience. Biological Report 5.

Eisler, R. 1988. Lead hazards to fish, wildlife, and invertebrates; A synaptic review. U.S Fish Wildl. Serv. Biol. Rep 85.

Gerhard, I., Monga, B., Waldbrenner, A. and Runnenbaum, B. 1998. Heavy metals and fertility. J. toxicol. Environ. Health. 54:593 – 611.

Godley, B.J., Thompson, D.R. and Furness, R.W. 1999. Do heavy metal concentrations pose threat to marine turtles from Mediterranean Sea? Mar. Pollut. Bull. 38:497 – 502.

Gramentz, D. 1988. Involvement of loggerhead turtle with the plastic, metal and hydrocarbon pollution in the central Mediterranean. Mar. Pollut. Bull.. 19:11 – 13.

Ho, Y.B. 1990. Ulva lactuca as bioindicator of metal contamination in interdal waters in Hong Kong. Hydrobiologia 203: 73–81.

Jarup, L., Berglund, M., Elinder, C.G., Nordberg, G. and Vahter, M. 1998. Health effects of cadmium exposure: a review of the literature and a risk assessment. Scandinavian Journal of Work, Environment, and Health 24:3–52.

Kazantzis, G. 2004. Cadmium, osteoporosis and calcium metabolism. Biometals 17:493–498.

Khristoforva, N.K. and Kozhenkova, S.I. 2002. Ocean Polar Res., 24:325.

Kobayashi, N. and Okamura, H. 2004. Effects of heavy metals on sea urchin embryo development. Chemosphere 55:1403 – 1412.

Lam, J.C.W., Tanabe, S., Chan, S.K.F., Yuen, E.K.W., Lam, M.H.W. and Lam, P.K.S. 2004. Trace element residue in tissue of green turtles (*Chelonia mydas*) from South China waters. Mar. Pollut. Bull. 48:164 – 192.

Lam, J.C.W., Tanabe, S., Chan, S.K.F., Lam, M.H.W., Martin, M. and Lam, P.K.S. 2006. Levels of traceelements in green turtle eggs collected from Hong Kong: Evidence of risks due to Selenium and Nickel. Environmental Pollution 144:790 – 801.

Landis, W.G. and Yu, M.H. 2003. Introduction to Environmental Toxicology: Impacts of chemicals upon ecological systems. CRC Press, Lewis Publishers, Boca Raton, Florida.

Miller, J.D. 1997. Reproduction in sea turtles in; Lutz Pl, Musick JA (eds). The Biology of Sea Turtle. CRC Press, pp. 51 – 82.

Plotkin, P. 2003. Adult migrations and habitat use in ; Lutz Pl, Musick JA (eds). The Biology of Sea Turtle, vol.2. pp. 225 – 242.

Sakai, H., Ichihashi, H., Suganuma, H. and Tatsukawa, R. 1995. Heavy metal monitoring in sea turtles using eggs. Mar. Pollut. Bull. 30(5):347-353.

Sakai, H., Saeki, K., Ichihashi, H., Suganuma, H., Tanabe, S. and Tatsukawa, R. 2000. Species - specific distribution of heavy metals in tissues and organs of loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. Mar. Pollut. Bull. 40:701 – 709.

Serfor Armah, Y., Nyarko, B.J.B., Osae, E.K., Carboo, D. and Seku, F. 1999. Elemental analysis of some green and brown seaweeds from the coastal belt of Ghana. J. Radioanal. Nucl. Chem. 242:193-197.

Singhal, R.L., Vijayvargia, R. and Shukla, G.S. 1985. Toxic effects of Cd and lead on reproductive functions. Endocrine Toxicology. Raven Press, New York. Pp.149 – 179.

Sivalingam, P.M. and Ismail, R. 1981. Japan J. Phycol (Sorui). 29:171.

Stoneburner, D.L., Nicora, M.N. and Blood, E.R. 1980.Heavy metals in loggerhead sea turtles eggs (*Caretta caretta*): Evidence to support the hypothesis that demes exists in the Western Atlantic population. Journal of Herpetology 14:171 – 175.

Storelli, M. and Marcotrigiano, G.O. 2003. Heav ymetal residue in tissue of marine turtles. Mar. Pollut. Bull. 40:397 - 400.

Sundermann, Jr. F.W., Kataryzna, A. and Antonijezuk,.A. 1995. *Xenopus lipovitellin 1* is a $Zn^{2+}$and Cd^{2+} binding protein. Mol. Reprod. Develop. 42:180 – 187.

U.S.EPA (1976). Effects of exposure to heavy metals on selected fresh water fish: Toxicity of Copper, Cadmium, and Lead to eggs and fry of 7 fish sp. Environmental Research Laboratory, Office of Research and Development 600:76 – 105.

Valchos, V., Critchley, A.T., Bannatyne, T.E. and Von Holy, A.. 1998. S. Afr. J. Bot. 64:233.

Wright, D.A. and Welbourn, P. 2002. Environmental Toxicology. Cambridge University Press, Cambridge, U.K.

WWF-Malaysia. 2009. Survey of marine turtle egg consumption and trade in Malaysia. Petaling Jaya, Malaysia: World Wide Fund.