Supplemental Material

Materials and Methods

Rationale for calculating Hazard Quotients

Although the effects of POPs on the health of free ranging loggerhead sea turtles (*Caretta caretta*) has been well investigated (Keller et al. 2004; Keller et al. 2006a; Keller et al. 2006b), there was only one previous study on the toxicological effects of POPs on sea turtle embryonic development (Podreka et al. 1998). However, there are a number of studies on freshwater turtles that have investigated the effects of POPs on embryonic sex reversal. Based on these studies, the PNECs could be determined for *p,p'*-DDE, dieldrin and PCBs. The PNEC for *p,p'*-DDE was estimated as 543 ng g^{-1}, based on a study on *C. mydas* that found no sex reversal of developing embryos up to a concentration of 543 ng g^{-1} (Podreka et al. 1998). A study on red-eared slider turtles (*Trachemys scripta*) found no significant sex reversal when embryos were dosed with 5 μL of 2.6 μM dieldrin (Willingham and Crews 1999). Based on an estimated penetration of 30% and an average egg mass of ~ 10 g, this dose represented egg concentration of ~ 40 ng g^{-1} dieldrin. Therefore, after applying an uncertainty factor of 10 for inter-species differences, the PNECs for dieldrin were estimated at 4 ng g^{-1}. The effects of PCBs on sex reversal have also been studied on *T. scripta* (Bergeron et al. 1994). In this study, the topical administration of 10 μg of single PCB compounds to *T. scripta* eggs produced no sex reversal. Based on an estimated penetration of 30% and an average egg mass of 11.4 g, these doses represented egg concentrations of ~ 260 ng g^{-1}. Therefore, after applying an uncertainty factor of 10 for inter-species differences, the PNEC for a single PCB was 26 ng g^{-1}.

Information on the toxicological effects of heavy metals on sea turtles is limited to a single study on the effects of mercury on the immune function of free ranging juvenile loggerhead turtles, *Caretta caretta* (Day et al. 2007). Studies on the toxicological effects of heavy metals have generally focussed on invertebrates and fish and are scarce for oviparous reptiles and birds. However, due to the profound difference in ovipary of these species, the estimation of PNECs for *C. mydas* from invertebrate and fish studies was not considered valid. The PNECs in the present study were therefore limited to the toxic metals and selenium that were estimated from reptile and bird toxicological studies. The PNEC for lead was calculated from the no observed adverse effect level (NOAEL) determined for *T. scripta* by Burger et al. (1998). An uncertainty factor of 100 was applied to the NOAEL value of 100 $\mu g\ g^{-1}$ (x 10 for inter-species differences, x 10 for the use of a sub-chronic NOAEL). A PNEC of 1 $\mu g\ g^{-1}$ was therefore estimated for lead. There was no toxicological information on oviparous reptiles for selenium, cadmium or arsenic. The PNECs for selenium were therefore estimated as 0.34 and 6 $\mu g\ g^{-1}$, based on assumptions and calculations from bird egg studies detailed in Lam et al. (2006). The PNEC for cadmium was estimated from the NOAELs of two dosing studies on broiler chickens. In studies by Voleda et al. (1997) and Leach et al. (1979), egg cadmium concentrations of 1.3 $\mu g\ g^{-1}$ and 0.14 $\mu g\ g^{-1}$, respectively, had no influence on embryo mortality or eggshell thinning. An uncertainty factor of 100 was applied to each of these NOAELs to account for inter-species differences and the use of sub-chronic exposure. This resulted in lower and upper estimates for the PNEC for cadmium to be 0.0014 and 0.013 $\mu g\ g^{-1}$, respectively. The PNEC for arsenic was estimated from a feeding study on mallard hens (Stanley et al. 1994). There were no observable adverse effects on duckling production, mortality or hatch success in eggs with arsenic concentrations of 1.8

μg g^{-1}. Therefore, after the uncertainty factor of 100 was applied to this NOAEL, the PNEC for arsenic was estimated at 0.018 μg g^{-1}.

Results

Supplemental Material, Figure 1. Locations of markets in Peninsular Malaysia selling *Chelonia mydas* eggs for human consumption (top) and the nesting locations from where the eggs were collected (bottom).

References

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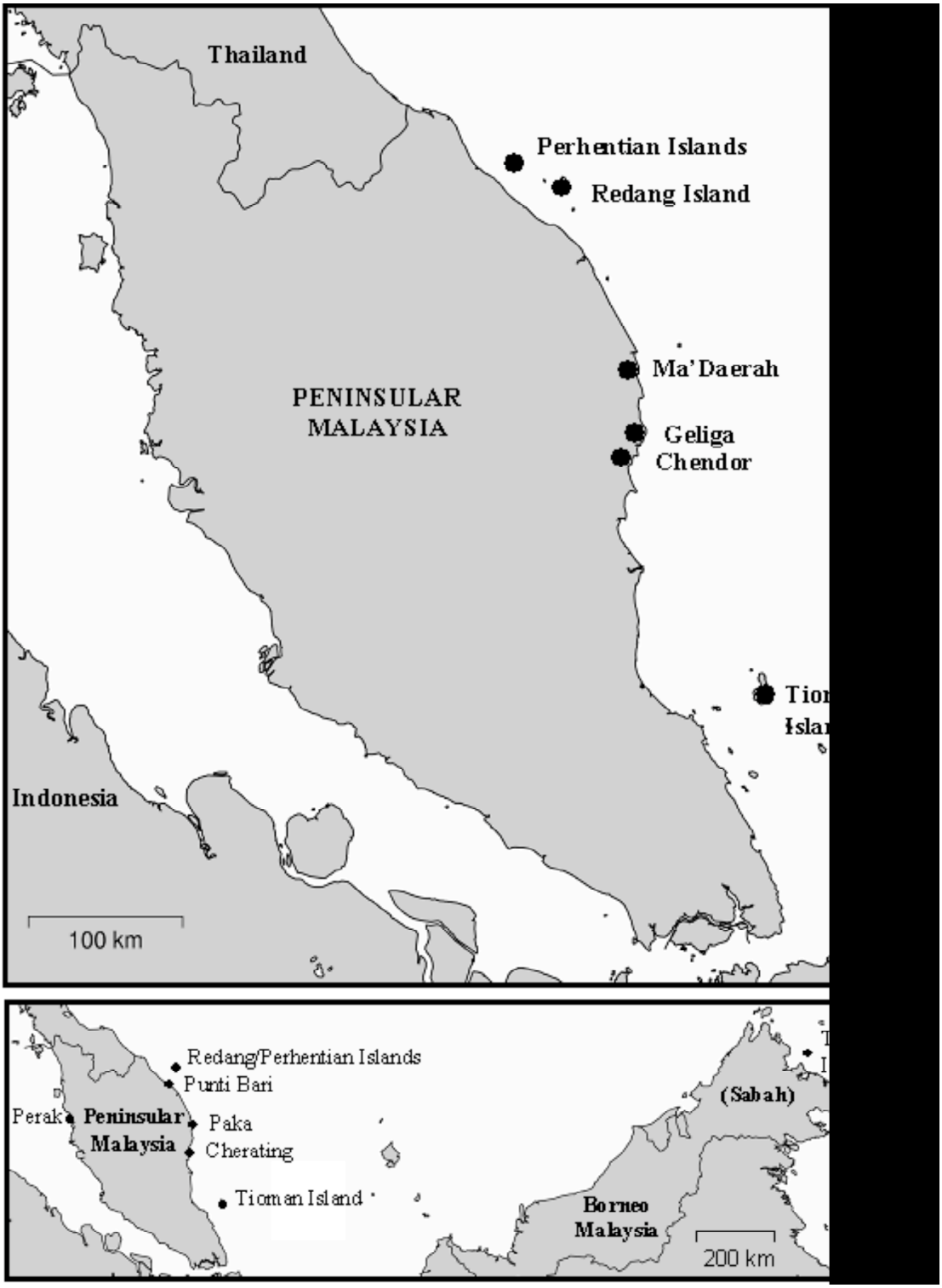
Thailand
Perhentian Islands
Redang Island
Ma' Daerah
PENINSULAR
MALAYSIA
Geliga
Chendor
Indonesia
100 km
Redang/Perhentian Islands
Puntti Bari
Perak
Peninsular
Malaysia
Paka
Cherating
Tioman Island
(Sabah)
Borneo
Malaysia
200 km