Montara Well Release:

Report on necropsies from a Timor Sea green turtle

August 2010

Curtin University

Preface

This report was prepared by Associate Professor Marthe Monique Gagnon and Dr Christopher Rawson from the Department of Environment and Agriculture, Curtin University. The green turtle specimen was received frozen at Curtin University, and dissection and necropsies collection proceeded in the Ecotoxicology laboratories at Curtin University.

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Summary of Results

- Necropsies were collected from a green turtle (*Chelonia mydas*) collected from Ashmore Reef in November 2009.
- A total of eleven samples (4 muscle tissue samples, 2 gut content samples and 5 swab samples from external and internal surfaces) were analysed for the presence of total petroleum hydrocarbons (TPHs - gas chromatography with flame ionisation detection) and polycyclic aromatic hydrocarbons (PAHs – gas chromatography-mass spectrometry).
- Expert examination of the chromatographic pattern produced in the TPH analysis allowed the qualitative assessment of whether the source of the compounds was of petrogenic or biological (e.g., fatty acids, cholesterol) origin, as both hydrocarbon types co-extract in the sample extraction process.
- Where TPH was detected in the samples the chromatographic patterns indicated that these were from biogenic origin.
- PAHs were not detected in any of the samples analysed.
- There was no evidence that the sea turtle had been exposed to crude oil from the Montara well release either *pre-* or *post mortem*.

Table of Contents

Background

In August 2009 the Montara well in the Timor Sea released crude oil and gas condensate to the surrounding environment causing concern over the impacts of exposure to petroleum compounds on wildlife. In the following months a number of deceased animals suspected of being impacted by the release were collected from the region. One of these animals was a green turtle (*Chelonia mydas*).

Associate Professor Marthe Monique Gagnon from Curtin University was contracted by the Australian Government Department of Environment, Heritage, Water and the Arts (DEWHA) to receive the deceased animal suspected of exposure to the Montara well head hydrocarbons, and collect appropriate necropsies for chemical analysis to confirm such exposure, and possibly determine the cause of death.

Sample received

The green turtle specimen was received frozen at Curtin University. The turtle was accompanied by information regarding the location of collection (West Ashmore Reef) but no information on the date of collection or the collector (Table 1). The specimen remained frozen until dissection.

Collection Location

Figure 1 shows the location of collection of the green turtle specimen delivered for necropsy in relation to the West Atlas drilling rig.

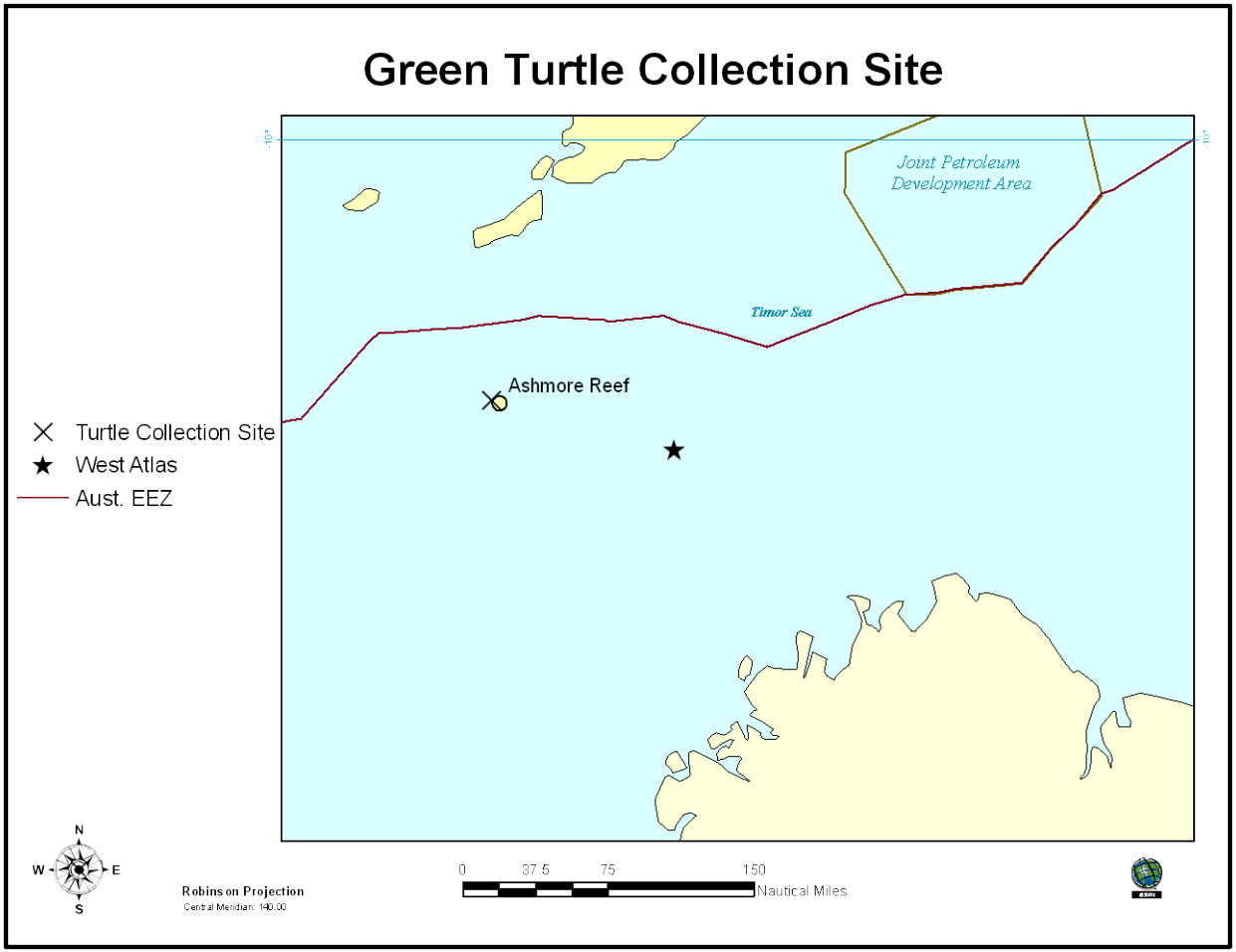

Figure 1. Location of the West Atlas drilling platform and the location where the green turtle was found (West Ashmore Reef).

Table 1. Information associated with the green turtle specimen. U = Unknown.

Common Name	ID	Date Collected	Collected by	Location	GPS	Comments
Green Turtle	GT1	U	U	Ashmore Reef	12.2415 S 122.96413 E	Nil.

Table 2. Observations made during dissection of the green turtle. CCL = Curved carapace length, CCW = curved carapace width.

Common Name	ID	CCL (mm)	CCW (mm)	Head Width (mm)	Head Length (mm)	Plastron length (mm)	Plastron Width (mm)	Total Wt (g)	Comments
Green Sea Turtle	GT1	481	431	57	127	370	385	11200	Skin lifting on back left flipper (may be due to defrosting or exposure to petroleum) (Figure 2). No signs of exposure on the outside. Water in the lungs. 1-2 days post-mortem max. Appears in good condition. Lots of fat. No muscle atrophy. Tissues look good. Good skin colour. Stomach is full of seagrass (*Thalassia* sp.). All intestines were opened during dissection. No sign of plastic ingestion or any pathology.

Figure 2. Skin lifting on the right flipper of the green turtle.

Necropsies Collected

The turtle was dissected on clean dissection mats using rinsed (HPLC-grade hexane) dissection tools. Swabs were taken using sterile cotton Livingstone swabs. All tissue samples and swabs were wrapped in hexane-rinsed aluminium foil and stored at -20^{o}C.

The turtle was thawed for 18hrs prior to collection of biopsies. There was no evidence of oil on the outer surface of the animal. External swabs were taken from the left back flipper (skin was lifting at this location), left front flipper, the plastron and the top carapace. The plastron was removed and the following necropsies collected for analysis;

- Swabs from the mouth cavity,
- Swabs from the oesophagus,
- Swabs from the trachea,
- Lung tissue,
- Liver tissue,
- Muscle from left pectoral,
- Kidney tissue,
- Stomach and intestinal contents

Chemical Analysis

A total of eleven samples (4 tissue samples, 5 swab samples, 2 intestinal content samples) were transferred to Advanced Analytical laboratories for chemical analysis of total petroleum hydrocarbons (TPHs: C10-14, C15-28, C29-36) and 19 individual polycyclic aromatic hydrocarbons (PAHs). The methods used for TPH and PAH quantifications were Advanced Analytical methods 04-020 and 04-077, respectively. The samples were extracted (acetone: hexane) and analysed using gas chromatography with flame ionization detection (GC-FID) for the presence of TPHs.

The extraction of petroleum hydrocarbons from biological samples results in the co-extraction of biological compounds (e.g., fatty acids, cholesterol) and the presence of these co-extractives can interfere with the detection of petroleum hydrocarbons. These biological extractives have characteristic chromatographic patterns and examination of individual chromatographs of each extract allows the identification of the presence of either petroleum hydrocarbons, biological extractives or both. The TPH concentrations reported are the sum of the petroleum hydrocarbons and the biological extractives since their co-extraction renders their separate quantification not possible. Individual PAHs were quantified using GC-MS. Limits of reporting are shown in Table 3.

Table 3. Limits of reporting for chemical analytes. The limits of reporting vary according to the amount of sample provided and the level of matrix interference.

		Tissue	Stomach/ Intestinal Contents	Swab (µg/swab)
TPH (mg/ kg)	TPH C 10 – 14	200	200	125
	TPH C 15 – 28	200	400	250
	TPH C 29 - 36	200	400	250
PAH (µg / kg)	Naphthalene	50	50	10
	1-Methylnaphthalene	50	50	10
	2- Methylnaphthalene	50	50	10
	Acenaphthalene	50	50	10
	Acenaphthene	50	50	10
	Fluorene	250	250	10
	Phenanthrene	50	50	10
	Anthracene	50	50	10
	Fluoranthene	50	50	10
	Benz(a)anthracene	50	50	10
	Chrysene	50	50	10
	Benzo(b)&(k)fluoranthene	100	100	10
	Benzo(a)pyrene	50	50	10
	Indeno(1,2,3-c,d)pyrene	250	250	10
	Dibenz(a,h)anthracene	250	250	10
	Benzo(g,h,i)perylene	250	250	10
	Coronene	50	50	10
	Benzo(e)pyrene	50	50	10
	TOTAL PAH	1000	1000	10

Results and Interpretation

Sea turtles are long-lived, slow-maturing reptiles with an iconic status. Any potential anthropogenic impact on their health warrants investigation. This study examined a green turtle found dead at Ashmore Reef in 2009 following the Montara well release. Ashmore Reef is located about 90 NM west of the source of the release.

Overall the animal was observed to be in good condition. The full stomach indicated that it was feeding well close to the time of death. A thick fat layer suggested no reduction in food consumption over an extended period.

None of the samples taken from the green turtle had petroleum hydrocarbon patterns matching that of crude oil. Where hydrocarbons were detected they followed the chromatographic pattern expected for biogenic compounds (fatty acids, cholesterol). There was no oil of petrogenic origin detected on the external surfaces of the turtle. There is therefore no evidence that this animal was exposed to crude oil from the Montara well release before or after death.

Where oil is released to an environment containing sea turtles the risk of exposure exists. Turtles must surface to breathe and where fresh or weathered oil remains on the surface there is a risk of oil adhering to body surfaces and of oil inhalation. A controlled laboratory study described severe dermal pathologies (particularly in the softer skin of the neck) associated with exposure to crude oil through surfacing behaviour which decreased over a matter of weeks following removal from the oil (Lutcavage et al., 1995). Such pathologies were not noted in the animal examined in the current study. The study described above also noted an increase in white blood cells (stress response) and a failure of the salt gland in a small number of impacted turtles (Lutcavage et al., 1995). Neither of these responses could be examined on the deceased animal in the current study.

Table 4. Total hydrocarbons and PAHs in green turtle samples. Samples in bold denote those where hydrocarbons were reported. In each of these cases the chromatographic patterns indicated that the hydrocarbons were biological extractives and were not of petrogenic origin. All individual and total PAH measurements were below the assay detection limits. Tissue sample concentrations are expressed in mg/ kg for TPH and µg/ kg for ∑PAH, swabs are expressed in µg/swab (TPH and ∑PAH).

Common Name	ID	Sample	TPH C10-C14	TPH C15-C28	TPH C29-36	∑PAH
Green Sea Turtle	GT1	Lung	<200	**3700**	**1600**	<1000
		Liver	<200	**38000**	**3700**	<1000
		Stomach contents	<200	**1300**	**530**	<1000
		Muscle	<200	**750**	**740**	<1000
		Intestinal contents	<200	**11000**	**2500**	<1000
		Kidney	<200	**7100**	**2700**	<1000
		Skin swab	<125	<250	<250	<10
		Plastron swab	<125	<250	<250	<10
		Mouth cavity swab	<125	<250	<250	<10
		Oesophagus swab	<125	<250	<250	<10
		Trachea swab	<125	<250	<250	<10

Sea turtles may also be exposed to crude oil via dietary pathways. This can be via direct feeding on tar mistaken for food or to incidental feeding through grazing in sea grass (e.g., *Thalassia* spp.) meadows contaminated with oil residues (tar). Indirectly, turtle health may be impacted by the loss of such meadows through heavy contamination. In a study of juvenile logger head turtles (*Caretta caretta*) 20% had ingested weathered crude oil (Witherington, 2002). Oros et al. (2005) investigating the death of 93 sea turtles over 4 years estimated that two of these died from ingestion of crude oil. Neither of these studies focussed on a specific spill incident but it is clear that dietary exposure to oil is common and can be fatal for sea turtles.

While the current study found no petroleum hydrocarbons in the necropsied tissues other studies have shown that following oil exposure, tissue contamination can and does occur. Following the Ixtoc I oil spill (1979) a number of live and deceased sea turtles were collected from nearby locations (Hall et al., 1983). Two deceased green sea turtles (*Chelonia mydas*) were dissected and similar necropsies to those in the current study (kidney, liver, pectoral muscle) collected to establish a cause of death. Both animals were judged to be in poor condition. From chemical analysis of the organ necropsies the authors concluded that both turtles had petroleum hydrocarbons in all organs examined but that some compounds had been selectively eliminated (particularly the short chain hydrocarbons) (Hall et al., 1983).

Vertebrates are able to metabolise some of the toxicants associated with crude oil (especially PAHs) resulting in little or no accumulation in organs or tissues. Turtles have been shown to have a well developed hepatic system of enzymes (cytochocrome P450-1A) to metabolise organic contaminants and aid in elimination from the body (Yawetz et al., 1998). Glutathione transferases (a cellular defence against electrophilic DNA damage by such toxicants as PAHs) have also been isolated from green sea turtles (Richardson et al., 2009). Therefore, when turtles are exposed to PAHs in crude oil, endogenous mechanisms exist to enhance elimination of xenobiotics compounds out of the organism.

In conclusion, there is no evidence suggesting that the green turtle found on Ashmore Reef has been exposed to petroleum hydrocarbons originating from the Montara well release. The specimen was in good physical condition, seemed healthy and was feeding well up to the time of death.

References

Hall, R. J., Belisle, A. A., Sileo, L., 1983. Residues of petroleum hydrocarbons in tissues of sea turtles exposed to the Ixtoc I oil spill. Journal of Wildlife Diseases. 19**,** 106-109.

Lutcavage, M. E., Lutz, P. L., Bossart, G. D., Hudson, D. M., 1995. Physiologic and clinicopathologic effects of crude oil on loggerhead sea turtles. Archives of Environmental Contamination and Toxicology. 28**,** 417-422.

Oros, J., Torrent, A., Calabuig, P., Deniz, S., 2005. Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain. Diseases of Aquatic Organisms. 63**,** 13-24.

Richardson, K. L., Gold-Bouchot, G., Schlenk, D., 2009. The characterization of cytosolic glutathione transferase from four species of sea turtles: Loggerhead (*Caretta caretta*), green (*Chelonia mydas*), olive ridley (*Lepidochelys olivacea*), and hawksbill (*Eretmochelys imbricata*). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 150**,** 279-284.

Witherington, 2002. Ecology of neonate loggerhead turtles inhabiting lines of downwelling near a Gulf Stream front. Marine Biology. 140**,** 843-853.

Yawetz, A., Woodin, B. R., Stegeman, J. J., 1998. Cytochromes P450 in liver of the turtle *Chrysemys picta picta* and the induction and partial purification of CYP1A-like proteins. Biochimica et Biophysica Acta (BBA) - General Subjects. 1381**,** 12-26.