# Montara Well Release:

# Report on necropsies from a Timor Sea horned sea snake

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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. One horned sea snake (*Acalyptophis peronii*) was dissected and necropsies collected in the Ecotoxicology laboratories at Curtin University.

## *Acknowledgements*

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## Summary of Results


- One horned sea snake (*Acalyptophis peronii*) was collected in the Timor Sea on 29th September 2009 and transported to Curtin University for dissection and necropsy.
- A total of five samples (1 muscle tissue sample, 1 gut content sample, 1 bile sample, 1 swab sample from skin and 1 swab from the inner lung ) were analysed for the presence of total petroleum hydrocarbons (TPHs, by gas chromatography with flame ionisation detection) and polycyclic aromatic hydrocarbons (PAHs, by 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 petroleum hydrocarbons and/ or biological co-extractives (e.g., fatty acids, cholesterol).
- The horned sea snake had petroleum hydrocarbons in its stomach contents but none in any other necropsies (muscle, skin, trachea, lung). This indicates exposure through prey ingestion. The absence of petroleum hydrocarbons in the trachea and in the lung suggests that inhalation of floating crude oil did not occur.

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# Background

In August 2009 the Montara well in the Timor Sea released crude oil and gas condensate to in 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. A specimen of horned sea snake (*Acalyptophis peronii*) was one of these animals.

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 sea snake collected in the Timor Sea. The aim of the investigation was to assess if this sea snake had been exposed to the hydrocarbons originating from the Montara well release, this assessment being made through the collection of necropsies on the deceased animal, and the subsequent chemical analysis of these necropsies.

## *Sample received*

The horned sea snake  specimen was received frozen at Curtin University accompanied by information regarding the date, time, collector and location of collection (GPS coordinates) (Table 1). The specimen remained frozen until dissection.

## *Collection Locations*

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

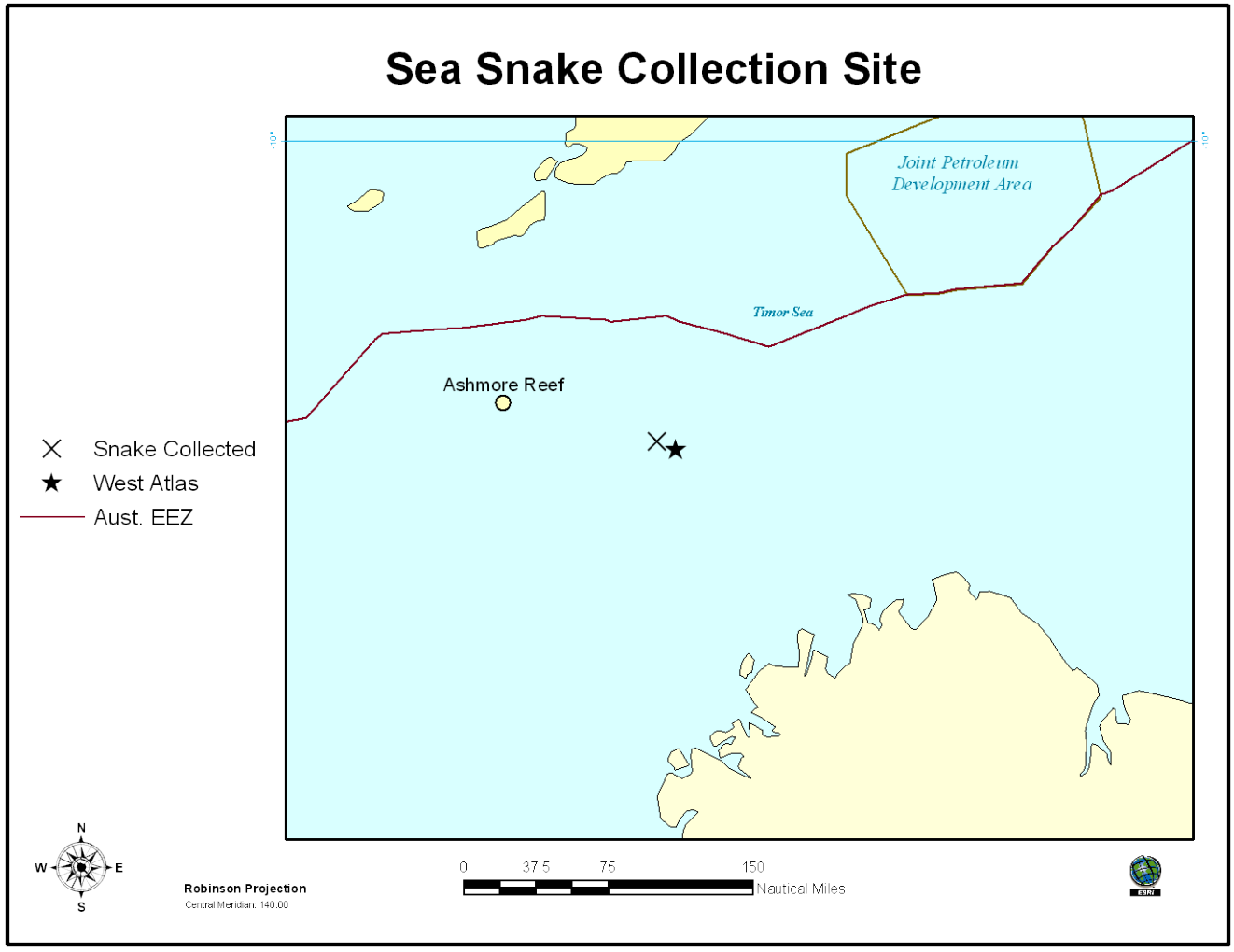


**Figure 1. Location of the West Atlas drilling platform and site at which the horned sea snake was collected on 29th September 2009.**

**Table 1. Information on the horned sea snake specimen received at Curtin University. U = Unnamed location.**

| Common Name | ID | Date Collected | Collected by | Location | GPS | Comments |
|---|---|---|---|---|---|---|
| Horned sea snake | SS1 | 29/09/2009 | James Watson | U | 10.6003 S 124.36875 E | Horned sea snake collected on 19/09/09 dead in an oil slick. |

**Table 2. Observations made during dissection.**

| Common Name | ID | Total Wt (g) | Length (mm) | Comments |
|---|---|---|---|---|
| Horned sea snake | SS1 | 874.91 | 115 | Snake looks in very good condition. Ice in lungs. No patch of oil observed in the lungs or on the body. |

### *Necropsies Collected*

The specimen was dissected on a clean dissection mat 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.

Skin swabs were collected prior to dissection. The specimen was dissected along the lateral line and opened for collection of necropsies;

- Swabs were taken inside the lung,
- Muscle was excised,
- Bile was collected,
- Stomach contents were collected.

## Chemical Analysis

A total of 5 samples (1 muscle tissue sample, 1 bile sample, 1 stomach contents sample, 1 skin swab and 1 inner lung swab,) were transported to Advanced Analytical Ltd. for quantification 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 total petroleum hydrocarbons. 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. See Table 3 for Limits of reporting for chemical analytes.

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

| | | Tissue | Bile | Stomach/ Intestinal Contents | Swab (µg/swab) |
|---|---|---|---|---|---|
| TPH (mg/ kg) | TPH C 10 – 14 | 500 | 1000 | 500 | 196 |
| | TPH C 15 – 28 | 1000 | 2000 | 1000 | 393 |
| | TPH C 29 - 36 | 1000 | 2000 | 1000 | 393 |
| PAH (µg / kg) | Naphthalene | 50 | 250 | 100 | 10 |
| | 1-Methylnaphthalene | 50 | 250 | 100 | 10 |
| | 2- Methylnaphthalene | 50 | 250 | 100 | 10 |
| | Acenaphthalene | 50 | 250 | 100 | 10 |
| | Acenaphthene | 50 | 250 | 100 | 10 |
| | Fluorene | 250 | 1250 | 500 | 10 |
| | Phenanthrene | 50 | 250 | 100 | 10 |
| | Anthracene | 50 | 250 | 100 | 10 |
| | Fluoranthene | 50 | 250 | 100 | 10 |
| | Benz(a)anthracene | 50 | 250 | 100 | 10 |
| | Chrysene | 50 | 250 | 100 | 10 |
| | Benzo(b)&(k)fluoranthene | 100 | 500 | 200 | 10 |
| | Benzo(a)pyrene | 50 | 250 | 100 | 10 |
| | Indeno(1,2,3-c,d)pyrene | 250 | 1250 | 500 | 10 |
| | Dibenz(a,h)anthracene | 250 | 1250 | 500 | 10 |
| | Benzo(g,h,i)perylene | 250 | 1250 | 500 | 10 |
| | Coronene | 50 | 250 | 100 | 10 |
| | Benzo(e)pyrene | 50 | 250 | 100 | 10 |
| | TOTAL PAH | 1000 | 5000 | 2000 | 10 |

## Results and Interpretation

Chemical analysis indicated the presence of crude oil in the stomach of the horned sea snake indicating exposure to oil via prey ingestion. Horned sea snakes generally feed on small burrowing fish (e.g., gobies) and shrimp (Voris and Voris, 1983) rather than surface prey. In this present case, the prey items appeared to have been exposed to petroleum hydrocarbons prior to being ingested by the horned sea snake.

There was no oil in the trachea or lungs of the snake and no oil detected in external surface swabs. This finding suggests that it is unlikely that the snake was exposed to petroleum hydrocarbons during recent normal surfacing behaviour. There was also no accumulation of TPH or PAHs in the muscle tissue of the animal.

There is a paucity of literature available on the toxic impacts of oil contamination on sea snakes. In the absence of such information assigning a cause of death is tentative. While this sea snake had swallowed items contaminated with crude oil or crude oil itself it is unclear whether this was the cause of death or not. Further, the snake was in good physical condition with no visible external or internal pathologies to indicate chronic toxic impact on foraging success or major trauma associated with any oil exposure. Although crude oil was detected in the gut contents there were no impacts on the physical gut structure or any blockage to digestion. These facts along with the negative results for TPH and PAHs in all tissue samples and external and internal swab samples suggest that it is unlikely that crude oil was the primary cause of death of this snake.

**Table 4. Total hydrocarbons in sea snake samples. Samples highlighted denote those where petroleum hydrocarbons were reported. All individual PAH measurements were below the assay detection limits. Tissue samples 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 |
|---|---|---|---|---|---|---|
| Horned sea snake | SS1 | Bile | <1000 | <2000 | <2000 | <5000 |
| | | lung swab | <196 | <393 | <393 | <10 |
| | | Skin swab | <196 | <393 | <393 | <1000 |
| | | Stomach contents | <500 | **1900** | **1700** | <2000 |
| | | Muscle | <500 | <1000 | <1000 | <1000 |

## References

Voris, H. K., Voris, H. H., 1983. Feeding Strategies in Marine Snakes: An Analysis of Evolutionary, Morphological, Behavioral and Ecological Relationships. Amer. Zool. 23**,** 411-425.