REPORT ON BIOPSY COLLECTIONS FROM SPECIMENS COLLECTED FROM THE SURROUNDS OF THE WEST ATLAS OIL LEAK - SEA SNAKE SPECIMEN

Curtin University of Technology

15th October 2009

Photo: Spotty the Sea Snake

RESULTS AT A GLANCE:

1. The sea snake inhaled and ingested petroleum compounds, as evidenced by very high TPH and PAH levels in the lung & trachea swabs, as well as in the stomach contents.

2. High PAH levels were also found in the muscle of the sea snake, suggesting that this animal has been exposed to petroleum compounds for several days.

3. The likely cause of death for the sea snake is exposure to petroleum hydrocarbons.

4. Sea snake skin swabs did not indicate direct dermal contact with petroleum compounds.

5. The yellow substance collected from the lung of the dead sea snake showed no presence of the chemical dispersants Slickgone or Adrox 6120.

6. During the oil spill, surface animals are more at risk of being affected by exposure to petroleum hydrocarbons than are deep sea fish.

1. Background

- A deceased sea snake was landed in Broome WA, on September 4th, 2009, by a commercial fisherman. The sea snake was stored temporarily in Broome and subsequently sent to Perth via Centurion Transport.
- Associate Professor Marthe Monique Gagnon, Ecotoxicologist at Curtin University, was contacted on Monday 7th September 2009 by WA Transport on behalf of AMSA, and requested to conduct biopsy collection and assessment of cause of death on a sea snake.
- The sea snake was collected at the Centurion Transport depot on Lewis Road, Kalamunda, by M. M. Gagnon on Monday September 14th, 2009.
- Biopsy collection was conducted on 15th September, 2009. Biopsies were collected in duplicate, where enough biological material was available. The first set of biopsies was tested for total petroleum hydrocarbons (TPHs) and polycyclic aromatic hydrocarbons (PAHs). The second set of biopsies, along with the carcass, has been sent offsite for long-term storage.

2. Sea Snake

- The following biopsy collections were performed on the sea snake specimen:
  - Two skin swabs using sterile Livingstone cotton tip sterile applicators;
  - Two trachea swabs using Livingstone cotton tip sterile applicators;
  - Two mid-lungs swabs using Livingstone cotton tip sterile applicators;
  - Stomach contents placed in an analytical grade hexane-double-rinsed glass jar, sealed with hexane-rinsed aluminium foil, two samples;
  - Biopsies of red muscle collected and placed in analytical-grade hexane-rinsed aluminium foil, two samples.

- The sea snake was identified using taxonomic keys as being a specimen of *Hydrophis elegans*. The snake was examined externally, weighed and measured prior to autopsy.

- The sea snake tissues were carefully sectioned from the vent towards the trachea. The head of the snake was wrapped in aluminium foil to avoid accidental contact between the poisonous fangs and the hands of the staff handling the specimen. It was assessed that the stomach was less than ¼ full capacity, but did contain fresh prey.

Specimen Identification and External Measurements

Common name	Elegant sea snake
Latin name	*Hydrophis elegans*
Total weight	186.15 g
Vent length (head to vent)	770 mm
Total length (head to end of tail)	856 mm

Fig 1. The sea snake specimen *Hydrophis elegans* landed in Broome by a commercial fisherman on September 4th, 2009.

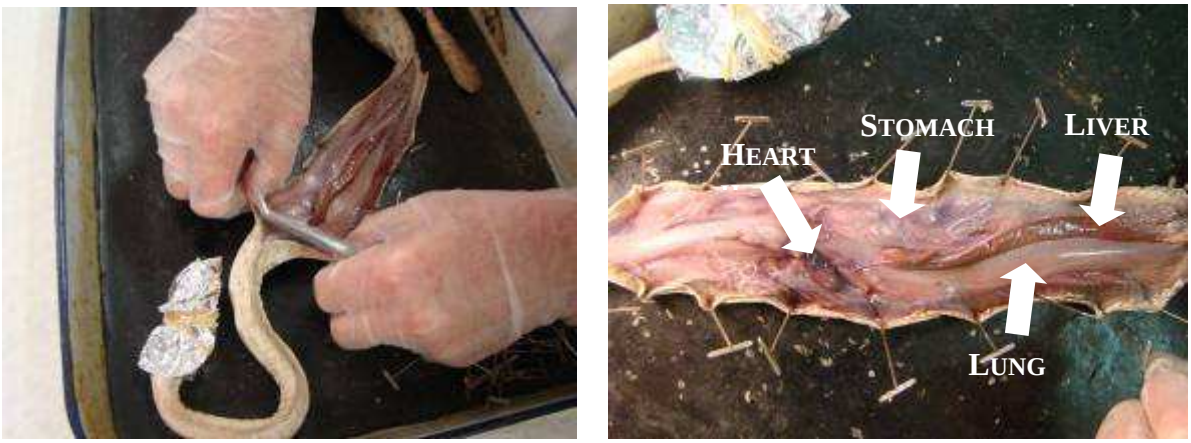

Fig 2. The specimen was carefully dissected in order to expose various organs.

- There was evidence of recent feeding (within the past 24 hours) in the stomach of the sea snake, with fish pieces easily identifiable. The partially digested stomach content indicates that the sea snake was feeding shortly before its death, which suggests that the animal was healthy enough to perform successful predation.

- A yellow substance was observed in the middle lung – swabs of the substance were taken.

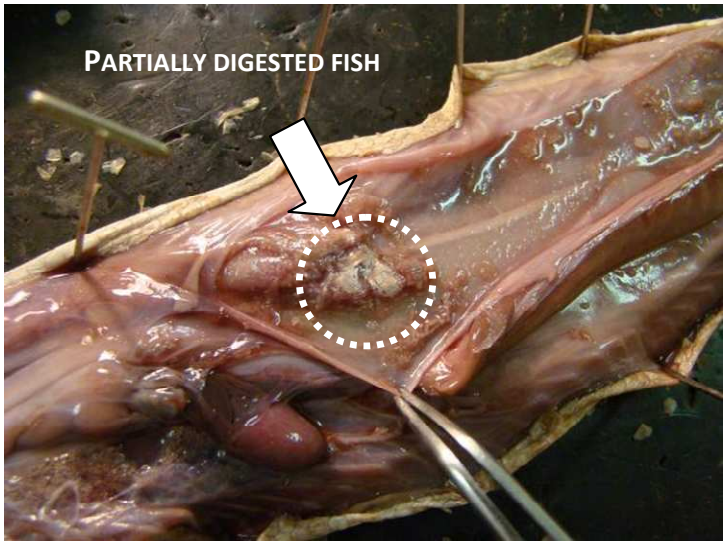

Fig 3. Detail of partially digested fish in the stomach of the sea snake

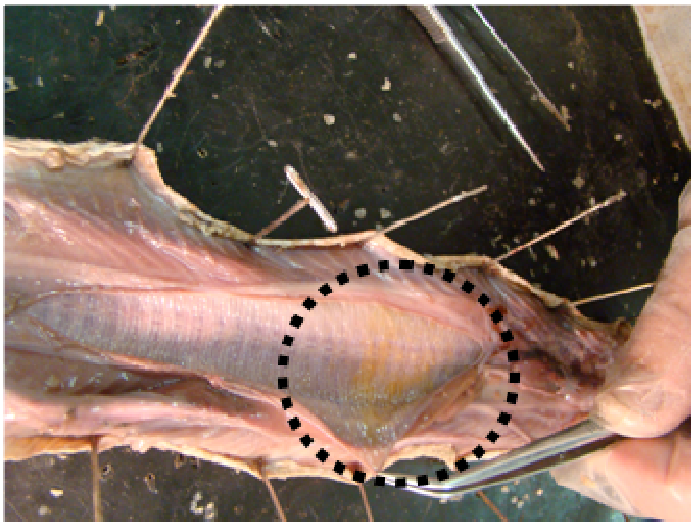

Fig 4. Yellow substance was found inside the lung of the sea snake. The lung swab collected this substance.

2.1 Results from chemical analysis of sea snake *Hydrophis elegans* biopsies:

Summary of Chemical Analysis

Compound / Sample	Skin swab	Lung swab	Trachea swab	Stomach content	Red muscle
TPH (mg/g)	8	802	240	9.46	0.955
Total PAHs (mg/g)	Not detected	14	4	0.083	0.011

- Unless TPH levels are accompanied by petroleum specific PAH compounds, it is considered that the TPH levels represent biological oils naturally occurring in animals and in their prey (person. comm., A. Tottszer, Advanced Analytical, Brisbane).
- Analysis of the skin swab indicated low levels of TPHs (8 mg/g), and no PAHs on the external surface of the snake. Naturally occurring biological oils are most likely responsible for this TPH measurement.
- The lung swab which collected the yellow substance contained very high levels of petroleum compounds, with TPH levels of 802 mg/g and PAH levels of 14 mg/g.

- The trachea swab also indicated the presence of high levels of petroleum compounds in the airways, with 240 mg/g and 4 mg/g for TPHs and PAHs, respectively.

- The TPH and PAH levels of the stomach content indicate that the sea snake had ingested petroleum-contaminated products, with levels of 9.46 mg/g and 0.083 mg/g for TPHs and PAHs, respectively.

- The red muscle sample from the sea snake had a much lower levels of 0.955 mg/g TPHs as well as 0.011 mg/g PAHs.

- It was not possible to locate published literature where TPH and PAH levels were measured in sea snakes exposed to spilt crude oil. It is clear, however, that TPH and PAH levels observed in the lung and trachea swabs, stomach content, and muscle are exceptionally high relative to levels measured in other taxa. For example:
    - the maximum PAH concentration in wild fish flesh shortly after the Braer oil spill was 0.003 mg/g (Law and Hellou, 1999).
    - the PAH levels measured in invertebrates one month after the Nakhodka oil spill was 0.000 044 mg/g (Koyama et al. 2004). Relatively speaking, the stomach content of the sea snake which could be expected to contain small vertebrates and invertebrates has a PAH content of 0.083 mg/g.

2.2 Results from chemical analysis for the presence of dispersants in the lung swab:

- Two dispersant samples were provided in order to assess if the yellow substance in the sea snake lung contained chemical dispersants. These dispersant samples were labelled "Slickgone" and "Adrox 6120".
- The analytical protocol profiled the two individual dispersants as well as the lung swab extract by gas chromatography-mass spectrometry (GC-MS) and by liquid chromatography-mass spectrometry (LC-MS).
- The analysis concluded that the yellow substance collected from the lung of the dead sea snake showed no presence of the dispersants Slickgone or Adrox 6120.

2.3 Additional observations:

- Two puncture holes were observed on the body of the sea snake; a first one in the middle of the body (left picture, fig 5), and the second one close to the vent (right picture, fig 5).
- The two puncture holes (1-2 mm wide) perforated the skin and were both associated with internal bruising of the muscle, indicating that the punctures occurred while the snake was still alive.
- However, the punctures did not reach internal organs, and no internal bleeding was observed in the puncture areas, indicating that the punctures were not life threatening and were not the cause of death for this snake. It is possible that the snake was attacked while drifting (but still alive) at the surface of the water.
- The likely cause of death for this sea snake is exposure to petroleum hydrocarbons.

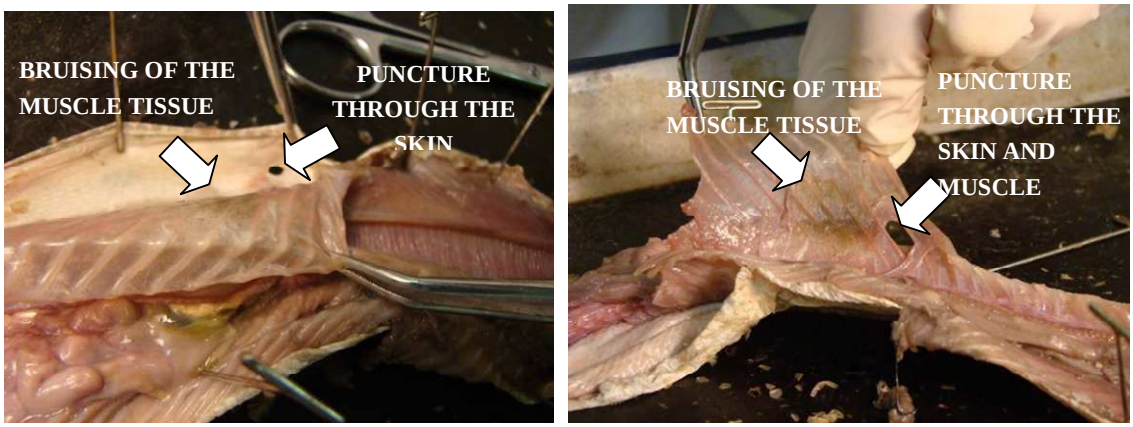

Fig 5. Details of the punctured skin and muscle wall noted in the sea snake specimen.

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2.4 Additional observations on the sea snake:

- No fat reserves were observed in the snake.
- Although the gonads were not fully developed, they appear to indicate that the specimen was a young female.
- The liver was of healthy colour, and appeared in good condition.
- No other pathologies were observed in the sea snake specimen.

3. References

1. Law RJ, Hellow J (1999) Contamination of fish and shellfish following oil spill incidents. Environmental Geosciences, 6: 90-98.
2. Koyama J, Uno S, Kohno K (2004) Polycyclic aromatic hydrocarbon contamination and recovery characteristics in some organisms after the Nakhodka oil spill. Marine Pollution Bulletin 49:1054-1061.
3. Hartung R (1995) Assessment of the potential for long-term toxicological effects of the Exxon Valdez oil spill on birds and animals. IN: Exxon Valdez oil spill: Fate and Effects I Alaskan waters, ASTM, pp. 693-725.

4. Acknowledgements

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