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**REPORT ON BIOPSY COLLECTIONS FROM  
SPECIMENS COLLECTED FROM THE SURROUNDS  
OF THE WEST ATLAS OIL LEAK -  
FISH SPECIMENS**

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*Photo: fish specimens collected during the West Atlas oil leak*

## **RESULTS AT A GLANCE:**

1. Skin swabs on four fish species did not indicate exposure to petroleum hydrocarbons.
2. Fish stomach contents indicate no ingestion of petroleum hydrocarbons.
3. Fish white muscle indicates no trace of petroleum hydrocarbons.
4. During the oil spill, surface animals are more at risk of being affected by exposure to petroleum hydrocarbons than are deep sea fish.
5. It is imperative to commence a monitoring program of fish health during the oil spill, and continue monitoring after the oil leak has been solved.

## **1. Background**

- Four fish specimens, all of different species, were landed in Broome on September 4<sup>th</sup>, 2009, by a commercial fisherman. The fish were 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 7<sup>th</sup> September 2009 by WA Transport on behalf of AMSA, and requested to conduct biopsy collection on the fish specimens.

- The four fish specimens were collected at the Centurion Transport depot on Lewis Road, Kalamunda, by M. M. Gagnon on Monday September 14<sup>th</sup>, 2009.

- Two specimens of the commercially important goldband snapper were purchased at Sealane fish market, Fremantle WA, in order to compare the chemical analysis of store-bought fish samples relative to those from the fish provided by the fisherman. The store-bought snappers were of similar size to the field-collected one.

- Biopsy collection was conducted on 10<sup>th</sup> and 16<sup>th</sup> 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 all carcasses, have been sent offsite for long-term storage.

## 2. Fish Autopsy

Four fish specimens were provided, with one individual of each of the following species:

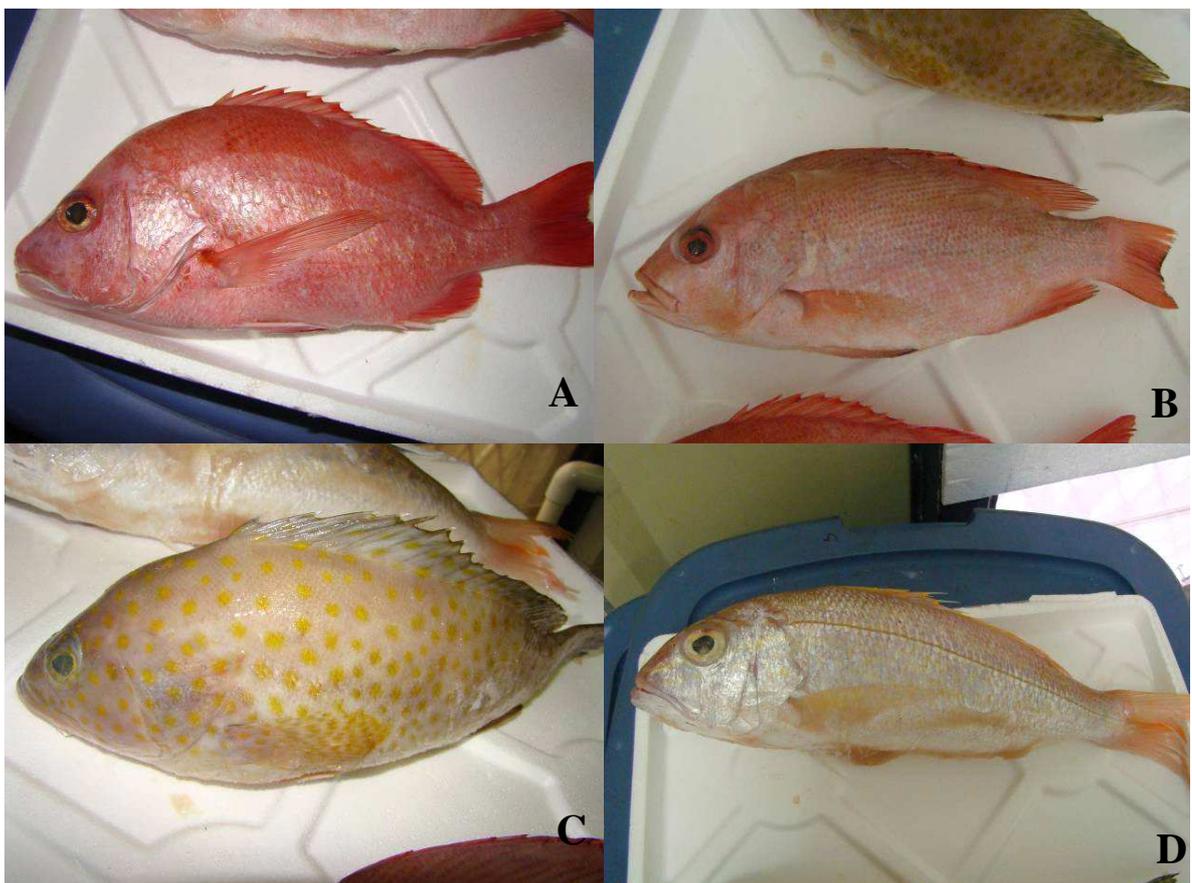
Saddletail snapper (*Lutjanus malabaricus*)

Crimson snapper (*Lutjanus erythropterus*)

Yellowspotted rockcod (*Epinephelus areolatus*)

Goldband snapper (*Pristipomoides multidens*)

- All fish were in good physical condition. No residues of oil were observed in the mouth, gills or on the skin of the specimen.



**Fig 1.** Fish provided for collection of biopsies. A. saddletail snapper; B. crimson snapper; C. yellowspotted rockcod; D. goldband snapper.

- Because the samples had been frozen prior to the autopsy, it was not possible to collect bile to assess if these fish have been exposed to petroleum compounds. Bile, blood and other biopsies can be collected on freshly captured specimen only. However, stomach content and white muscle was collected for all specimens and wrapped in HPLC-grade hexane-rinsed aluminium foil for subsequent chemical analysis.

### ***3.1 Physical characteristics of individual fish, and samples collected:***

#### **Saddletail snapper:**

- Lengths: standard, fork, total: 275, 315, 330 mm
- Total weight: 617.17 g
- Sex: immature individual with undeveloped gonads
- White muscle: 2 samples
- Skin swabs: 2 samples
- Stomach content: 1 sample only.

#### **Crimson snapper:**

- Lengths: standard, fork, total: 267, 310, 325 mm
- Total weight: 449.22 g
- Sex: immature individual with undeveloped gonads
- White muscle: 2 samples
- Skin swabs: 2 samples
- Stomach content: included two 8 mm long toothed jaws from a small predatory fish, 1 stomach content sample only
- Note: surface injury observed on the left side by the dorsal fin. Probably injured during capture as very little internal bruising associated with the injury.

#### **Yellowspotted rockcod:**

- Lengths: standard, fork, total: 280, 323, 330 mm
- Total weight: 484.43 g
- Sex: female, gonad development stage 2 (early stage of egg formation)
- White muscle: 2 samples
- Skin swabs: 2 samples
- Stomach content: included two 20 mm long crab claws with attached legs, and crab shell fragments; 2 stomach content samples collected.

#### **Goldband snapper:**

- Lengths: standard, fork, total: 345, 367, 418 mm
- Total weight: 927.65 g
- Sex: female, gonad development stage 2 (early stage of egg formation)
- White muscle: 2 samples
- Skin swabs: 2 samples
- Stomach content: 1 sample only.

### 3.2 Results of chemical analysis of fish biopsies

#### Results from chemical analysis of Fish Skin Swabs

Compound / Sample	Saddletail snapper	Crimson snapper	Yellowspotted rockcod	Goldband snapper	Goldband snapper #1 store-bought	Goldband snapper #2 store-bought
TPHs (mg/g)	not detected	not detected	not detected	4.00	not detected	2.60
Total PAHs (mg/g)	not detected	not detected	not detected	not detected	not detected	not detected

#### Results from chemical analysis of Fish Stomach Contents

Compound / Sample	Saddletail snapper	Crimson snapper	Yellowspotted rockcod	Goldband snapper	Goldband snapper #1 store-bought	Goldband snapper #2 store-bought
TPHs (mg/g)	14.65	15.30	2.47	8.50	10.26	8.96
Total PAHs (mg/g)	not detected	not detected	not detected	not detected	not detected	not detected

#### Results from chemical analysis of Fish Muscle

Compound / Sample	Saddletail snapper	Crimson snapper	Yellowspotted rockcod	Goldband snapper	Goldband snapper #1 store-bought	Goldband snapper #2 store-bought
TPHs (mg/g)	0.10	not detected	not detected	not detected	not detected	0.11
Total PAHs (mg/g)	not detected	not detected	not detected	not detected	not detected	not detected

- The TPH results for all samples are biased by the presence of organic compounds of biological origins.
- Unless TPH levels are accompanied by petroleum specific PAH compounds, it is considered that the TPH levels represent biological oils naturally occurring in fish and in their prey (person. comm., A. Tottszer, Advanced Analytical, Brisbane). In the present case, petroleum-specific PAHs have not been detected in any of the fish samples and consequently, it is considered that these commercially important deep sea fish species have not accumulated petroleum hydrocarbons.
- Bile samples collected upon capture of fish can accumulate petroleum hydrocarbons up to 1000-times higher levels than in fish white muscle and consequently, collection and analysis of fish bile would be a more sensitive parameter in informing on possible exposure of fish to petroleum hydrocarbons.

Contrarily to the samples analysed for the present investigation, samples collected during other oil spill events have been found to contain high molecular weight petroleum hydrocarbons. For example:

- the maximum concentration of PAHs in wild fish flesh shortly after the 1993 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 (1997) was 0.000 044 mg/g (Komaya et al 2004). Comparatively, no PAHs were detected in the stomach contents of the deep sea fish collected by the commercial fisherman in the vicinity of the oil slick. No PAHs were detected either in the stomach content of the store-bought fish.

### ***3.3 Additional notes on fish sampling:***

- PAHs are readily accumulated in clams and mussels, but not in fish, birds and mammals because vertebrate species are capable of metabolising PAHs at rates that prevent significant bioaccumulation (Hartung 1995). However, ill-effects associated to chronic (long-term) exposure to crude oil have been commonly observed in wildlife, including in fish (Budzinski et al. 2004; Marty et al 2003; Barsiene et al 2006). It is imperative to commence a monitoring program of fish health during the oil spill, as well as after the oil leak has been solved. A fish health monitoring program should continue until there is no sign of exposure or ill-effects in fish.
- Petroleum body burden in wildlife is expected to reduce rapidly following the cessation of exposure. For example, invertebrates exposed to the Nakhodka oil spill saw their PAHs content reduced from 44 ng/g to 5 ng/g over 4 weeks following the end of the exposure (Koyama et al. 2004).
- It is important to note that only one sample per biopsy has been analysed for TPHs and PAHs.

### 3. References

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### 4. Acknowledgements

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