Montara Well Release

Olfactory analysis of Timor Sea fish fillets

November 2011

Curtin University

Preface

This report was prepared by Christopher Rawson and Marthe Monique Gagnon from the Department of Environmental and Agriculture, Curtin University and Hannah Williams from the Food Science & Technology Program, School of Public Health, Curtin University. This report describes the results of the olfactory analysis of fish samples collected in the Timor Sea following the Montara well release. The olfactory analysis was conducted in March 2011, in the Sensory Evaluation laboratories located in the School of Public Health, Curtin University.

Acknowledgements

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Executive Summary

Curtin University was contracted by PTTEP Australasia (PTTEPAA) to conduct an olfactory analysis of fish fillets collected in the Timor Sea following the Montara well release in August 2009. During 3 scientific monitoring cruises aboard the *FV Megan M* commercial fishing vessel, fillets were collected from a range of species at 9 different locations in the Timor Sea. During 2 of these cruises (S4A Phase I – November 2009; S4A Phase II – March 2010) fillets were collected by ecotoxicologists from Curtin University while on the other cruise (S4B – January 2010) fillets were collected by personnel from the Department of Fisheries, Western Australia.

Olfactory analysis was conducted on fillets collected from sites designated as “impacted” and “non-impacted” based on available information as to the location of hydrocarbons during the well release and immediately following the capping of the Montara H1 well. The commercially important species red emperor (*Lutjanus sebae*) and goldband snapper (*Pristimoides multidens*) were selected for olfactory testing.

The testing was conducted between the 14th and 22st March 2011 using the duo-trio method (Standards Australia, 2005). Trained panellists were used to determine if they could distinguish between an uncooked sample from a fish captured in an “impacted” location and a sample from a fish captured in a “non-impacted” location. The panellists were not aware of the origin of the test material, they were only requested to identify if differences existed between the portions. Panellists were further asked to provide qualitative comments on the olfactory qualities of the samples. The trial was repeated after the samples were cooked.

Panellists were able to identify differences between “impacted” and “non-impacted” samples for red emperor (but not for goldband snapper) captured in the first sampling period, immediately following the capping of the H1 well in November 2009. These differences were observed in both raw and cooked samples. No differences were identified between “impacted” and “non-impacted” samples of either species collected later (January 2010 and March 2010).

Qualitative responses of the trained panellists did not provide consistent opinion as to the possible source of the observed differences between “impacted” and “non-impacted” red emperor samples from November 2009. Similarly there was no consistent overall trend in the qualitative description of odours which would indicate the presence of particular contaminants. All descriptors by the panellists could be used to describe “normal” fish odours.

Given that all samples were collected, handled and stored similarly, the detected odour differences between the “impacted” and “non-impacted” red emperor fillets are likely due to site-specific characteristics at the time of capture. The fact that these differences were not evident in samples collected in March 2010 and November 2010 indicates that the source of these differences was absent at these later times.

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Background

The Montara H1 well release starting on Friday 21st August, 2009, resulted in the release of gas, condensate and crude oil into the Timor Sea. The incident occurred in an area utilised for commercial fishing activities (Northern Demersal Scale Fishery). Following the capping of the leaking H1 well in early November 2009 concern has been raised regarding possible impacts on commercial fisheries and the potential for commercial fishes to have been tainted by exposure to hydrocarbons resulting from the well release.

Immediately following the capping of the H1 well ecotoxicologists from Curtin University were in the vicinity of the well release collecting samples from commercially important species for biomarker-based analysis of exposure of fish to hydrocarbons. This was Phase I of Study S4A under the Monitoring Plan for the Montara Well Release agreement between the then Department of Environment, Water, Heritage and the Arts (now Sustainability, Environment, Water, Populations and Communities – SEWPaC) and PTTEP Australasia (PTTEPAA). PTTEPAA requested that as part of this sampling trip Curtin University staff collect samples of fish fillets from each sampling site for possible olfactory or taste evaluation at some future point. Samples were also collected in January 2010 by WA Fisheries personnel during a sampling event for Study S5 of the Monitoring Plan for the Montara Well Release and by Curtin University personnel in March 2010 during sampling for Phase II of Study S4A.

In early 2011 Curtin University was contracted by PTTEPAA to conduct a sensory assessment of the fish fillets collected from the Timor Sea in the 3 sampling periods described above. Sensory assessment of seafood has been established as a sensitive technique for identifying taint by petroleum products even when the product is considered “acceptable” in terms of food safety (Yender et al. 2002)

Project Aims

- The aim of this evaluation was to use trained assessors to determine if differences existed in the olfactory status of fish fillet samples collected from sites designated as “impacted” and “non-impacted”.
- By allowing assessors to submit qualitative evaluation (comments) any consistent descriptors might provide an indication of possible source(s) of specific odours.
- By using samples from all 3 sampling periods it was expected that any persistence of olfactory taint could be identified.

Methodology

Fish Sampling Sites

During studies S4A and S5, fish were collected from a total of 9 sites. During Study S4A Phase I six sites were sampled and each was designated as either “impacted” or “non-impacted” on the basis of information available at the time regarding the location and direction of visible hydrocarbons or residue. This designation was retained throughout studies S5 and S4A Phase II with only the addition of supplementary “non-impacted” sites. Where sites have the same identifying number actual sampling location is likely separated by a small distance as localised fish populations may not have re-established. Hence, traps were generally set as close as practically possible to previous locations.

Sample Transport and Storage

During S4A Phase I and II fillet samples were collected, wrapped in hexane rinsed aluminium foil and placed in a snap-lock plastic bag immediately following capture. These were frozen aboard the *Megan M* and transported frozen to the Aquatic Toxicology laboratories at Curtin University immediately upon return to land. Collection of samples during Study S5 occurred after the return of the *Megan M* to Darwin in the laboratories of the NT Fisheries. These samples were stored in snap-lock plastic bags and transported frozen to Curtin University. All fillets were stored at –20°C until olfactory analysis was undertaken. It should be noted that the storage time, therefore, varied for samples collected during the 3 sampling periods. Significant attempts at minimising the impacts of this storage on the samples were made. In particular the careful, multi-layered wrapping described above was conducted in order to minimise desiccation, freezer damage or other deterioration associated with long-term storage.

Fish Sample Selection

For the purpose of the olfactory analysis, fish fillets were required from both “impacted” and “non-impacted” sites. Samples from the sites closest to the source of the well release were randomly selected from the available samples and designated as “impacted” samples. In each sampling period “impacted” samples were collected from fish captured within 2 NM of the West Atlas drilling rig (Table 1). “Non-impacted” samples were selected from fish collected at Site 5 (Study S4A Phase I and II) and Site 8 (Study S5) both of which lie further than 80NM from West Atlas. Within the fish sampled at each site, samples were randomly selected where there were greater than the required number available.

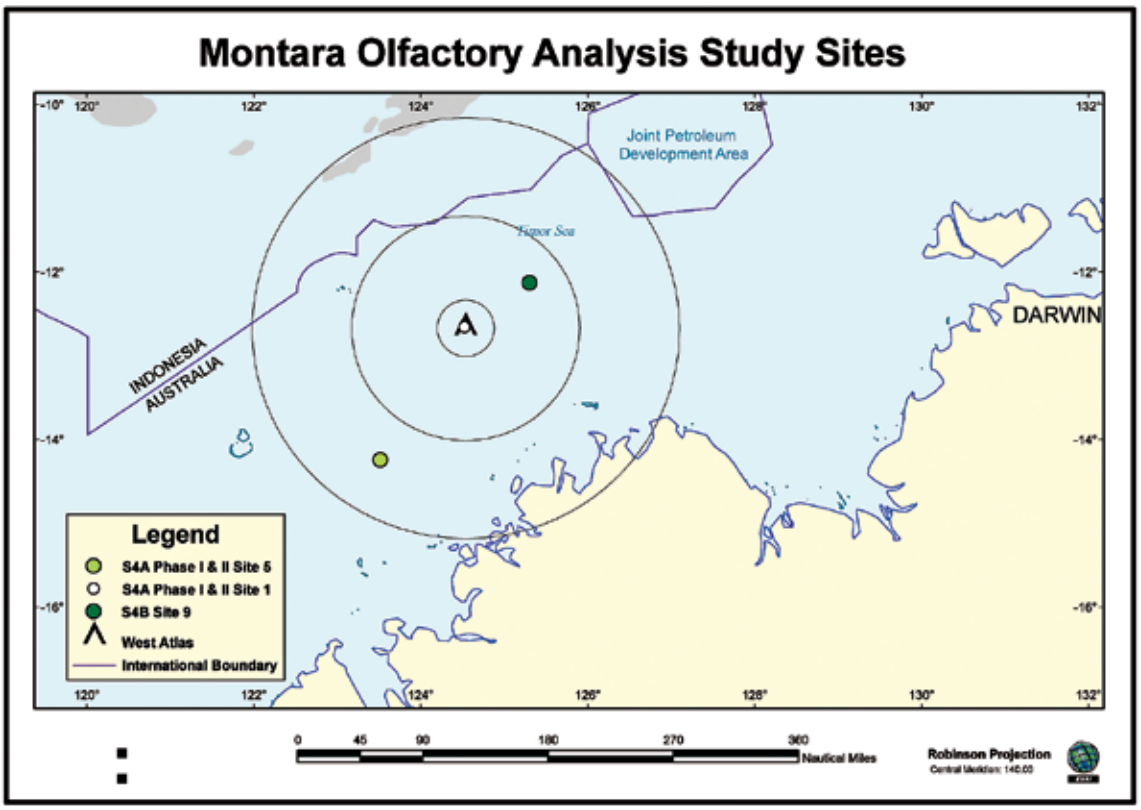

Figure 1. Map of sites sampled during Studies S4A and S4B which were selected for olfactory analysis. Concentric rings represent 20, 80 and 150 NM from the West Atlas drilling rig.

Table 1. Sites selected for inclusion in olfactory analysis of Timor Sea fish fillets.

Site No.	Site Desc.	Impact	Location		Distance from West Atlas rig (NM)	Depth (m)
			Lat (°)	Long (°)		
1	West Atlas	Impacted	12.9450 S	124.6900 E	<1 – 2	70
5	Browse Is.	Non-impacted	14.2563 S	123.5255 E	82	15 – 80
9	WAF Site 2	Non-impacted	12.1402 S	125.3135 E	54	75

Experimental Design

The experimental design of the olfactory analysis is described in detail in Appendix A. Ten red emperor and 10 goldband snapper fillets from "impacted" sites and 10 red emperor and 10 goldband snapper fillets from "non-impacted" sites were selected for analysis from each of the 3 sampling periods (Study S4A Phase I, Study S5, Study S4A Phase II) (Table 2). Since there was a difference in storage time for samples collected at different sampling periods each was tested separately (i.e., no comparison between sampling periods is made).

Table 2. Experimental design for olfactory testing of Timor Sea fish fillets.

Sampling Period	Species	Impact	Number	Treatment
S4A (November 2009)	Red emperor	Impacted	10	Raw, cooked
		Non-Impacted	10	Raw, cooked
	Goldband snapper	Impacted	10	Raw, cooked
		Non-Impacted	10	Raw, cooked
S5 (January 2009)	Red emperor	Impacted	10	Raw, cooked
		Non-Impacted	10	Raw, cooked
	Goldband snapper	Impacted	10	Raw, cooked
		Non-Impacted	10	Raw, cooked
S4B (March 2010)	Red emperor	Impacted	10	Raw, cooked
		Non-Impacted	10	Raw, cooked
	Goldband snapper	Impacted	10	Raw, cooked
		Non-Impacted	10	Raw, cooked

Olfactory Testing Methodology

Testing methodology is described in detail in Appendix A. Testing was conducted by the duo-trio method in accordance to the Australian Standard (Standards Australia , 2005). This method asks panellists to identify which sample of two (one "impacted" and one "non-impacted") is most similar to a control sample (either "impacted or "non-impacted"). The panellists were seafood industry employees recruited then trained at Curtin in the sensory evaluation of seafood quality parameters. They were screened prior to the study and did not include smokers, asthmatics, pregnant women or those with allergies to seafood. The same panellists were used throughout the study. The panellists were not informed of any potential source of contamination and were not aware of species being tested or their origin. Binomial statistics based on "correct" answers was used to statistically analyse the results.

Results

The results of the analysis are presented in Appendix A. In summary:

- There was a detectable difference between “impacted” red emperor fillets and “non-impacted” collected immediately following the capping of the Montara H1 well (November 2009). The differences were detected in both raw and cooked samples.
- At the same sampling period there was no detectable difference between “impacted” and “non-impacted” samples of goldband snapper.
- There were no detectable difference between “impacted” and “non-impacted” samples of either species collected in the later January 2009 and March 2010 sampling periods.
- Qualitative descriptions of odours of the samples showed no consistent trend.

Conclusions

In the first sampling period following the well release (November 2009) there were detectable olfactory differences between the red emperor samples collected at Site 2 (<2 NM from the drilling rig) and those collected at a reference site (Site 5 – 82 NM from the West Atlas). There were no distinctive taints recognised by the panellists that could be related to the presence of oil or dispersants in the environment where these fish were collected. Further, it was difficult to interpret the descriptors used by the panellists as positive or negative and therefore difficult to say whether they regarded the fish samples collected in the impacted area as of better or worse quality than the fish samples originating from reference area. The testing protocol does not (for reasons of confounding bias) allow for the researcher to ask the panellists for further information on their comments. While a statistically significant difference was detected between the fish collected at the two sites all samples were collected, handled and stored similarly. Hence, the difference in odour appears related to the site specific characteristics at the time of collection.

It is not possible to conclusively identify the source(s) of these olfactory differences. It is possible that the detected differences were due to the fish at Site 2 being exposed to hydrocarbons from the Montara well release (and/or dispersants sprayed in the area following the well release) to a greater degree than those collected at Site 5. This explanation seems to explain both the geographic difference between the samples and the fact that the difference is not detected in later sampling periods (an indication that the source of the variation had been removed). However this is an extremely tentative interpretation of the data. Based on the lack of consistent descriptors relatable to exposure to hydrocarbons a number of other site specific factors are equally likely to be responsible. Variations in food sources, biological age of the animals, home range, the physical activity surrounding the normal operations of the drilling rig and the activity in the area following the well release could explain the olfactory differences detected between sites. The temporary intensive marine activity immediately following the Montara well release (but not associated with exposure to oil) could also explain the temporal trends in the results of the olfactory assessment.

References

Standards Australia, (2005). Method 2.4 Specific methods – Duo Trio. In *Australian Standard 2542: Sensory analysis*, Sydney Standards Australia.

Yender, R., J. Michel, et al. (2002). Managing seafood safety after an oil spill. Seattle, Hazardous Response Division, Office of Response and Restoration, National Oceanic and Atmospheric Administration: 72pp.

APPENDIX A –
Sensory evaluation results report

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Olfactory assessment of fish fillets

Description:

The well release in the Timor Sea on Friday 21st August 2009 resulted in the release of gas and condensate. While the spill is not believed to pose threat to the quality of the impacted fisheries evidence is still required. The aim of this work is to determine if any sensory difference can be detected between fish caught in the impacted region and fish caught elsewhere. Samples were collected at three time points over a 5 month period to allow for build-up and/or dilution of any impacting factors.

Planned start date: 01/03/11 ***Planned finish date***: 20/04/11

Aim:

The aim of this research is to compare the consumers' perception of odour for 2 species of fish caught at each time point and identify significant odour differences between fish captured in an area impacted by the well release and those captured from a reference area.

Methods

A panel of 8 to 10 trained seafood sensory panellists from the seafood industry was used to assess the samples. The low number of panellist was justified based on the limited size of the samples available and their expertise in sensory evaluation of fish. The panellists are seafood industry employees recruited then trained at Curtin in the sensory evaluation of seafood quality parameters. They were asked to attend three sensory sessions to assess the fish samples. Sessions were held over 3 days with only samples collected at the same time point being assessed in each session (Table 1). Panellists were given no information about the samples that they were testing in order to prevent the development of false expectations.

Table 1: experimental design

Sampling point	Species	Exposure	number	Treatment
Sample set 1: S4A Phase 1 (November 2009)	Red emperor	Impacted	10	raw, cooked
		Non impacted	10	raw, cooked
	Goldband snapper	Impacted	10	raw, cooked
		Non impacted	10	raw, cooked
Sample set 2: S4B (January 2010)	Red emperor	Impacted	10	raw, cooked
		Non impacted	10	raw, cooked
	Goldband snapper	Impacted	10	raw, cooked
		Non impacted	10	raw, cooked
Sample set 3: S4A Phase 2 (March 2010)	Red emperor	Impacted	10	raw, cooked
		Non impacted	10	raw, cooked
	Goldband snapper	Impacted	10	raw, cooked
		Non impacted	10	raw, cooked

Total to be tested: 120 samples to be assessed by olfaction, first tested raw, then cooked.

Panel sessions were held in the sensory evaluation laboratories at Curtin University. Blue-green lights were used in the sensory booths to mask colour differences and enable panellists to focus solely on odour as the differentiating characteristic. Panels were run late in the afternoon to reduce the likelihood of interfering odours from other laboratory activities biasing the results and to facilitate panellist attendance.

Each fish portion was divided into pieces of a standard size and thickness. The samples were presented on a white china plate labelled with a random 3 digit code (between 100 and 999) to ensure blind assessment. Each sample was assessed in its raw state and again in its cooked state. Every impacted sample was paired with a non-impacted sample from the same sampling period and assessed for difference using a duo trio test (Standards Australia. 2005). In this test each panellist receives two samples and an identified control sample. The balanced reference technique was used for all samples. For half of the tests the control sample is the impacted sample and for the other half of the tests the control sample is the non impacted sample. The panellists are then asked to identify which of the samples is the same as the control based on the olfactory properties alone. Qualitative data of differences noted was also collected. An example of the data collection sheet is shown in appendix A.

The number of times the sample that is different to the control is correctly identified in each test run (irrespective of sample order) is calculated. The results were analysed using the binomial distribution tables (Standards Australia 2005). The tables show that the proportion of panellists able to detect the different sample must meet the level shown in Table 2 to declare significance at the alpha levels given (Standards Australia, 2005).

Table 2: proportion of correct responses required for significance

Alpha level (p)	Proportion of correct responses
0.2	0.75
0.1	0.88
0.05	0.88

Results

N.B. Significant differences are highlighted in red text

Table 3: results for Sample set 1

Trial 1	Goldband raw	Goldband cooked	Red Emperor raw	Red Emperor cooked
Proportion Detected	0.63	0.5	**0.75 (P=0.2)**	**0.88 (p=0.05)**
Comments				
Impacted	chemical odour, mild, no smell, flat, alkaline	wooden, mild	stronger, sweeter, fresher	dried fish, bland neutral, flat
Non Impacted	stronger smell, seaweed, neutral	seaweed, salty,	savoury, briny, seaweed, fishy	sweet, lingering, metallic

Table 4: results for Sample set 2

Trial 2	Goldband raw	Goldband cooked	Red Emperor raw	Red Emperor cooked
Proportion Detected	0.63	0.63	0.63	0.63
Comments				
Impacted	stronger, raw lettuce	oily, honeyed, bacon, corn, neutral	stronger, fishy , honeyed, bland, neutral	stronger, fishy , honeyed, bland, neutral
Non Impacted	slight fish smell, pungent old eggs, sour, rotting seaweed	stronger, fishier, rubber	stronger, milder, linseed	stronger, burning, harsher, waxy, oily, linseed

Table 5: results for Sample set 3

Trial 3	Goldband raw	Goldband cooked	Red Emperor raw	Red Emperor cooked
Proportion Detected	0.67	0.67	0.33	0.11
Comments				
Impacted	stronger, washing powder, metallic	bland, fresher, stronger, metallic, acid	seaweedy, metallic	neutral
Non Impacted	fresh seaweed, hot neutral, tea	chemical, stronger, salty, berries, rancid	pungent fishy, neutral	sweet, caramel

Based on the results displayed in tables 3 to 5 the study shows that only the samples from Sample set 1 (November 2009) demonstrate a significant difference in odour. Of the two species sampled there is no significant differences in odour detected in Goldband ($p>0.2$), however significant differences in odour perception were detected in the Red emperor samples ($p \leq 0.2$). The differences in odour were more readily detected in the cooked samples than the raw samples (cooked, $p=0.05$, compared to raw, $p=0.2$). The release and increase in odours may be related to increased volatilisation of volatile compounds upon heating. The odour descriptors show that the odour profile of the Red Emperor samples was neutralised in the impacted sample i.e. it lacked the distinctive notes associated with the non impacted samples. The difference in odour between the samples may be due to the presence of compounds that interact with the odoriferous chemicals present to alter the aromatic profile but do not add significant identifiable notes to it.

It must be noted that the age of the samples, their packaging and storage techniques make it impossible to compare these results with freshly harvested fish samples. Several of the descriptors used by the panel are indicators of spoilage and fat rancidity (linseed, rubber, oily (rancid vegetable/fish oil odours)). In addition it is possible that the aluminium foil used to wrap some of the samples resulted in the metallic notes found as the foil had significantly degraded on some samples.

Conclusions:

The earliest samples collected showed detectable differences in odour. The difference was not maintained through to the later samples at a statistically significant level. No distinctive taints were recognised by the panellists and all descriptors used can be applied to normal fish odours.

References:

Standards Australia, 2005. Method 2.4 Specific methods- Duo Trio. In *Australian Standard 2542: Sensory analysis*, Sydney Standards Australia

Appendix A

Duo Trio Test sheet

Test Code: RE1 CK01

AROMA ASSESSMENT

Panellist Code: TPN1 Date:

Instructions: DO NOT EAT THE FISH SAMPLES

You have been given three samples to assess. The sample on the left is the reference sample, one of the samples is the same as the reference and one is different. Smell the samples carefully using small 'bunny' sniffs and select the different or odd sample and indicate by ticking the box next to that sample's code. If you cannot decide please guess!!!

	Sample Code	
Reference	440	☐
	100	☐
	672	☐

Please describe any differences in aroma that you detected as fully as possible

_____

_____

_____

_____

Thank you.