Montara Well Release:

Report on necropsies from birds collected in the Timor Sea

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. Bird specimens collected from various sites during and after the Montara well release were shipped to Curtin University where they were dissected and necropsies collected in the Ecotoxicology laboratories, Curtin University.

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Summary of Results

- Necropsies were collected from 16 birds (13 common noddy, 1 lesser frigatebird, 1 wedge-tailed shearwater, 1 brown booby) collected in the Timor Sea during and following the Montara well release.
- A total of 72 samples (38 samples of lungs, breast tissue or gizzard contents, 32 swab samples from feathers and trachea, 2 bile samples) were analysed for the presence of total petroleum hydrocarbons (TPH - gas chromatography with flame ionisation detection) and polycyclic aromatic hydrocarbons (PAH – 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).
- One common noddy (collected deceased) from the vicinity of the Montara incident had crude oil on its feathers, in its gizzard contents, in its lungs and in its breast tissue indicating significant exposure to crude oil. Since this bird was very decomposed upon receipt (thoracic cavity open and intestine missing) such exposure could have been *pre-* and/ or *post-mortem.*
- One common noddy collected at Ashmore Reef had crude oil in its lungs but in no other samples (feather swab, trachea swab, breast tissue, gizzard). The fact that external swabs and intestinal tract samples were negative for the presence of crude oil suggests non-recent exposure.
- The remaining 14 birds submitted for analysis had no signs of crude oil in the feathers, in the gizzard contents, in the lungs or in the breast tissues.
- The majority of birds submitted for analysis appeared in poor physical condition and are likely to have died of natural causes.

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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 birds suspected of being impacted by the release were collected from the region. A small number of live birds which later died in captivity were also collected.

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 deceased birds suspected of exposure to the products of the well release and collect appropriate necropsies for chemical analysis to confirm if exposure to petroleum compounds had occurred.

Collection Locations

Specimens were received with varying degrees of information regarding sampling location, date of collection and the identity of the collector. Most of the specimens received at Curtin University were accompanied by GPS coordinates. Other specimens were accompanied by named locations of collection allowing the GPS coordinates to be gathered from online geographic databases. A small number of specimens were received with no information on the location of collection. Figure 1 shows the known locations of collection of the specimens received for necropsy.

Samples received

Bird specimens were received at Curtin University between November 2009 and March 2010. All samples were received frozen. This information is summarised in Tables 1. Briefly the specimens received were 13 common noddy (*Anous stolidus*), one wedge-tailed shearwater (*Puffinus pacificus*), one lesser frigatebird (*Fregata ariel*) and one brown booby (*Sula leucogaster*).

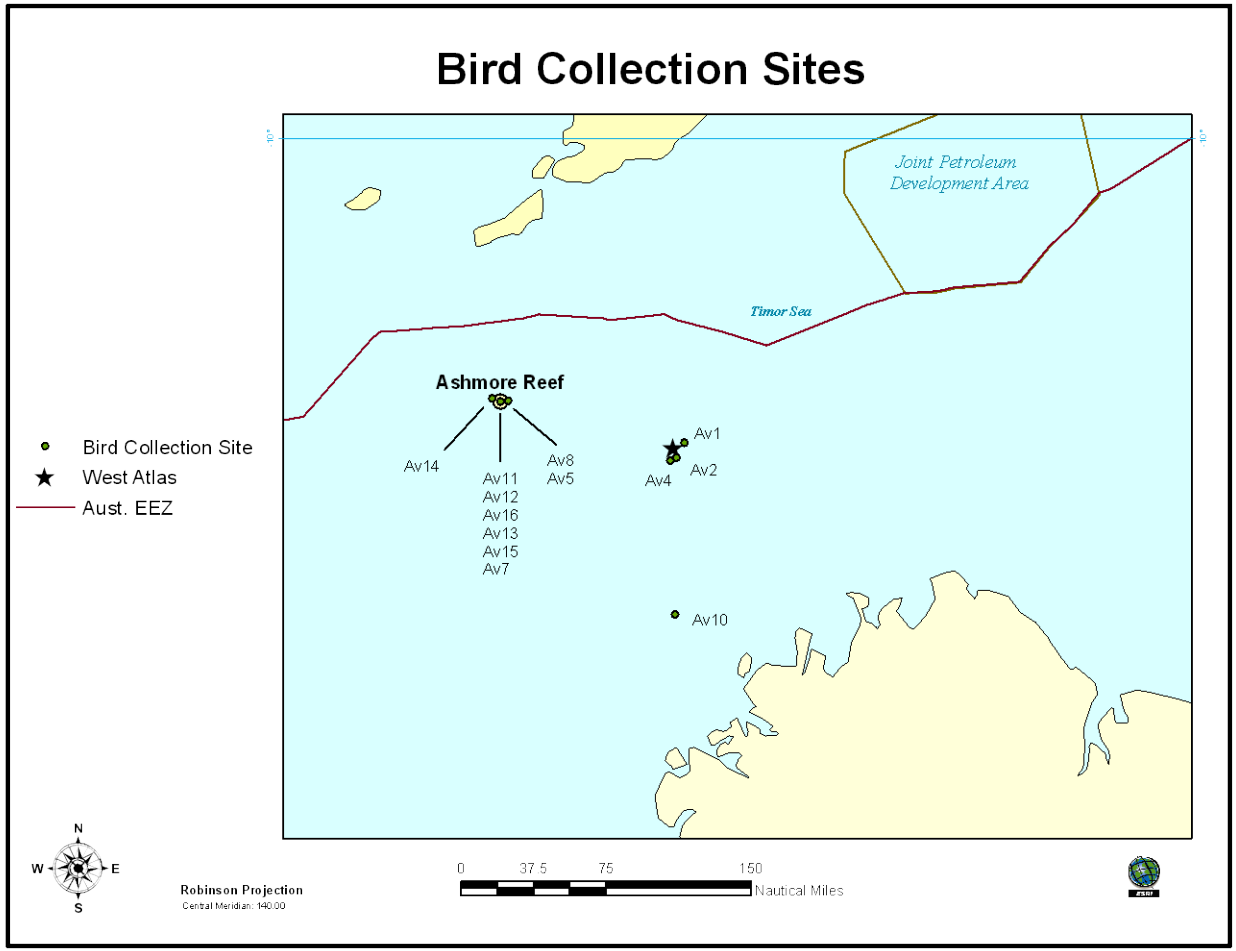

Figure 1. Location of the West Atlas drilling platform and the locations where the samples necropsied were collected. Samples Av3, Av6 and Av9 were received with no collection location information.

Table 1. Information on bird specimens received at Curtin University. Specimens are presented according to the collection date. U = Unknown (information not received with specimen).

Common Name	ID	Date Collected	Collected by	Location	GPS	Comments (with specimen)
Common noddy	Av1	30/09/2009	James Watson		12.62757 S 124.62811 E	Collected at sea alive floating in an oil slick. Died 10 hours post collection.
Common noddy	Av2	1/10/2009	Damian Tedon		12.75650 S 124.56000 E	Nil
Common noddy	Av11	13/10/2009	Phillipa Wilson	Middle Island	12.27158 S 123.04118 E	Label: Common noddy #16. Middle Is. 13/10/09 1700hrs. With possible oil contamination (dorsal surface). Weight = 100g. Fed but not washed. Dead 14/10/09 0700hrs.
Common noddy	Av12	14/10/2009	Phillipa Wilson	Middle Island	12.27158 S 123.04118 E	Found dead on Middle Is. 14/10/09 0830hrs. Montara oil spill. Possible oil contamination.
Common noddy	Av16	14/10/2009	Phillipa Wilson	Middle Island	12.27158 S 123.04118 E	Common noddy. Found dead on Middle Is. 0830 14/10/09.
Common noddy	Av14	23/10/2009	A. Williams	West Island	12.24324 S 122.97030 E	Label: AW 23-10-01. Found deceased near West Is. Ashmore Reef 23/10/09
Wedge-tailed shearwater	Av4	23/10/2009	T. Budd		12.78317 S 124.50683 S	Bird recovered near surface oil. Transferred from *First Class* to *Sea Spirit* (25/10/09). Received from *Sea Spirit* 25/10/09 Barbara Ross
Common noddy	Av13	27/10/2009	David Bond-Smith	Middle Island	12.27158 S 123.04118 E	Found 27/10/09 Middle I. North side. GPS co-ords unknown. Found on high tide mark. Oil suspected. Found dead.
Common noddy	Av15	27/10/2009	David Bond-Smith	Middle Island	12.27158 S 123.04118 E	Label: DBS-01. Common noddy. Found 27/10/09. Middle Is. East side. Not able to give GPS. Found dead on water line. Oil suspect on head and breast.
Common noddy	Av8	31/10/2009	Suzy Roworth	East Island	12.26583 S 123.10865 E	Label: Common noddy 17. East Island. Hydrated and washed. Found dead 01/11/09 in bird cage.
Lesser frigatebird	Av7	10/11/2009	Marissa Spiers	Middle Island	12.27158 S 123.04118 E	Label: MS-01. Collected eastern side of Middle Is. Found washed up. Very freshly dead. Highly doubt it is oiled but collected as precaution.
Common noddy	Av10	14/11/2009	Marissa Spiers	Browse Island	14.11068 S 124.54848 S	Label: MS-04. Found half-way up beach slope ion southern side of Browse Island.
Common noddy	Av3	U	U	U	U	Nil

Table 2. (cont.)

Common Name	ID	Date Collected	Collected by	Location	GPS	Comments (with specimen)
Common noddy	Av5	U	U	East Island	12.26583 S 123.10865 E	Nil
Common noddy	Av6	U	U	U	U	Nil
Brown booby	Av9	U	U	U	U	Nil

Necropsies Collected

All specimens were 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.

External swabs of feathers were taken initially. Primary incisions down the ventral midline allowed access for the remainder of the necropsies. Some specimens were clearly collected some time *post mortem* as evidenced by desiccation, the presence of maggots and by body damage caused by scavengers. In some instances it was not possible to collect all necropsies as some organs had been consumed by predators, scavengers or decomposers. Where possible;

- Swabs were taken in the trachea (close to the lung),
- Bile was collected
- Lung tissue was excised,
- Breast muscle was excised,
- The contents of the gizzard were collected were possible.

Chemical Analysis

A total of 72 samples (38 samples, 32 swab samples, 2 bile samples) were transported to Advanced Analytical Ltd. for the 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 can bias the results. These biological extractives have characteristic

chromatographic patterns and examination of individual chromatographic patterns of each extract in which hydrocarbons are detected 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 2.

Table 2. Limits of reporting for chemical analytes. The limits of reporting vary according to the amount sample provided and the level of matrix interference.

		Tissue / Gizzard contents	Bile	Swab (µg/swab)
TPH (mg/ kg)	TPH C 10 – 14	200 - 2500	2500 -12500	125
	TPH C 15 – 28	400 - 5000	5000 - 25000	250
	TPH C 29 - 36	400 - 5000	5000 - 25000	250
PAH (µg / kg)	Naphthalene	50 - 500	500 - 2500	10
	1-Methylnaphthalene	50 - 500	500 - 2500	10
	2- Methylnaphthalene	50 - 500	500 - 2500	10
	Acenaphthalene	50 - 500	500 - 2500	10
	Acenaphthene	50 - 500	500 - 2500	10
	Fluorene	250 - 2500	2500 -12500	10
	Phenanthrene	50 - 500	500 - 2500	10
	Anthracene	50 - 500	500 - 2500	10
	Fluoranthene	50 - 500	500 - 2500	10
	Benz(a)anthracene	50 - 500	500 - 2500	10
	Chrysene	50 - 500	500 - 2500	10
	Benzo(b)&(k)fluoranthene	100 - 1000	1000 - 5000	10
	Benzo(a)pyrene	50 - 500	500 - 2500	10
	Indeno(1,2,3-c,d)pyrene	250 - 2500	2500 - 12500	10
	Dibenz(a,h)anthracene	250 - 2500	2500 - 12500	10
	Benzo(g,h,i)perylene	250 - 2500	2500 - 12500	10
	Coronene	50 - 500	500 - 2500	10
	Benzo(e)pyrene	50 - 500	500 - 2500	10
	TOTAL PAH	1000 - 10000	10000 - 50000	10

Table 3. Observations made on bird carcasses during dissection.

Common Name	ID	Total Wt (g)	Head to Bill (mm)	Wing L (mm)	Observations during dissection
Common noddy	Av1	123.51	74.52	262	Feathers look disrupted, barbules not interlocked (probably due to washing while alive). Many parasitic worms in proventriculus. Stomach empty. Very digested contents of intestine.
Common noddy	Av2	150.88	75.24	269	Very decomposed. Thoracic cavity open at bottom. Intestine missing. Stomach empty. Feathers very oily.
Common noddy	Av11	76.21	72.72	255	Bird appears to have been starved (green and white cloaca, breast bone very exposed). Tissues fresh and not desiccated.
Common noddy	Av12	83.55	75.44	258	No sign of oil. Slightly decomposed.
Common noddy	Av16	51.88	76.18	267	Bird has been dead for a long time. Maggots have consumed the lungs. Abdominal cavity is empty and desiccated. Desiccated breast tissue collected.
Common noddy	Av14	81.40	75.51	253	No visible oil on/in this bird. Animal probably dead for several days (lungs brownish).
Wedge-tailed shearwater	Av4	228.62	94.84	292	No visible oil.
Common noddy	Av13	94.18	76.30	257	No visible oil but bird has wet feathers. Acceptable to poor body condition. Slightly skinny. Looks freshly dead.
Common noddy	Av15	78.90	78.90	272	No visible oil. One lung damaged but cause of decomposition unknown.
Common noddy	Av8	57.77	71.93	260	Bird is very skinny (looks starved). Almost no breast tissue. No stomach contents. No intestinal contents. No sign of oil on feathers.
Lesser frigatebird	Av7	890.00	164.09	570	No sign of oil. Coarse white sand in wings.
Common noddy	Av10	56.53	79.25	269	No sign of oil on feathers. Maggots present. Desiccated.
Common noddy	Av3	80.97	72.87	246	Feathers dry. No visible oil. Shell grit covering body. Half decomposed. Very smelly. Abdominal cavity open. Lungs very decomposed - no biopsy of gizzard contents, breast or lungs.
Common noddy	Av5	88.60	76.85	265	Feathers clean and fluffy. Parasites (white, 15mm long, stiff) in the proventriculus
Common noddy	Av6	136.52	83.93	282	Feathers dirty with excrement. Otherwise clean
Brown booby	Av9	258.85	156.69	370	Likely juvenile (bluish green feet). Very decomposed. Thoracic cage rotten and open. Maggots present. Desiccated. Very sandy (fine and coarse sand). Bird has been dead for several weeks. No muscle tissues or internal tissues left. Only bones and feathers.

Results and Interpretation

Tissues samples from all birds sampled had reportable concentrations of total hydrocarbons. However, expert examination of the chromatographs revealed that most of the birds had chromatographic patterns indicating the presence of biological extractives (fatty acids and cholesterol) with no evidence of petroleum hydrocarbons.

Tissue (breast, lung) samples and gizzard contents from one common noddy (Av2) had chromatographic patterns suggesting the presence of crude oil. The gizzard contents and the breast tissue collected on this bird also contained measurable concentrations of PAHs. The swab of the feathers of this bird similarly suggested the presence of crude oil. When this bird was examined the presence of large amounts of an oily substance was noted on all surfaces and, based on the degree of decomposition this bird had been dead for some time prior to collection. The fact that crude oil was found in the gizzard indicates that the bird had ingested the oil or that the oil had found its way inside the body *post mortem*. With the degree of decomposition noted it is not possible to suggest which of these scenario is the most likely to have occurred.

The analysis of lung tissue from an additional common noddy specimen (Av13) collected at Ashmore Reef resulted in chromatographic patterns indicating the presence of crude oil. This specimen showed no evidence of crude oil on the feathers suggesting no (or very little) external exposure. Further, no crude oil was picked up in the swab of the trachea indicating that the crude oil in the lung tissue is unlikely due to recent exposure. It is likely that this bird had inhaled floating crude oil while foraging in the weeks preceding its death however, it is not possible to ascertain if exposure to crude oil is the cause of death.

According to information supplied with the specimen, Av1 was collected while alive and floating in an oil slick. It is not clear from the information supplied whether this bird was later washed prior to death. Since analysis of the feather swabs taken from Av1 indicated no presence of petroleum hydrocarbons, this is likely the case. Gizzard content and lung necropsies taken from Av1 detected no evidence of exposure to petroleum hydrocarbons indicating that the bird had not ingested or inhaled petroleum hydrocarbons.

It was not clear from the information provided whether Av4 was collected alive or dead. It was stated that the bird was recovered "near surface oil". None of the necropsies collected on Av4 were positive for petroleum hydrocarbons. As the external swabs did not show evidence of petroleum hydrocarbons either the bird was collected alive and washed prior to death or had not been exposed to the surface oil near which it was collected.

PAHs are readily accumulated in aquatic filtering organisms such as 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 (Budzinski et al. 2004; Marty et al 2003; Barsiene et al 2006). In seabirds, chronic effects such as increased DNA damage (Laffon et al., 2006) and altered metabolism (Oropesa et al. 2007) were observed following the *Prestige* oil spill in 2002. In the year following the *Prestige* spill, monitoring of the reproductive activities of adults birds showed that effects observed in exposed adults were much reduced in the next generation of chicks (Alonso-Alvarez et al. 2007).

Petroleum body burden in wildlife is expected to reduce rapidly following the cessation of exposure. For example, blood concentrations of PAHs in seabirds oiled by the *Prestige* incident have reduced 3-fold in just one year, down to background levels observed in reference bird populations (Perez et al, 2008).

The prestige oil spill involved a heavy fuel-oil which generally causes more intense and profound effects on wildlife than lighter crude oils would. The Montara well release involved light crude oil and condensate which are very volatile and thus, would have dispersed rapidly in the environment.

In summary, chemical analysis of the 16 specimens suspected of being exposed to crude oil from the Montara well release revealed that only two individuals had detectable levels of petrogenic hydrocarbons in or on their body. The majority of birds submitted for analysis appeared in poor physical condition and are likely to have died of natural causes.

Table 4. Total hydrocarbons in bird samples. Bile samples are excluded as all were below the detection limits. Samples in bold are measured as above the detection limit. Highlighted samples denote those where chromatographic patterns indicated the presence of petroleum hydrocarbons. Tissue samples are expressed in mg/ kg for TPH and swabs are expressed in µg/swab.

		Gizzard			Breast			Lung			Feather Swab			Trachea Swab		
Common Name	ID	C10-14	C15-28	C29-36	C10-14	C15-28	C29-36	C10-14	C15-28	C29-36	C10-14	C15-28	C29-36	C10-14	C15-28	C29-36
Common noddy	Av1	<500	**5600**	**2900**	<500	**11000**	**1400**	<2500	**6600**	**10000**	<125	<250	<250	<125	<250	<250
Common noddy	Av2	<1000	**7200**	**4700**	<1000	**6200**	**2500**	<2500	<5000	<5000	**890**	**50000**	**6500**	<125	<250	<250
Common noddy	Av11	<5000	**2700**	**3000**	<200	**2700**	**1300**	<1000	**2300**	**7600**	<125	<250	<250	<125	<250	<250
Common noddy	Av12	<500	**3800**	**3000**	<200	**2300**	**1200**	<500	**3600**	**6200**	<125	<250	<250	<125	<250	<250
Common noddy	Av16				<200	**2400**	**1500**				<125	<250	<250	<125	<250	<250
Common noddy	Av14	<500	**2300**	**2900**	<200	**3300**	**1700**	<1000	<2000	**7000**	<125	<250	<250	<125	<250	<250
Wedge-tailed shearwater	Av4	<500	**2500**	**3300**	<200	**3000**	**2300**	<1000	**2700**	**8800**	<125	<250	<250	<125	<250	<250
Common noddy	Av13	<500	**2500**	**2700**	<200	**4800**	**1700**	<1000	**2600**	**6900**	<125	<250	<250	<125	<250	<250
Common noddy	Av15	<200	**870**	**1300**	<200	**5200**	**1500**	<1000	<2000	**6500**	<125	<250	<250	<125	<250	<250
Common noddy	Av8	<200	**2200**	**3600**	<500	**2600**	**2000**	<1000	**2400**	**7500**	<125	<250	<250	<125	<250	<250
Lesser Frigatebird	Av7				<500	**2800**	**1100**	<500	<1000	**3800**	<125	<250	<250	<125	<250	<250
Common noddy	Av10				<2500	<5000	<5000	<1000	**3000**	**3900**	<125	<250	<250	<125	<250	<250
Common noddy	Av3										<125	<250	<250	<125	<250	<250
Common noddy	Av5	<500	**2100**	**2600**	<500	**4300**	**1500**	<2500	**5700**	**9000**	<125	<250	<250	<125	<250	<250
Common noddy	Av6	<500	**2800**	**2600**	<200	**12000**	**1400**	<2500	<5000	**6600**	<125	<250	<250	<125	<250	<250
Brown booby	Av9										<125	<250	<250	<125	<250	<250

Table 5. PAH analysis in bird samples (mg/ kg for tissue samples, µg/ kg for swab samples). PAHs not shown were all below assay detection limits (naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, fluoranthene, pyrene, benz(a)anthracene, benzo(b)&(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene, coronene, benzo(e)pyrene). Highlighted samples denote those where petroleum hydrocarbons were reported.

Common Name	ID	Sample	1-methylnaphthalene	2-methylnaphthalene	Phenanthrene	Chrysene	Total PAHs
Common Noddy	Av1	Gizzard	<100	<100	<100	<100	<2000
		Bile	<2500	<2500	<2500	<2500	<50000
		Breast	<50	<50	<50	<50	<1000
		Lung	<500	<500	<500	<500	<10000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10
Common noddy	Av2	Gizzard	**1600**	**2400**	**6200**	**300**	**11000**
		Breast	**2100**	**3100**	**7500**	**400**	**13000**
		Lung	**950**	**1400**	**33000**	<500	<10000
		Feather swab	**14**	**10**	**50**	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10
Common noddy	Av11	Gizzard	<100	<100	<100	<100	<2000
		Breast	<50	<50	<50	<50	<1000
		Lung	<250	<250	<250	<250	<5000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea swab	<10	<10	<10	<10	<10
Common noddy	Av12	Gizzard	<100	<100	<100	<100	<2000
		Breast	<50	<50	<50	<50	<1000
		Lung	<100	<100	<100	<100	<2000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10
Common noddy	Av16	Breast	<50	<50	<50	<50	<1000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea swab	<10	<10	<10	<10	<10
Common noddy	Av14	Gizzard	<100	<100	<100	<100	<2000
		Breast	<50	<50	<50	<50	<1000
		Lung	<250	<250	<250	<250	<5000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10

Table 5. cont.

Common Name	ID	Sample	1-methylnaphthalene	2-methylnaphthalene	Phenanthrene	Chrysene	Total PAHs
Wedge-tailed shearwater	Av4	Gizzard	<50	<50	<50	<50	<1000
		Bile	<500	<500	<500	<500	<10000
		Breast	<50	<50	<50	<50	<1000
		Lung	<250	<250	<250	<250	<5000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10
Common noddy	Av13	Gizzard	<100	<100	<100	<100	<2000
		Breast	<50	<50	<50	<50	<1000
		Lung	<250	<250	<250	<250	<5000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10
Common noddy	Av15	Gizzard	<50	<50	<50	<50	<1000
		Breast	<50	<50	<50	<50	<1000
		Lung	<250	<250	<250	<250	<5000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10
Common noddy	Av8	Gizzard	<100	<100	<100	<100	<2000
		Breast	<100	<100	<100	<100	<2000
		Lung	<250	<250	<250	<250	<5000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10
Lesser Frigatebird	Av7	Breast	<50	<50	<50	<50	<1000
		Lung	<50	<50	<50	<50	<1000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea swab	<10	<10	<10	<10	<10
Common Noddy	Av10	Lung	<250	<250	<250	<250	<5000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10

Table 5. cont.

Common Name	ID	Sample	1-methylnaphthalene	2-methylnaphthalene	Phenanthrene	Chrysene	Total PAHs
Common noddy	Av3	Breast	<250	<250	<250	<250	<5000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10
Common noddy	Av5	Gizzard	<100	<100	<100	<100	<2000
		Breast	<100	<100	<100	<100	<2000
		Lung	<500	<500	<500	<500	<10000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10
Common noddy	Av6	Gizzard	<50	<50	<50	<50	<1000
		Breast	<50	<50	<50	<50	<1000
		Lung	<250	<250	<250	<250	<5000
		Feather Swab	<10	<10	<10	<10	<10
		Trachea Swab	<10	<10	<10	<10	<10
Brown Booby	Av9	Feather Swab	<10	<10	<10	<10	<10
		Trachea swab	<10	<10	<10	<10	<10

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