Project 2b-2 - Ecotoxicology of Rena Oil, Dispersant and Cryolite on Crayfish, Snapper and Wrasses

Nick Ling

Executive Summary

An experimental laboratory study of acute effects of environmentally realistic exposure durations and concentrations was undertaken to examine major pollutants of the Rena shipwreck. This report presents preliminary data from this research project which is ongoing until June 2014. Effects of Rena heavy fuel oil either singly or in combination with Corexit 9500 dispersant, and aluminium hexafluoroaluminate (Cryolite) were examined in sub-adult spotted wrasse (Notolabrus celidotus), snapper (Pagrus auratus) and red rock lobster (Jasus edwardsii) for periods up to 96 h duration. Analysis of fish bile indicated rapid and significant uptake of PAH compounds from oil, however, uptake did not appear to be enhanced by combined exposure to Corexit 9500. Minor effects of oil and cryolite were observed on some haematological parameters of both spotted wrasse and snapper but may reflect a generalised stress response to toxicant exposure rather than a specific toxicant effect.
1. Introduction

As part of the RENA long-term ecological recovery research programme, an experimental study of ecotoxicological effects was initiated to examine potential effects of major pollutants discharged from or associated with the Rena shipwreck. This study aimed to examine impacts on major kai moana species associated with both rocky and soft-shore marine habitats. Exposures to contaminants are being tested using larger individuals than would typically be tested in laboratory toxicity tests in order to measure a greater range of sublethal physiological or histological parameters and to measure effects on animals likely to enter the human food chain and which are significant commercial, recreational or kai moana species. The research programme was aligned to support a graduate student research thesis and is therefore ongoing with completion scheduled for June 2014.
2. Methods

Initial experiments have examined exposures of sub-adult snapper (*Pagrus auratus*: mean ±SD; length 202 ± 28 mm FL, weight 174 ± 44 g), spotted wrasse (*Notothenia cellidotes*: mean ±SD; length 146 ± 31 mm TL, weight 55 ± 27 g) and red rock lobster (*Jasus edwardsii*: mean ±SD; length 70.5 ± 7.0 mm OCL, weight 304 ± 82 g) to environmentally relevant concentrations (0.1 g/L) and exposure durations (up to 96 h) for *Rena* heavy fuel oil (HFO), *Rena* HFO combined with Corexit 9500 dispersant, and cryolite (sodium hexafluoroaluminate).

Snapper and spotted wrasse were collected by angling and rock lobster were collected by SCUBA divers from areas not impacted by the *Rena* oil spill and were acclimatised to laboratory captivity for at least two weeks prior to experiments.

Animals were exposed individually to toxicant dilutions in artificial seawater in 10 L glass aquaria (constructed without silicon glue which can adsorb hydrophobic compounds in water). Aquaria were aerated using glass tubes and toxicant solutions were partially replaced (50%) daily. Three individuals were subjected to each exposure duration (24, 48, 96 h) along with 96 h toxicant-free controls to account for potential stress effects resulting from confinement in small aquaria.

Toxicants were prepared as follows:

Heavy fuel oil – water accommodated fraction (WAF) was prepared according to the method of Singer et al. (2000) at a concentration of 1g/L HFO in seawater. 2 g of *Rena* HFO was added to 2 L of filtered (0.45 μ) artificial seawater in a 2 L borosilicate glass bottle and mixed with sufficient energy to produce a 25% depth vortex. This preparation was stirred for 24 h and the WAF diluted 10-fold in seawater to give a final exposure concentration of 1:10,000 (HFO:seawater). WAF was aspirated from below the surface of mixing vessels using a glass tube to avoid removing residual oil.

Heavy fuel oil + Corexit 9500 – chemically enhanced water accommodated fraction (CEWAF; Singer et al. 2000) was prepared as above with the addition of 100 μL/L of Corexit 9500 (1:10, dispersant:HFO). After 24 h mixing, CEWAF was diluted 1:10 in seawater as above.

Cryolite – powdered cryolite was mechanically dry sieved to remove larger particles (>100 μ) and a saturated suspension in seawater was prepared by vortex mixing for 24 h as for WAF above at a concentration of 1 g/L. This suspension was further diluted to produce a final exposure concentration of 0.1 g/L.

Exposure tests plan to use 6 animals per test for durations of 0, 24 h, 48 h & 96 h in addition to further animals that will be exposed for 96 h and then allowed to recover in toxicant-free seawater for either 4 d or 10 d to examine either delayed effects or recovery from acute effects. All tests have been approved by the University of Waikato Animal Ethics Committee.

Following exposures, fish were euthanized with an overdose of anaesthetic (0.1 g/L benzocaine) and lobster by hypothermia followed by brain ablation. Animals were sampled for blood by caudal venepuncture (fish) or cardiac puncture (lobster). Fish blood was mixed with anticoagulant (heparin 5000 i.u., 1:100) and lobster blood was mixed with 10% buffered formalin fixative (1:1). Fish and lobsters were then necropsied and tissues (fish; gill, liver, spleen, gonad where present: lobster; gill, hepatopancreas, gonad where present) were fixed in 10% buffered formalin for histological analysis. Liver samples were split with half taken for histology and the remainder stored at -20°C for later analysis of PAHs. Bile was sampled from the gall bladders of fish with a 0.5 mL syringe (29 G fixed needle) and stored at -20°C for later analysis of PAH concentration.
Standard haematological parameters (packed cell volume (PCV), haemoglobin concentration [Hb]) were obtained using standard methods (capillary centrifugation and cyanmethaemoglobin methods, respectively). Total red blood cell counts (RBCC) were performed for fish and haemocyte counts (HC) for lobster blood using a haemocytometer. Fish blood was diluted 1:200 in cell counting fluid and lobster blood was counted without further dilution. Derived haematological variables (mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), mean cell volume (MCV)) were calculated from these parameters as follows:

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\text{MCHC (g/L)} = \frac{[\text{Hb}]}{(\text{PCV}/100)}
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\text{MCH (g/cell)} = \frac{[\text{Hb}]}{\text{RBCC}}
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\text{MCV (L/cell)} = \frac{(\text{PCV}/100)}{\text{RBCC}}
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Fish bile was analysed for PAH (conjugated hydroxypyrene) concentration using synchronous fluorescence spectroscopy. Frozen bile was thawed and diluted 1:500 in ethanol:water (50:50) and analysed for pyrene-1-glucuronide by synchronous fluorescence spectrometry (SFS) using a Shimadzu RF-5301 scanning spectrofluorometer and a 1 cm path-length quartz cuvette. SFS spectra were scanned from 263 to 413 nm (excitation wavelength), scanning both monochromators simultaneously at a fixed wavelength difference (\(\Delta\lambda\)) of 37 nm and bandwidth of 5 nm. Quantification of pyrene-1-glucuronide concentration was determined by measuring the net peak area of the SFS spectrum from 375 to 390 nm (emission wavelength). Because pyrene-1-glucuronide standards are not available, peak areas were calibrated against a series of unconjugated 1-hydroxypyrene (Toronto Research Chemicals Inc.) standards and corrected for the greater fluorescence intensity and blue shifted emission spectrum (by 5 nm) of pyrene-1-glucuronide using a calibration factor of 2.2 (Ariese et al. 1993).
3. Results

Results to date represent a preliminary series of exposures to WAF, CEWAF and cryolite for periods of up to 96 h, although not all time points have been completed and sample size is currently limited to 3 animals per exposure. No mortality occurred for any species to any of the 3 toxicants tested during up to 96 h exposure to 0.1 g/L *Rena* HFO as WAF or 0.1 g/L cryolite, or for 24 h exposure to CEWAF.

Exposure to WAF resulted in rapid uptake and partitioning of PAH compounds into fish bile (Figure 1) but the addition of oil dispersant did not appear to increase PAH accumulation. Preliminary results indicate the potential for WAF and CEWAF to cause reductions in either packed red blood cell volume or whole blood haemoglobin concentration or both for spotted wrasse (Figure 2) and snapper (Figure 3). One-way ANOVA indicated significant effects of oil on spotty PCV and Hb, and on snapper Hb and MCHC (p<0.05). However, the small sample size and variability in most parameters limits statistical interpretation of these data. CEWAF does not appear to induce any difference in the nature of response to WAF but may enhance its effects. Haematological responses of fish to oil vary with studies variously reporting either increases (Zbanszék & Smith 1984) or decreases (Alkindi et al 1996) in PCV and whole blood haemoglobin concentration.

No significant effects were observed for haemocyte counts of rock lobster exposed to WAF or CEWAF (Figure 4). There are indications of an increase in haemocyte count but further data is required to confirm this effect.

Similar haematological effects of WAF and CEWAF were observed in spotted wrasse (Figure 5) and snapper (Figure 6) following exposure to cryolite suggesting that these effects represent a generalised stress response rather than a specific effect of each toxicant. Contrary to the effect of WAF and CEWAF on lobster, there are indications of a reduction in haemocyte count due to cryolite exposure (Figure 7) but once again the effect is not significant and further data is required.
Figure 1 Concentration of conjugated hydroxypyrene in fish bile following exposure to 0.1 g/l Rena HFO for periods of up to 96 h. A = spotted wrasse. B = snapper. C9500 = HFO with the addition of Corexit 9500 dispersant. Bars are mean values ± SEM. 0= 96 h toxicant-free controls.
Figure 2: Haematological values of spotted wrasse exposed to 0.1 g/L Rena HFO in seawater for periods up to 96 h. C9500 = HFO with the addition of Corexit 9500 dispersant. Bars are mean values ± SEM. O = 96 h toxicant-free controls. PCV = packed red cell volume, volume, Hb = whole blood haemoglobin concentration, RBCC = red blood cell count, MCHC = mean red cell haemoglobin concentration, MCH = mean red cell haemoglobin content, MCV = mean red cell volume.
Figure 3 Haematological values of snapper exposed to 0.1 g/L Rena HFO in seawater for periods up to 96 h. C9500 = HFO with the addition of Corexit 9500 dispersant. Bars are mean values ± SEM. 0 = 96 h toxicant-free controls. PCV = packed red cell volume, Hb = whole blood haemoglobin concentration, RBCC = red blood cell count, MCHC = mean red cell haemoglobin concentration, MCH = mean red cell haemoglobin content, MCV = mean red cell volume.
Figure 4 Haemocyte counts for rock lobster exposed to WAF or CEWAF for periods up to 96 h. Bars are mean values ± SEM. 0 = 96 h toxicant-free controls.
Figure 5 Haematological values of spotted wrasse exposed to 0.1 g/L cryolite in seawater for periods up to 96 h. Bars are mean values ± SEM. 0 = 96 h toxicant-free controls. PCV = packed red cell volume, Hb = whole blood haemoglobin concentration, RBCC = red blood cell count, MCHC = mean red cell haemoglobin concentration, MCH = mean red cell haemoglobin content, MCV = mean red cell volume.
Figure 6 Haematological values of snapper exposed to 0.1 g/L cryolite in seawater for periods up to 96 h. Bars are mean values ± SEM. 0 = 96 h toxicant-free controls. PCV = packed red cell volume, Hb = whole blood haemoglobin concentration, RBCC = red blood cell count, MCHC = mean red cell haemoglobin concentration, MCH = mean red cell haemoglobin content, MCV = mean red cell volume.
Figure 7 Haemocyte counts for rock lobster exposed to cryolite (0.1 g/L) for periods up to 96 h. Bars are mean values ± SEM. 0 = 96 h toxicant-free controls.
4. References


