© GÜLSAH DOGRUER / ENTOX / WWF-AUS

RIVERS TO REEF TO TURTLES PROJECT TECHNICAL REPORT AUGUST 2016

INTRODUCTION

Green turtles (*Chelonia mydas*) are an iconic species found on the Great Barrier Reef (GBR). They play an important role in maintaining healthy ecosystems, showing strong site fidelity to their foraging and breeding grounds. This strong site fidelity exposes coastal foraging populations to potential impacts from land-based sources of pollution due to the close connectivity between marine and terrestrial systems.

The GBR catchment has experienced extensive land use change in its central and southern catchments since the mid-20th century. Extensive clearing, predominantly for agriculture, has led to substantial increases in the amount of sediment and nutrients entering coastal waters as well as additional pollutants such as pesticides, heavy metals and other contaminants from agricultural, urban and industrial development. The effect of this increased pollutant load on GBR turtle populations is not well understood.

The 'Rivers to Reefs to Turtles' (RRT) project was instigated following a mass stranding and mortality event of Green turtles in Upstart Bay in 2012 and 2013, the cause of which remains unknown. The project is a collaborative partnership that aims to improve the understanding of the potential consequences of coastal pollution on marine turtles through identifying correlations between green turtle health and coastal water quality. The partner organisations are: WWF-Australia, TropWATER (James Cook University), Entox (National Research Centre for Environmental Toxicology, The University of Queensland) and Vet-MARTI (The University of Queensland), Griffith University, Queensland Government, the Great Barrier Reef Marine Park Authority (GBRMPA), and local community and traditional owner groups. This project is supported by Banrock Station Environmental Trust.

METHODS

Overview

The first two years of sampling incorporated a broad screening approach followed by more targeted analysis to characterise contaminant exposure in green turtles and their environment. Health parameters were also assessed to explore possible and plausible associations with any contaminant exposure.

Three study locations were selected for the project: Upstart Bay where the undiagnosed mass stranding of green turtles in 2012 and 2013 occurred; Cleveland Bay in suitably close geographical proximity to Upstart Bay; and, the Howicks Group of Islands (the Howicks Group), selected as a relatively unimpacted control site. More broadly, the three sites were also considered to represent catchments characterised by different land-use. Upstart Bay characterised primarily as agricultural, Cleveland as urban/industrial and the Howicks considered to be relatively unaffected by the adjacent land-use.

During the first two years of sampling, a total of 2,423 turtles were caught using the "turtle rodeo" method across the three study locations (Figure 1). Sub-adult turtles were selected for analysis to minimise variability due to age, gender, breeding migrations and parity, while maximising past exposure at the foraging site (approx. 20-35 years). Both blood and scute samples were collected for contaminant exposure assessment, and plasma and whole blood smears were used for biochemistry and haematology, respectively.

At each site, environmental samples were collected for analysis including benthic sediment, above ground seagrass biomass (forage), and water grab samples. Passive water samplers were also deployed across each study location, sampling for metals (DGT's) and organic chemicals (ED's and PDMS's). The crop contents of captured turtles were also archived for future analysis.

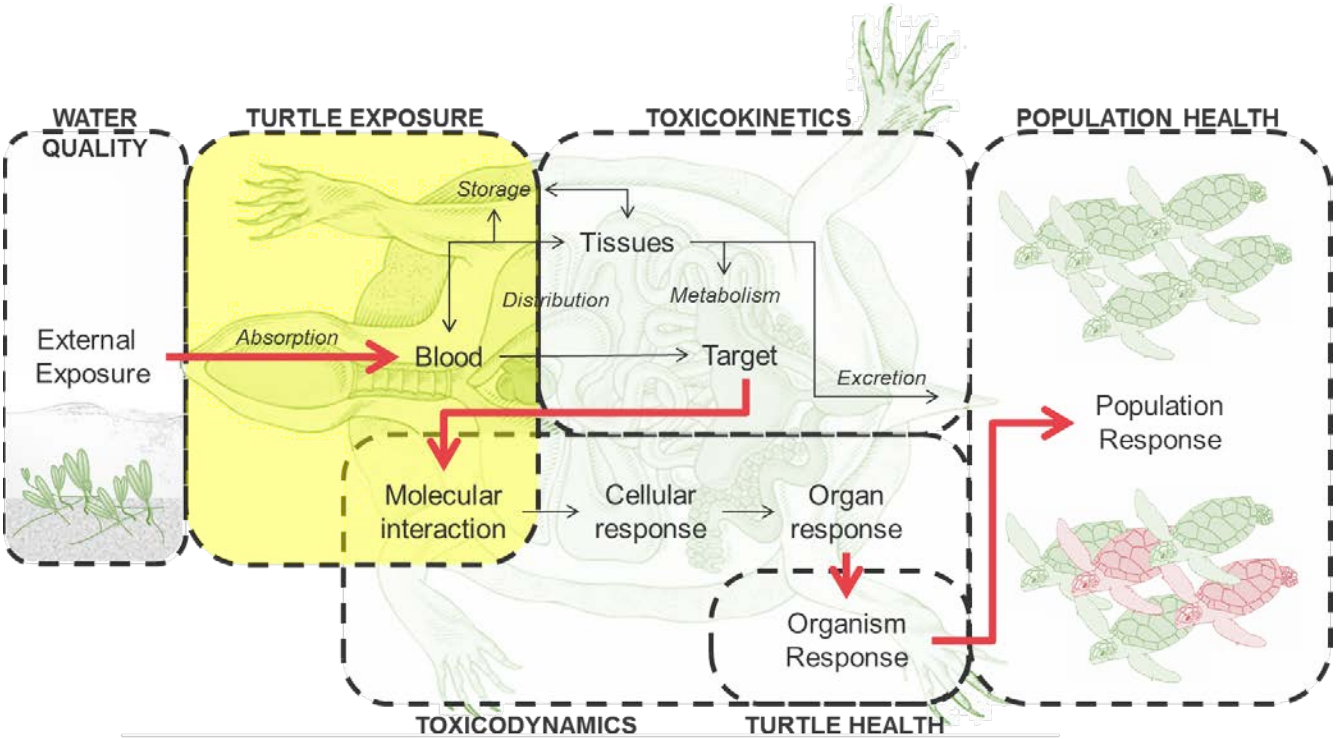


Figure 1. Conceptualised framework of the individual components of the RRT program, and links (red arrows) of the Turtle Exposure with other project components (ENTOX).

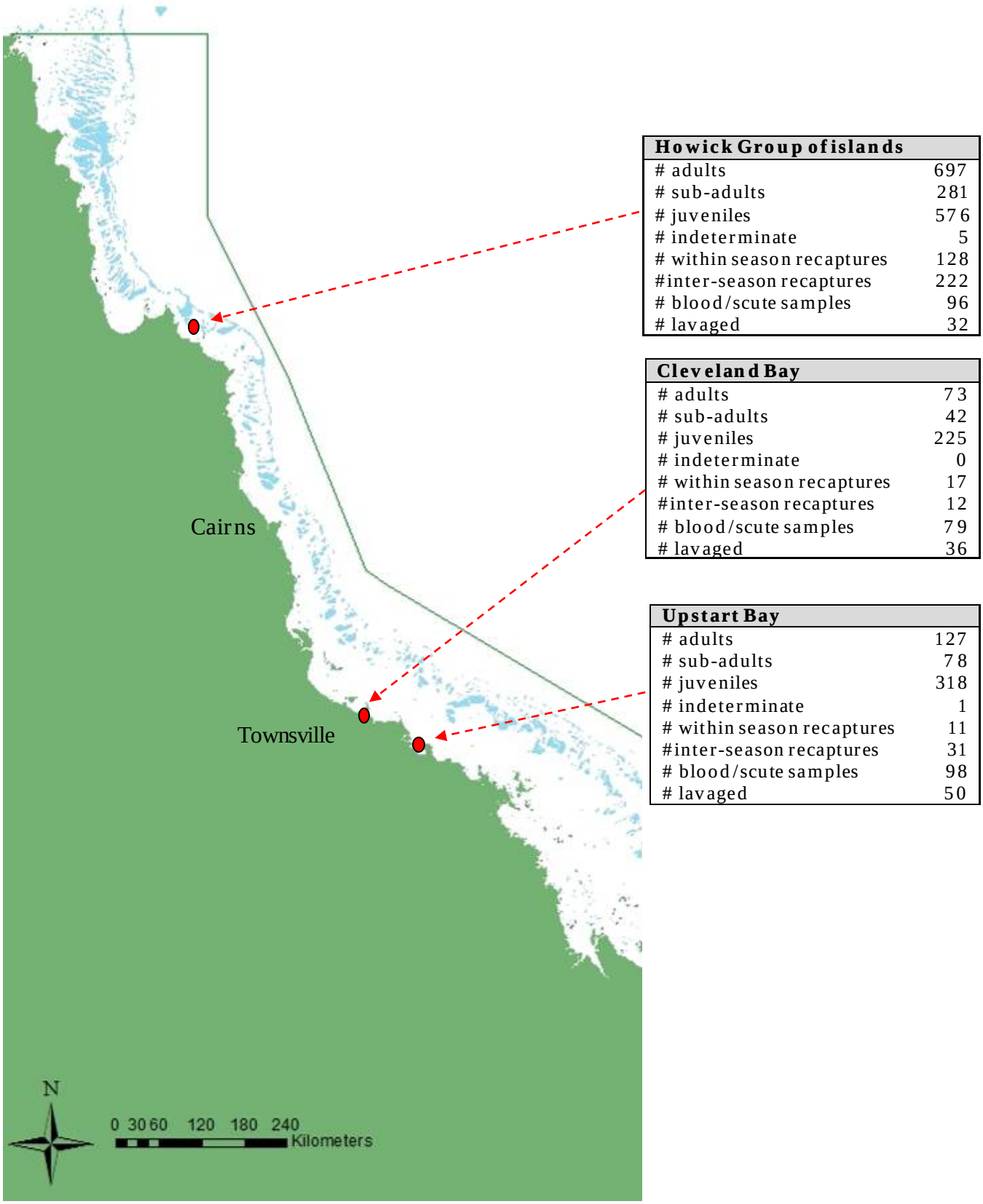


Figure 2: Study locations and samples collected during years 1 and 2

Analytical Methods

Turtle Genetics

Genomic DNA was isolated from all samples using standard manufacturer protocols for 1 of 4 extraction processes: phenol chloroform (modified from Sambrook et al. 1989), sodium chloride (modified from Miller et al. 1988), a modified DNEasy ® Qiagen extraction kit, or an X-tractor Gene robot (Corbett Robotics). The control region of the mitochondrial genome was amplified using polymerase chain reaction (PCR) methodologies to obtain ~800 bp using primers LCM-15382 (5' GCT TAA CCC TAA AGC in each PCR to detect any contamination. The PCR products were confirmed visually on 2% agarose gels stained with ethidium bromide (Maniatis et al. 1982). Purification of PCR products was done using a Qia - quick PCR Purification kit (Qiagen) or by combining 5 µl of PCR product with 2 µl of an Exonuclease I and Shrimp Alkaline Phosphatase solution (USB). Both strands were cycle sequenced using an ABI ® Big Dye Terminator v3.1 or v3.1 ABI Prism Terminator Cycle Sequencing Kit and analysed with Applied Biosystems® (models 3130 and 3730) automated genetic analyzers. Sequences were aligned, edited, and cropped at a standard cropping site (Frey et al. 2009) of 775 bp using the program SeqScape v2.5 (Applied Biosystems ®). Haplotypes were designated by comparing the generated sequences to a reference library of long 775 bp haplotypes representing published and unpublished green turtles from the Pacific Ocean.

The program BAYES (Pella & Masuda 2001) was used to estimate contributions from 28 known green turtle rookeries for turtles at the Howicks. A total of 20000 Markov Chain Monte Carlo steps were run for 4 chains. Each chain was started at different starting points. A burn-in of 10000 runs was used to calculate the posterior distribution. The Gelman and Rubin shrink factor diagnostic was computed to ensure that all chains had converged, as was indicated by a shrink factor of less than 1.2 for each chain (Pella & Masuda 2001). Individuals with haplotypes that had not been previously observed in any of the MUs (known as 'orphan' haplotypes) were removed from the analysis by the program as these are non-informative; however, they may indicate new MUs not yet sampled or undersampling of MUs.

Turtle Health

Clinical assessment

Body condition was determined based on visual assessment by experienced researchers and by assessing the index derived from the ratio of curved carapace length to weight. Turtles in good body condition, displaying no clinically apparent abnormalities (Herbst and Jacobson 2003), including neurological deficits (Chrisman et al. 1997) or lesions of the carapace or plastron (Flint et al. 2010), were classified as 'clinically healthy'. Animals being of 'average body condition' were classed as healthy or poor depending on lesions and other recorded abnormalities. Turtles in 'poor body condition' and/ or exhibiting clinical abnormalities were classified as 'clinically unhealthy' and examined in detail to determine cause of disease.

Blood collection and preparation

Blood samples were collected via the external jugular in the cervical dorsal sinus, prepared and examined using previously described techniques (Owens and Ruiz 1980, Flint et al. 2010), with minor modifications. Blood samples (>20 mL) were collected from turtles using an 18 G 38 mm needle attached to a 50 mL syringe. For the blood biochemistry and haematology, voided samples were transferred to a heparinised evacuated tube, and then placed in coolers (4 °C), separated and serum frozen at -80 °C until assessment of blood biochemistry and haematology, which occurred within 12 months of collection.

Blood biochemistry

Serum samples warmed to room temperature were run on a calibrated (using coefficients of variance) autoanalyser (Olympus AU400) in accordance with test methodology outlined by the manufacturer. The following blood variables were measured: albumin, amylase, aspartate transaminase (AST), creatinine kinase, cholesterol, creatinine, protein, gamma-glutamyl transpeptidase (GGT), globulin, alkaline phosphatase (ALP),

alanine aminotransferase (ALT), urea, glucose, total bilirubin, sodium, calcium, magnesium potassium, triglyceride, chloride, and phosphorous.

Haematology

Smears were made and air-dried at the time of blood collection. They were stained using H&E and sealed with a coverslip. Slides were manually read. Haematological measures (of leukocytes) were calculated for thrombocytes, lymphocytes, heterophils, eosinophils, basophils and monocytes and used to estimate total white cell counts (TWCC) (Work et al. 1998, Flint et al. 2010).

In Year 2 it was proposed to take a step forward in the approach to sampling sea turtles for clinical and anatomical pathology. Whereas in Year 1 the baselines were confirmed for the populations using standard health measures, Year 2 restricts sampling to recaptures from Year 1 to confirm suspicious cases of clinically ill turtles based on the toxicological screening results presented by UQ's Entox. To tease out the effect of these suspected anomalies on the individual, in addition to the standard biochemistry and haematology, (i) plasma protein electrophoresis (PPE) of suspicious cases based on toxicology and recaptures and (ii) physical examinations and necropsy examinations were conducted on any suitable green sea turtle carcasses found within the study areas (Flint et al. 2009, Flint et al. 2015).

PPE involves assessing samples by the biuret method using a calibrated autoanalyser (Olympus AU400, Olympus). Agarose gel electrophoresis is performed on each sample using commercial Helena Laboratories TITAN GEL Serum Protein System kits (Helena Laboratories, Beaumont, Texas). 3.6 µl sample is diluted 1:4 in buffer, loaded onto the gel using the manufacturer supplied template, and the gel run at 120V for 15 min. The gel is then fixed, stained and dried ready for scanning. The gels are scanned using a laser densitometer (Helena Laboratories Electrophoresis Data Center, Beaumont, Texas) at a wavelength of 595 nm to determine plasma protein fractions. Fractions are compared with the established reference ranges (Flint et al. 2015b).
In Year 2, a potential disease syndrome was also identified affecting the eyes of the examined coastal.

Ocular examination

Ocular examination included the sampling of abnormal growths seen in approximately two dozen animals' eyes of Upstart and Cleveland Bays. Presentations between the two Bays were suggested to differ. The growth was gently manipulated with forceps and if it could be lifted from the cornea without causing trauma, it was sampled by sharp dissecting the lesion from the cornea and being placed immediately into 70% alcohol. Alcohol was chosen as the fixative to allow for histological and molecular examination. Given the thinness of the tissue, adequate penetration for fixation was presumed to be easily achievable. In addition, swabs of the eye with a sterile cotton swab were taken and placed in a sterile transport media at < 15°C until it could be prepared for cytology. Cytology was prepared by placing two drop of ethanol on the swab and transferring any product to a glass slide. Slides were fixed and stained with H&E for examination under light microscopy.

Standard necropsy examination

Standard necropsy examination involved placing the turtle in sternal recumbency and removing the plastron using a circumferential incision around the suture line of the plastron with the soft tissue and carapace. Retracting and removing the plastron, the forelimbs can be removed to expose the organs of the coelom. From here, each organ is systematically examined, removed, opened and findings reported. Samples from each organ are obtained and stored in 10% neutral buffered formalin for histology and/or samples are wrapped in an inert foil or glass jar and frozen for toxicological or microbial screening. Any appropriate smears or slides are made at the time of the dissection (Flint et al. 2009).

Environmental Exposure

Metals

Surface Sediment

Sediments were processed for total extractable metals using microwave assisted extraction in a CEM MARS 6 system, as per EPA Method 3051A. Briefly, up to 0.5 g of homogenised wet sediment was directly weighed into a TFM PTFE digestion vessel, followed by the addition of 9 mL concentrated nitric acid and 3 mL concentrated hydrochloric acid. Vessels were placed into the microwave system and heated to 175°C, maintained at 175°C for at least 4.5 minutes, and then allowed to cool. Extraction solutions were transferred to polypropylene tubes, centrifuged, and an aliquot of the supernatant transferred to a polypropylene tube and diluted 50-fold with Milli-Q water. Diluted extract solutions were analysed for metals with an Agilent 7900 ICP-MS operated in collision mode with He as the cell gas for kinetic energy discrimination (KED) interference removal. Concentrations were converted from wet weight to dry weight basis using the measured moisture content of each sample obtained by weighing a sub-sample of wet sediment, oven-drying at 105°C to a constant weight, and reweighing. Independent quality control solutions (10 µg L-1) were analysed regularly throughout each analytical run. A certified reference material of stream sediment (NCS DC 73309) was analysed (n=6) to verify the efficiency of the extraction procedure and the accuracy of the ICP-MS analysis. Recoveries were between 84 – 119% of certified values, indicating that the measured concentrations of trace metals were within acceptable limits.

Aboveground Forage

Forage samples were processed for total metals using microwave assisted digestion in a CEM MARS 6 system, as per EPA Method 3052. Briefly; forage was first washed in deionised water to remove adhered particulate matter and then oven-dried at 60°C. Dried samples were ground in an agate mortar and pestle and homogenised. A 0.25 g sample of dried and ground forage was directly weighed into a TFM PTFE digestion vessel, followed by the addition of 9 mL concentrated nitric acid, 2 mL of concentrated hydrochloric acid, and 1 mL of concentrated hydrogen peroxide. Vessels were placed into the microwave system and heated to 180°C, maintained at 180°C for at least 9.5 minutes, and then allowed to cool. Visual inspection of the digestion solution confirmed complete dissolution of the sample, with no solid material remaining. An aliquot of each sample was diluted 50-fold with Milli-Q water prior to analysis by ICP-MS, as described in the previous section.

Diffusive Gradients in Thin-films Passive Samplers (DGTs)

DGT samplers were disassembled inside a Class A laminar flow hood, located in a Class B clean room, to minimise trace metal contamination. The binding gel from each DGT sampler was eluted first in 1 mL of 1 mol L-1 ultra-pure HNO_3, followed by 1 mL of 1 mol L-1 NaOH. Eluents were combined, diluted 10-fold with 2 % ultra-pure nitric acid, and analysed by ICP-MS (Panther et al. 2013). Time-integrated concentrations were determined using the DGT equation, as described in Zhang and Davison (1995).

Organic Chemicals

Extraction Methods

Water

500 mL of water from each sampling location was measured and spiked with a mixture of isotope labelled herbicides and personal care products (PPCPs). Field blanks and laboratory blank water samples were also included. Samples were loaded onto Strata X 200mg 6cc cartridges (Phenomenex) that had been pre-conditioned with 4 mL methanol and 4 mL of MilliQ water. Cartridges were dried under vacuum for approximately 1 hr and eluted with two x 2 mL of methanol. Eluants were reduced in volume under N2 to approximately 1 mL, filtered (0.2 µm; RC) and adjusted to a final volume of 500 µL (20% methanol; 80% water) in a 2 mL LC vial. Isotope labelled recovery standards were spiked into each sample prior to analysis, however it must be noted results have not been recovery corrected at this stage.

Passive samplers and PDFMs

For all sampling campaigns, naked Empore discs (EDs) were selected for extraction due to the short deployment periods, and the likelihood that chemicals had accumulated at a faster rate than with routine EDs. Sampler

housings were disassembled and the disks blotted with a kim wipe and placed into an acetone-rinsed glass tube. A laboratory blank sampler was included for extraction. Disks were spiked with a mixture of isotope labelled herbicides and PPCPs. Disks were extracted via sonication first in 5 mL acetone (5 min) followed by 5 mL of methanol (5 min). The two extracts were combined and reduced in volume under N2 to approximately 1 mL. Concentrated extracts were filtered (0.2 µm; PTFE) and adjusted to a final volume of 500 µL (20% methanol; 80% water) in a 2 mL LC vial. Isotope labelled recovery standards were spiked into each sample prior to analysis however it must be noted results have not been recovery corrected at this stage.

For all sampling campaigns, two PDMS strips from each sampling location were selected for extraction. Laboratory blank samples were also included for extraction. Any remaining strips remained frozen. PDMS strips were cleaned with water and kim wipes to remove mud and detritus. A solution of an isotope labelled standard was spiked onto the surface of each strip, and the two strips from each location placed into acetone-rinsed jars (750 mL). 400 mL of hexane was added to each jar and samplers left to extract at room temperature on a bench top shaker for 2 x 24 hr, replacing the 400 mL of hexane after 24 hrs. The combined extracts were concentrated using rotary evaporation to < 5 mL and then passed through a Na2SO4 column to remove residual water. Extracts were reduced to approximately 1 mL and filtered (0.2 µm; PTFE) into a solvent rinsed gel permeation chromatography (GPC) tube, using dichloromethane (DCM) as the solvent. Extracts were subjected to further clean-up by GPC. Final extracts were reduced in volume under N2, transferred to a GC vial and adjusted to a final volume of 200 µL in hexane. Results have not been corrected for recoveries.

PFMs were weighed prior to deployment. Following deployment, PFMs were scrubbed to remove detritus, and were re-weighed and the average mass loss per day was determined (g/ day).

Sediments

Each pooled sample or sub-sample was thawed, thoroughly mixed and then an aliquot transferred into a 50 mL falcon tube. Samples were re-frozen and then freeze-dried to remove all water. 20g of sediment from each sampling location was weighed into pre-cleaned ASE cells with 5g of florisil and hydromatrix. Cells were spiked with a solution of an isotope labelled standard and exhaustively extracted using hexane: dichloromethane (1:1). Extracts were reduced in volume to approximately 3 mL and subjected to further cleanup using 3% deactivated silica and 6% deactivated almunium oxide, eluted with 40 mL of hexane: dichloromethane (1:1). Samples were reduced to a final volume of 200 µL in hexane. Results have not been corrected for recoveries.

Following an initial screen for organic pollutants, a more targeted 'in-cell' clean up approach was trialled (and continues to be evaluated), to increase the probability of detection of pollutants by using larger masses of sample, sulfuric acid clean up to remove interference and provide a quantitative analysis. Pooled freeze-dried samples were weighed into pre-cleaned ASE cells. ASE cells were packed with multiple layers of silica, sulfuric acid treated silica, copper, and florisil. Samples were spiked with a mixture of isotope labelled standards (pesticides, dioxins, PCBs and PBDEs) and extracted using hexane: dichloromethane (1:1). Method development included trial extractions using either 50 g, 25 g or 15 g portions of sediment, and between two and four extraction cycles to assess optimal extraction efficiency of isotope-labelled standards whilst minimising interference of the chromatograms by undesired co-extracted material.

Analytical Methods

Target analysis for herbicides and PPCPs in EDs and water samples was done using an AB Sciex QTRAP 6500 mass spectrometer (AB Sciex, Concord, Ontario, Canada) equipped with an electrospray (TurboV) interface coupled to a Shimadzu Nexera HPLC system (Shimadzu Corp., Kyoto, Japan).

Non-target screening for unknown chemicals in water samples was carried out using a Shimadzu Nexera X2 ultrahigh-pressure liquid chromatography (UPLC) system equipped with a binary pump and a reverse-phase Gemini-NX C18 column (3 µm × 2.0 mm × 50 mm, Phenomenex. The UPLC was coupled to a hybrid quadrupole time-of-flight mass spectrometer (QTOF-MS) system, (Triple-TOF 5600, Sciex), with an electrospray (ESI) interface operating in positive and negative ionization mode.

Target analysis for PDMS samplers was done using Thermo Fisher trace GC ultra-TSQ triple quadrupole Quantum XLS system for PCBs, PBDEs and pesticides and a Thermo Scientific DFS High Resolution GC/MS in splitless injection mode for PAHs, dioxins and furans. Non-target screens for chlorinated and brominated chemicals were done using a Shimadzu GC-2010 gas chromatography coupled with QP-2010 mass spectrometer operating in NCI-mode and for masses using a single quadrupole mass spectrometer (ISQ LT. Thermo Fisher

Scientific) operated in EI mode. For the advanced sediment clean up analysis, a Thermo Scientific DFS High Resolution GC/MS in splitless injection mode was used for the analysis of PBDEs, acid-resistant OCPs, PCBs, PCNs and PCDD/Fs.

For the target analysis, if the amount quantified in the field blank was <5% of the amount quantified in the sample, no blank correction was made. If the field blank amount was 5 - 20% of the sample amount, then it was subtracted from the sample prior to calculation of concentration. If the field blank amount was >20% of the sample amount, then the sample was assumed to be below the limits of quantification (<LOQ). It must be noted that corrections for losses during extraction have not been made.

Turtle Exposure

The analytical framework designed for this study encompassed a development phase during which novel and innovative screening methods were tested and applied to obtain non-selective exposure information across a wide range of chemical groups for both organic compounds (polar and non-polar) and trace elements (essential and nonessential). Each sample underwent specific extraction procedures that were developed specifically to allow minimal bias in chemical selectivity, followed by four different analytical screening techniques, each covering various chemical scopes, and together capturing the majority of biologically active compounds.

Effect-based screening

Cell-based bioassays were used to screen the presence of a wide range of chemicals in turtle blood in vitro, via induction of various modes of action. In 2015, we focused on three assays and in 2016 expanded these to cover a wider range from non-specific modes indicative of baseline toxicity (Microtox), reactive modes of action indicative of inflammation (NF-κb-bla), and DNA damage (p53), adaptive stress response pathways indicative of oxidative stress (AREc32), to receptor activation in hormone mediated modes of action indicative of estrogenicity (BG1Luc4E) and xenobiotic metabolism indicative of aryl hydrocarbon receptor activation (CAFLUX). These modes of action cover a comprehensive range of (known and unknown) endogenous and exogenous chemicals in the mixtures of chemicals expected to be present in blood (Table 1).

Table 1. Summary of the in vitro cell based bioassays used (adapted from Tang et al 2013 and Jin et al 2015a).

Assay	Cell line	Mode of action	Targeted chemicals	Measured end point	Reference compound
AhR- CAFLUX	Hepa1c1c7 mouse hepatoma cells	AhR activation indicative of xenobiotic metabolism	Dioxin-like chemicals including PAHs	AhR-dependent green fluorescent protein expression	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
BG-1	BG-1luc4E2 human ovarian cancer	ER activation indicative of xenoestrogens activation	Endocrine disruptors	ER-dependent luciferase expression	17ß-estradiol
AREc32 (Nrf2)	MCF-7 human breast cancer cells	Nrf2 activation indicative of oxidative stress response	Electrophiles and ROS-inducing compounds	Nrf2-dependent luciferase expression	tert-Butylhydroquinone (tBHQ)
p53-bla	HCT166 human colon cancer cells	p53 activation indicative of tumor suppressor gene	Genotoxic agents	p53-dependent beta-lactamase expression	Mitomycin
NF- κB-bla	THP1 human monocytic leukemia cells	ER activation indicative of represponse to inflammation	Drugs, endotoxins, immunemodulating compounds	NF-kB-dependent beta lactamase expression	Tumor necrosis factor-alpha_ (TNF-alpha)
Microtox	Bacteria: *V. fischeri*	Baseline Toxicity-Cytotoxicity	Non-specific, baseline toxicants	Inhibition of Bioluminescent	Phenol (QA/QC) "Virtual" baseline toxicant (Escher, B.I. et al., 2008) (Baseline-TEQ)

Similar approaches to those described in Lin et al 2015 were followed and initially validated all assays using small volumes of blood extracts to ensure the co-extracted matrix does not interfere with the assay response, and used a subset of samples from each site (n=5) to screen across all modes of action. Based on these data, assays were prioritised for method modification and re-validation in 2016 (particularly to increase the volume of extracted blood that can be dosed), and analysed a full suite of 30 blood samples per site for all assays where quality control and assurance was confirmed.

Blood equivalent volumes dosed to assays were 0.067 mL for the AREc32 assay, 0.5 mL for CAFLUX, 0.033 mL for Microtox, NF-κb-bla and p53, and up to 0.3 mL for BG1.
The bioanalytical equivalent concentration (BEQ) concept was used to quantify the biologically active chemical burden present in blood (BEQbio). BEQbio is the concentration of chemicals in blood causing the same effect as the equivalent concentration of an appropriate reference compound (Jin et al 2013).

Nonpolar chemical screening

A total of 56 analytes were analysed using a multi-residue technique similar to that described by Baduel et al (2015) on a gas chromatograph coupled to a triple quadrupole mass spectrometer. Analytes comprised 15 organochlorine pesticides (dieldrin, HCB, endosulfan, endosulfan sulfate, cis-chlordane, trans-chlordane, o,p-DDD, p,p-DDD, o,p-DDE, p,p-DDE, p,p-DDT, o,p-DDT, Aldrin, heptachlor, lindane), 4 non-ortho PCBs (PCB 77, 81, 126, 169), 8 mono-ortho PCBs (PCB 105, 114, 118, 123, 156, 157, 167, 189), 6 marker PCBs (PCB 28, 52, 101, 138, 153, 180 (+118)), 7 polybrominated diphenylethers (BDE 100, 154, 183, 153, 28, 47, 99), and 16 EPA-PAHs (acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[a]pyrene, benzo[b/j]fluoranthene, benzo[ghi]perylene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, fluoranthene, fluorine, indeno[1,2,3-cd]pyrene, naphthalene, phenanthrene, pyrene).

Polar chemical screening

Blood samples were screened via non-target approaches similar to those described in Rotander et al (2015), using a UHPLC coupled to a hybrid quadrupole time-of-flight mass spectrometer (TripleTOF) with electrospray ionization in positive and negative ionization modes. The analytical workflow was described in our previous report and involved a series of filtering strategies for data reduction, using HWK as a control site to identify endogenous or exogenous chemicals that differed in turtles from the two coastal sites. These criteria included considerations of p values (<0.05) and effect sizes (log fold-changes >0.5) and retention times and focused on monoisotopic masses (ignoring isotopes, adducts and ion products generated during the ionization processes). Subsequently, manual visual inspection of the profile plot of each mass was used to compare the relative intensity of a given mass at each of the three sites, taking into consideration procedural and field blanks. Masses were then ranked according to their intensity (i.e. elevation) and abundance in turtles from UPB and CLV compared to HWK. In some instances, initial filtering criteria had to be adjusted to reduce the number of masses requiring evaluation. As more time and resources become available, the criteria can be relaxed, and additional masses can be investigated.

Trace elements

For trace elements, we undertook initial screening analysis according to methods described in Villa et al 2015, using inductively coupled plasma mass spectrometry (ICP-MS). The method covers >70 elements. Using principle component analysis, the data allows semi-quantitative assessment of the trace element mixture in blood (mean error ±30%) using HWK as a control for selection of the elements that govern differences in exposure at each of the sites.

On the basis of these results, we selected key trace elements and included a suit of other potentially relevant elements (total n=26) for fully quantitative analysis. These included both essential and non-essential elements: sodium (Na), magnesium (Mg), aluminium (Al), potassium (K), calcium (Ca), titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), silver (Ag), cadmium (Cd), tin (Sn), antimony (Sb), barium (Ba), thallium (Tl), lead (Pb), thorium (Th) and uranium (U).

INITIAL RESULTS

Turtle Population Biology, Trends and Genetics

A total of 2,423 turtles have been caught across the three study locations. Of these, 897 were adults (716 females, 169 males, 12 indeterminate), 401 sub-adults, 1,119 juveniles, and 6 of indeterminate age class. Turtles in this study show strong site fidelity. Based on a small sample size of tag returns, no mixing within the sampled foraging sites or between the study locations occurs.

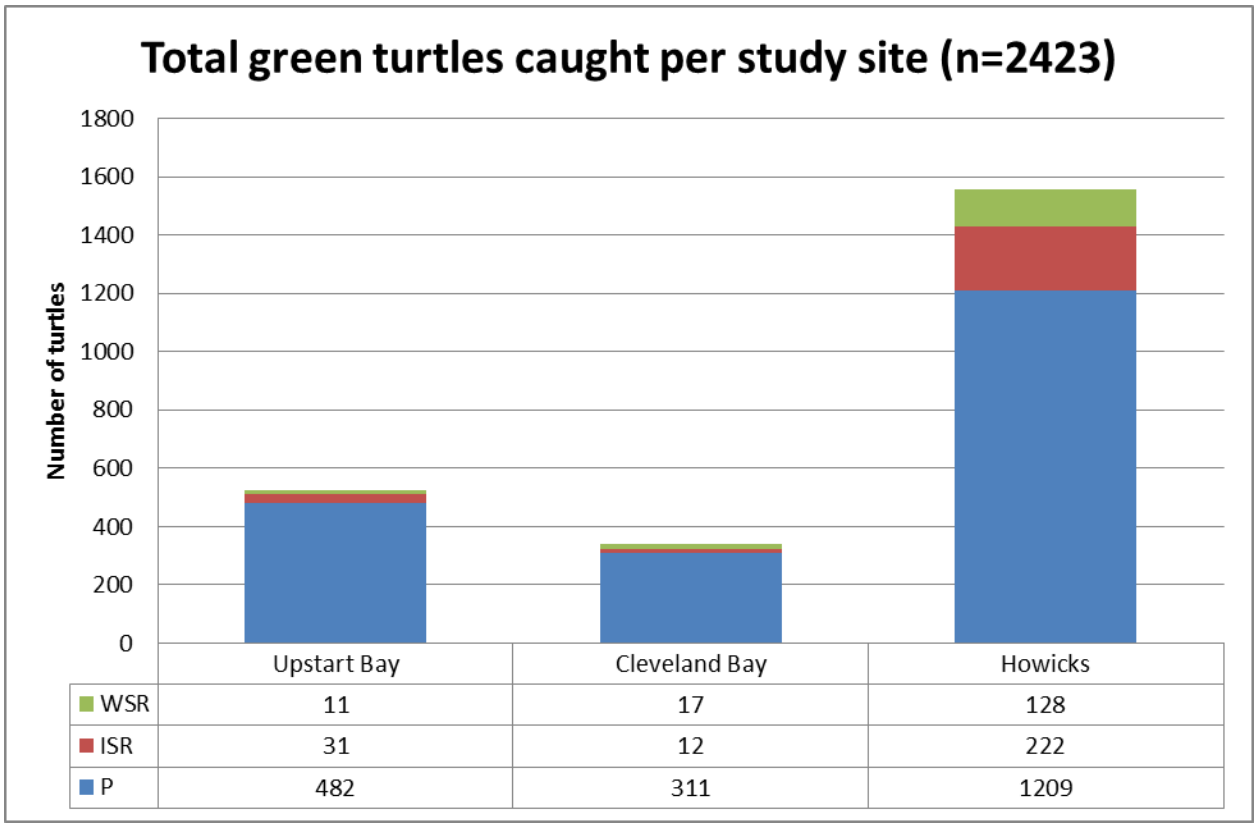


Figure 3: Total green turtles caught per site by tag status (P=Primary; ISR=Inter-season recapture; WSR=Within-season recapture)

At the Howick Group of Islands, preliminary mixed stock (genetic) analysis results indicate that the proportion of turtles originating from the northern GBR (nGBR) may have decreased further since the most recent study in 2008. The overall proportion (across multiple size classes) shows that 35% of the turtles originated from the nGBR and 51% from the southern GBR with the remaining turtles originating from non-GBR rookeries. It is important to highlight that these results need to be interpreted with caution until further analysis using larger sample sizes can be completed.

A low turtle recapture rate over all age classes precludes modelling trends in annual population abundance or survivorship. However, recapture rates of juvenile turtles found foraging on Ingram Island reef during the initial two years was sufficient to predict abundance of an assumed closed population. The juvenile population was estimated to be between 356 and 598 individuals. With continuous capture-recapture sampling at the same foraging ground, demographic parameters, such as survival, growth and movement rates may be estimated in the future. These demographic parameters are essential in understanding the health of the foraging population and movements of turtles among habitats.

Turtle Health

Health assessments, serum biochemistry and haematology performed throughout 2014 on 120 green sea turtles across all age classes, showed each site was having a different influence on its respective population of sea turtles. Although the data indicated no animals showed overt clinical signs of toxicosis, other renal or hepatic issues, parasitism, or external injuries, turtles within Cleveland Bay showed low frequency signs of an active systemic stressor while a large proportion (45%) of Upstart Bay turtles had a marked increase in inflammatory response components (heterophilia and monocytosis) consistent with phagocytosis. Turtles from the Howicks Group appeared to be the healthiest with only one animal identified as clinically unhealthy; which is considered normal for any healthy population.

At each of the study locations, age class, gender and body condition did not correlate with clinical health suggesting effects are acting at the population (site) level.

An observed lesion of the eye of 16 green turtles in Upstart Bay indicated an insidious disease process may be present in this region. Grossly, these lesions ranged in appearance from findings consistent with keratoconjunctivitis sicca (dry eye) through to full thickness ulceration of the cornea and conjunctiva. Histology and cytology are pending and subsequent microbiology will be performed, if required.

Additional targeted health assessments for Year 2 are not yet available, pending toxicology results for identifying at risk individuals and associated comparison controls.

Environmental Exposure

Analysis of environmental samples by Tropwater (JCU), Entox (UQ) and Griffith University showed several distinctions between the three study locations.

Aluminium and copper were detected at levels above Australian water quality guidelines at all locations. Similarly, cobalt was detected at levels above water quality guidelines at all locations except Home Hill. Six of the eleven elements assessed were enriched by at least 30 % in Upstart Bay relative to Howick Group water. The highest levels of enrichment were observed for aluminium, chromium, cobalt, and manganese (**Table 2**).

Sediment metal levels were within existing Australian sediment quality guidelines at all sites (guidelines are available for chromium, nickel, copper, zinc, arsenic, silver, cadmium, antimony, lead and mercury only, and have not been adjusted for hardness). Sediment from the Howicks Group generally showed lower metal levels than the two coastal sites. Differences were also observed between the Upstart Bay and Cleveland Bay coastal locations, which may be associated with either human activity or natural variations in catchment geologies. All metals assessed were elevated at Upstart Bay relative to Cleveland Bay except manganese, zinc, strontium, molybdenum, tin, and uranium.

Aluminium, vanadium, manganese, iron, cobalt, copper, zinc, molybdenum, silver, antimony, barium, lead, thorium and mercury were enriched by a factor of at least 30 % in forage from Upstart Bay and Cleveland Bay relative to forage collected from the Howicks. In addition, chromium, nickel, arsenic, and thallium were enriched by a factor of at least 30 % in Upstart Bay relative to Howicks forage. Strontium and cadmium was at least 30 % lower at Upstart Bay and Cleveland Bay compared to Howicks forage, and uranium was at least 30 % lower in Cleveland Bay relative to Howicks. All metals tested in forage were at least 30% higher in Upstart Bay compared to Cleveland Bay, except manganese, zinc, strontium, molybdenum, and antimony.

Table 2: Enrichment of three environmental matrices by metals at Upstart Bay and Cleveland Bay relative to the Howick Group of Islands.

Element	Code	Sediment		Forage		Water	
		Upstart	Cleveland	Upstart	Cleveland	Upstart	Cleveland
aluminium	Al	5.1	6.9	4.4	2.5	7.9	1.6
vanadium	V	2.1	2.8	3.7	1.9	0.3	0.5
chromium	Cr	0.7	1.0	2.1	1.2	5.2	6.4
manganese	Mn	19.1	26.9	32.4	34.5	2.8	7.1
iron	Fe	9.9	14.0	5.0	2.6	n.a.	n.a.
cobalt	Co	65.3	88.0	12.3	6.3	4.1	2.5
nickel	Ni	3.1	3.8	1.4	0.8	0.9	0.7
copper	Cu	10.6	10.7	2.2	1.5	1.8	1.8
zinc	Zn*	1.4*	2.0*	3.5	3.1	n.r.	n.r
arsenic	As	2.0	2.5	1.6	0.8	1.0	1.2
selenium	Se	2.5	3.3	1.2	0.8	n.a.	n.a.
strontium	Sr	0.02	0.03	0.2	0.2	n.a.	n.a.
molybdenum	Mo	<LOD	<LOD	2.5	2.3	n.a.	n.a.
silver	Ag	<LOD	<LOD	3.4*	2.2*	n.a.	n.a.
cadmium	Cd	<LOD	<LOD	0.6	0.3	0.3	0.2
antimony	Sb	<LOD	<LOD	1.4	1.8	n.a.	n.a.
barium	Ba	0.2	0.2	2.6	1.9	n.a.	n.a.
lead	Pb	4.2	5.2	4.3	2.5	1.4	0.6
thallium	Tl	<LOD	<LOD	2.0	1.1	n.a.	n.a.
thorium	Th	<LOD	<LOD	3.2	1.8	n.a.	n.a.
uranium	U	0.07	0.1	0.8	0.5	n.a.	n.a.
mercury	Hg	<LOD	<LOD	4.4	2.5	n.a.	n.a.

Notes: Forage samples are compared with Howick seagrass samples only; LOD = limit of detection; *Howick sample was <LOD, enrichment is estimated relative to the analytical LOD; n.r. = DGT zinc results are not reliable due to contamination of the device during deployment, retrieval, transport or storage; n.a. = not analysed.

The above results are all preliminary findings based on a screening approach of a single pooled sample per site and therefore cannot be considered representative. Higher levels of confidence in metal enrichment across environmental matrices will be attained when more samples have been analysed.

Similarly, differences were observed in exposure to polar and non-polar chemicals. Targeted analysis of herbicides, pharmaceuticals and personal care products in both polar passive samplers and grab water samples showed that herbicide exposure was (as expected) higher in the coastal sites than in the Howicks Group. Atrazine and diuron (both photosystem II inhibiting herbicides) were the most abundant herbicides sampled (both were also detected at the Howicks Group), although concentrations were overall very low (typically <1 ng/L). Water quality guideline values are not available for all detected contaminants however, for those that do have guideline values, none were exceeded, although the cumulative effects of exposure to mixtures of pollutants together with other local, regional or reef-wide stressors are unknown.

Screening of grab water samples from the first sampling campaign (pre-wet season 2014 only) against commercial spectral libraries containing >3,000 common pesticides, pharmaceuticals, personal care products and forensic compounds generated few positive matches with the exception of caffeine and paracetamol in Cleveland Bay, and amphetamine in Upstart Bay (the presence of which can only be confirmed with a chemical standard and further analysis). These exceptions may be due to contamination at the time of sampling.

Polycyclic aromatic hydrocarbons (PAHs) were detected in water (using passive samplers) and sediments at all sites at levels just above the analytical detection limits. There was a possible gradient from Cleveland Bay (6 in water, 10 in sediment) > Upstart Bay (5 in water, 6 in sediment) > Howicks Group (1 in sediment only) although statistical significance has not been assessed. Fluoranthene and pyrene dominated the PAH profiles at both Cleveland Bay and Upstart Bay, with a relatively higher concentration of perylene detected at Cleveland Bay

A screen of chlorinated and brominated chemicals in passive samplers and sediments (Figure 4) also showed distinct differences between sites which may be natural or anthropogenic in origin. Additional interrogation is required to draw further conclusions.

© GÜLSAH DOGRUER / ENTOX / WWF-AUS

© CHRISTINE HOF / WWF-AUS

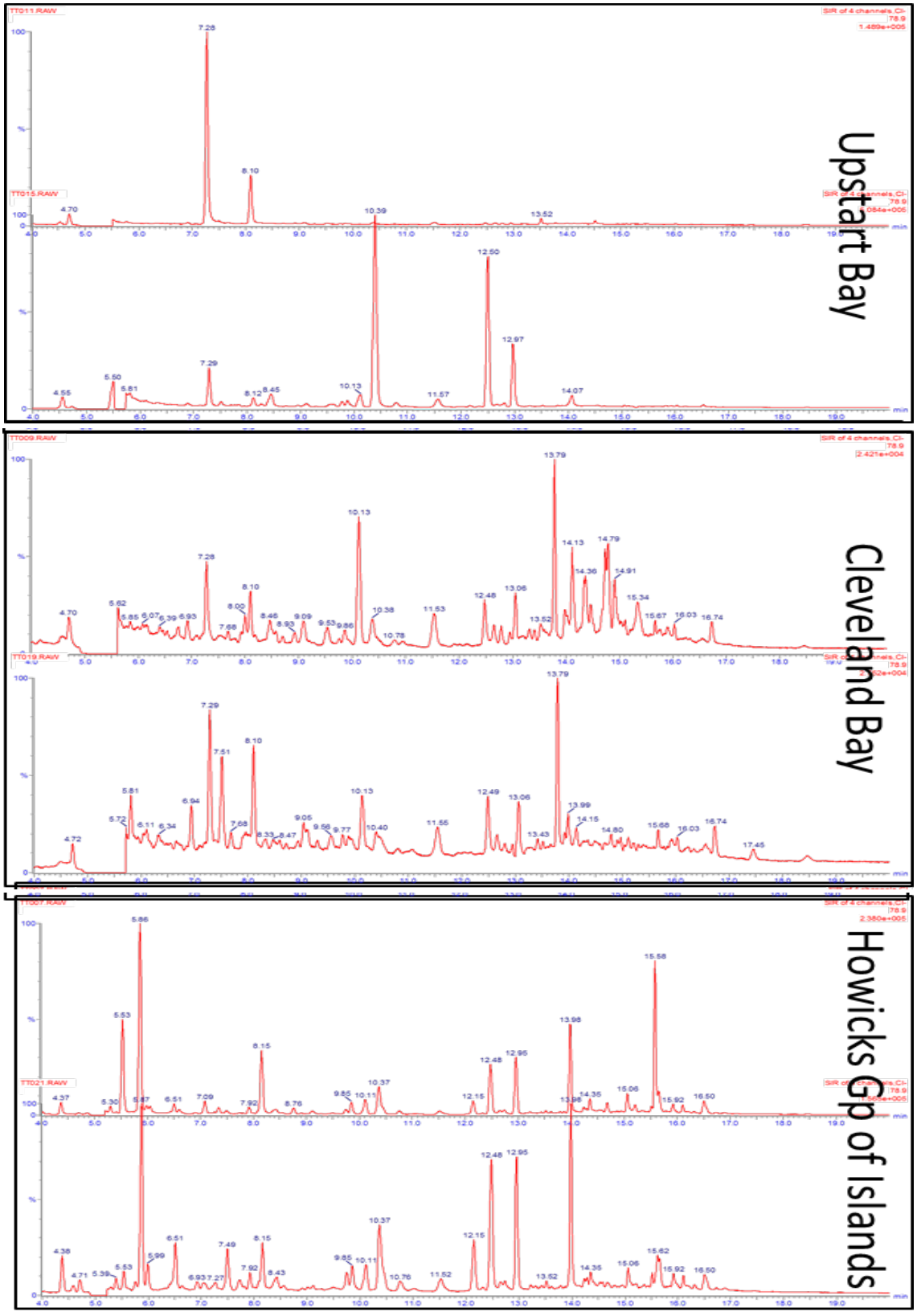


Figure 4: Comparison of chromatograms screening for brominated chemicals in sediments collected in the pre-wet and post-wet sampling periods (top and bottom of each section respectively)

Turtle Exposure

Multi-element screening of trace element concentrations in turtle blood and scute showed clear differences between the study locations, with the Howicks Group generally having lower concentrations than the two coastal sites. The data also showed that most trace elements, including rare earth elements, tend to be present at highest levels in turtles in Upstart Bay. The results from these screening analyses informed priorities for target analysis, and a set of 26 trace elements were investigated further.

Blood concentrations of trace elements in Howicks Group turtles were used to develop reference intervals (RIs) (Villa et al in review). The RIs reflect the species' healthy optima of essential elements, and a natural baseline for non-essential elements. In Upstart and Cleveland Bay, some elements clearly exceeded the RIs for a large proportion (>60%) of the populations (Figure 5). These were the essential elements Co, Mo, Mn, Mg and Na, and the non-essential elements As, and Sb. Mean Co concentrations were particularly elevated in Upstart Bay. Similarly, Sb, Mo, Mn and Na showed the highest concentrations in Upstart Bay turtles, while Cleveland Bay turtles contained the highest As levels. Significant differences in trace element blood concentrations in the geologically similar areas of Upstart Bay and Cleveland Bay suggest that the elevated exposure to Co, Sb and Mo at Upstart Bay may occur as a result of the area's anthropogenic activities, however further investigation is required to validate that conclusion.

Significant associations were observed between blood concentrations of Co and clinical health measures indicative of inflammatory responses in Upstart Bay. Significant correlations were also observed between Co, Sb, Mn and clinical markers of liver dysfunction in Cleveland Bay. These relationships between systemic stressors and acute inflammatory responses with the tested elements provide a strong argument that trace element exposure may be having an impact on the health of these coastal populations of sea turtles; be it a contribution to poor health in association with other stressors or a direct negative effect.

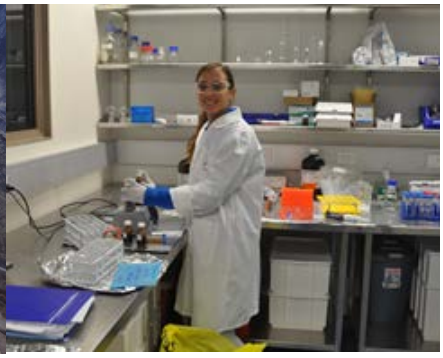
Non-target screening for the more polar contaminants also shows clear differences of chemical profiles in turtles between the three sites, mainly due to pharmaceuticals, industrial chemicals and endogenous biological compounds. Of particular interest was the detection of metabolites that relate to oxidative stress in Upstart Bay. This also corresponded with the identification of elevated levels of inflammatory cell components (heterophils and monocytes) for this study site. Further investigation of these compounds and their relationship with chemical exposure (particularly cobalt and other trace elements) and other stressors is required.

Screening for the more nonpolar chemicals suggests relatively low concentrations are present at all three sites. Of the detected compounds, concentrations were higher in Cleveland and Upstart Bay turtles. These levels are overall relatively low and similar to those reported from green turtles at other locations.

Through these analyses, the Howick Group of Islands is proving to be a suitable control site, and reflects baselines for contaminant exposure in a known healthy green turtle population.

© CAMERON LAIRD / ACCOLADE WINES AUSRALIA LIMITED / WWF-AUS

© CHRISTINE HOF / WWF-AUS



© CAROLINE GAUS / ETNOX

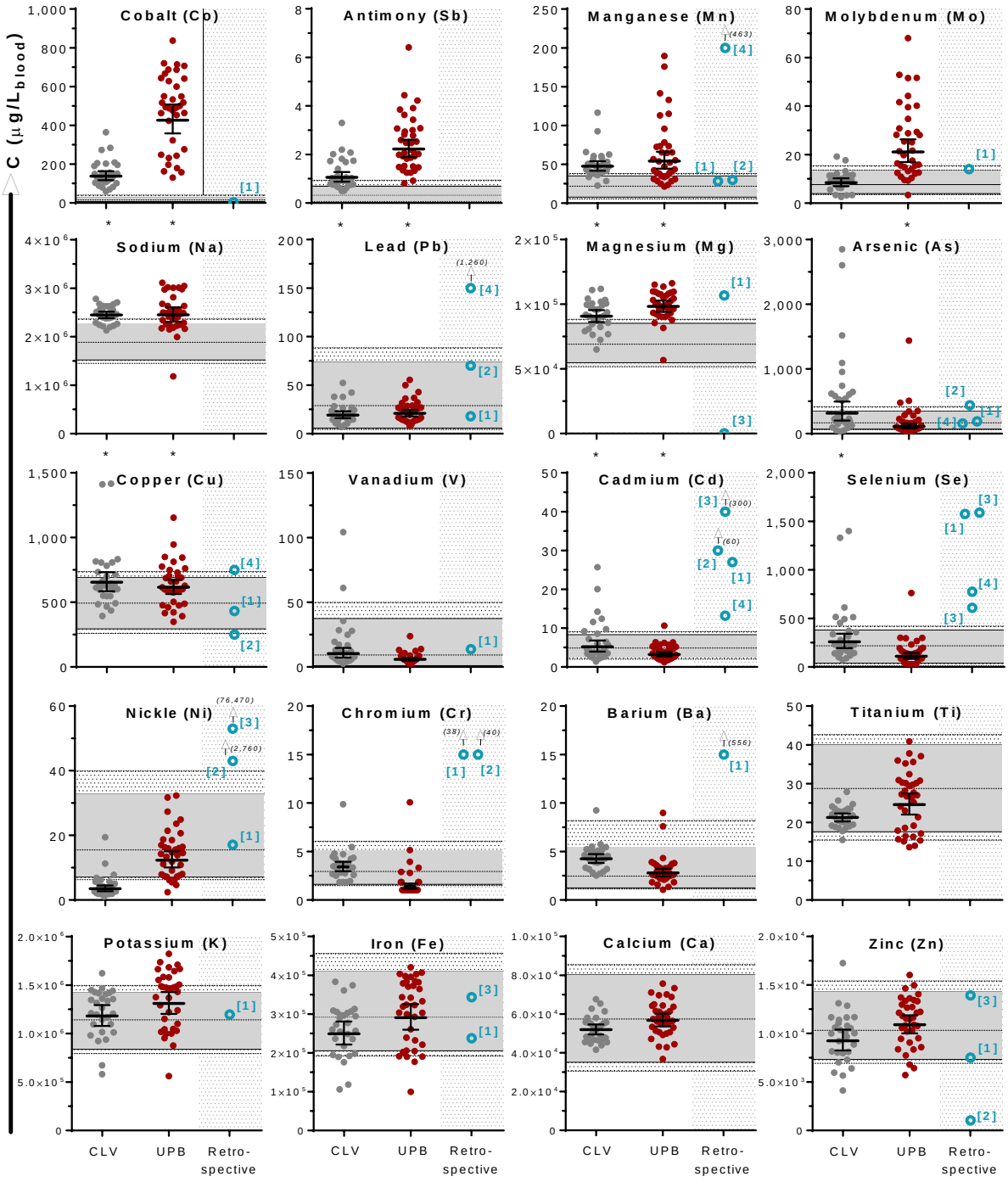


Figure 5. Reference Intervals and 90% CI (horizontal grey shaded area and dotted lines) compared to a) trace element concentrations (µg/L ww) in turtles from SHL, CLV and UPB coastal foraging grounds (left panel; lines = geometric mean and 95% CI), and b) mean levels previously reported from Palmyra Atoll by [1] McFadden et al. (2014); Cape Verde by [2] Camacho et al. (2014a); Punta Abreojos by [3] Labrada-Martagon et al. (2011); San Diego Bay by [4] Komoroske et al. (2011) (right panel). *Significantly different to RI and ≥ 60% of turtles exceed RIs.

Effect-based screening of turtle blood showed significantly higher responses for Upstart Bay in bioassays indicative of baseline toxicity and oxidative stress (Figure 6). This suggests the presence of a large spectrum of toxicologically relevant chemicals in the blood of Upstart Bay turtles when compared to turtles from Cleveland Bay or the Howicks. Based on the assays' modes of action, these chemicals may affect active transport and other ATP-dependent processes, and result in production of reactive oxygen species at harmful levels. Chemical

screening of blood biomarkers corroborates this with significantly higher levels and abundance of lipid oxidation products identified in samples from Upstart Bay compared to Cleveland Bay. Other chemicals identified in Upstart and Cleveland Bays include pharmaceuticals, industrial chemicals and endogenous biological compounds.

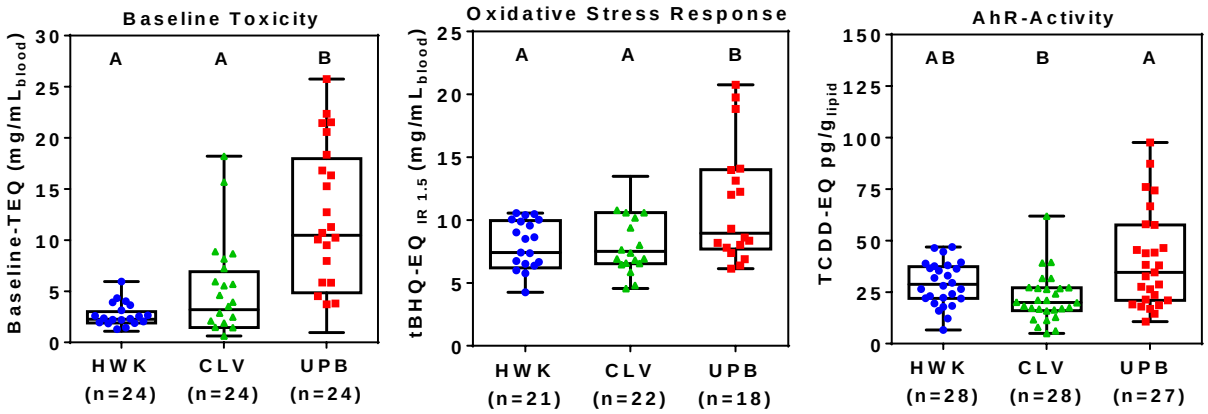


Figure 6. Comparison of the bioanalytical equivalent concentrations (BEQs) in blood of turtles from Howick Island (blue), Cleveland Bay (green) and Upstart Bay (red) for the Microtox (n=72 in duplicate), AREc32 (n=64 in triplicate) and AhR-CAFLUX (n=84 in triplicate) assays. Whiskers = range, boxes = 1st and 3rd quartiles, line = median; significant differences are indicated as letters (Microtox: $p < 0.0001$; AREc32: $p = 0.0038$; AhR-CAFLUX: $p = 0.0007$; Kruskal-Wallis test).

Effect-based screening also showed elevated dioxin equivalency (TCDD-BEQ) in some (~25%) turtles of Upstart Bay. The concentrations are similar to those identified in blood of green turtles from other coastal areas dominated by urban (e.g western Moreton Bay) and agricultural activities (e.g. Hervey Bay). Previous risk assessments indicated green turtles with dioxin concentrations at such levels have increased risks for chronic biochemical and immunological effects. Similar outcomes are expected for Upstart Bay turtles, but would require dioxin target analysis to confirm.

PRELIMINARY CONCLUSIONS

Sample analysis for the project has required the development of a range of new analytical methods and approaches. While resource limitation and logistical capability has impacted the ability to expand on some of the methods, and required some components to be reduced, the outcomes to date provide new insights into coastal green turtle populations.

Foremost among these is the validation of the project design through the use of the Howicks Group as a suitable "clean" comparison site. There were some initial concerns that the Howicks population may have been dominated by northern GBR stock, in contrast to the other two sites. Genetic analysis of the population, however, has confirmed that the majority of turtles within that feeding ground are from the southern GBR haplotype.

The second finding relates to the clear differences in the chemistry of the water, sediment, seagrass, turtle blood and scute across the three sites. More importantly, there are also differences in health indicators with good health at the Howicks Group, some minor systemic stressor at Cleveland Bay and a marked increase in inflammatory response in a high proportion of the population in Upstart Bay.

While analysis of organic chemicals has not yet identified a "smoking gun", analysis of metals has indicated that the elevated levels of Co in Upstart Bay in particular may be having an effect, correlating with clinical markers of inflammatory response and (together with Sb and Mn) liver dysfunction. Preliminary comparisons of Co in forage sampled from the three locations show that Upstart Bay is enriched with Co relative to both the Howicks and Cleveland Bay. This suggests that forage may be a significant exposure pathway for Co in local turtles, the source of which needs further investigation.

PROJECT CHALLENGES

A project of the breadth and scope of the Rivers to Reefs to Turtles project is not without several challenges that either hinder or limit the outcomes of the project. These include:

- resource or technical limitations preventing assessment of all variables (e.g. organic chemicals in seagrass, or quantification of sediment and water metal composition)
- ability to capture sufficient turtles to quantify population abundance and trends within the timeframe of the project
- the need to establish methodologies to analyse data
- greater understanding of the toxico-kinetics and dynamics to better connect toxicant and biological response.
- highly specialised technical equipment breakdowns
- the potential transience of pollutants that may have initiated acute toxic responses have since dispersed
- the interdisciplinary nature of the project extending the time-frame of deliverables due to one discipline awaiting results of another before being able to analyse data
- adequate access to resources such as carcasses and real-time disease and environmental events due to the remoteness of the study sites.

References

Baduel, C., Mueller, J. F., Tsai, H., & Ramos, M. J. G. (2015). Development of sample extraction and clean-up strategies for target and non-target analysis of environmental contaminants in biological matrices. Journal of Chromatography A, 1426, 33-47

Camacho M, Boada LD, Oros J, Lopez P, Zumbado M, Almeida-Gonzalez M, et al. (2014) Monitoring organic and inorganic pollutants in juvenile live sea turtles: Results from a study of Chelonia mydas and Eretmochelys imbricata in Cape Verde. Sci Total Environ., 481: 303-10

Chrisman, C. L., M. Walsh, J. C. Meeks, H. Zurawka, R. LaRock, and L. H. Herbst. (1997). Neurological examination of sea turtles. Journal of American Veterinary Medical Association 211:1043-1047.

Flint, M., B. J. Matthews, C. J. Limpus, and P. C. Mills. 2015. Establishment of reference intervals for plasma protein electrophoresis in Indo-Pacific green sea turtles, Chelonia mydas. Conservation Physiology 3:doi:10.1093/consphys/cov1037.

Flint, M., J. M. Morton, C. J. Limpus, J. C. Patterson-Kane, P. J. Murray, and P. C. Mills. (2010). Development and application of biochemical and haematological reference intervals to identify unhealthy green sea turtles (Chelonia mydas). The Veterinary Journal 185:299-304.

Flint, M., J. C. Patterson-Kane, C. J. Limpus, T. M. Work, D. Blair, and P. C. Mills. (2009). Post mortem diagnostic investigation of disease in free-ranging marine turtle populations: a review of common pathological findings and protocols. Journal of Veterinary Diagnostic Investigation 21:733-759.

Frey A, Dutton P, LaCasella E, LeRoux R, Abreu-Grobois A (2009) Standardizing the mtDNA control region fragment for sea turtles: How long do sequences need to be? In: Belskis L, Frick M, Panagopoulou A, Rees AF, Williams K (comps) Proc 29th Annu Symp Sea Turtle Biol Conserv. NOAA Tech Memo NOAA NMFS- SEFSC-630. NOAA, Miami, FL

Herbst, L. H., and E. R. Jacobson. (2003). Practical approaches for studying sea turtle health and disease. Pages 385-410 in P. Lutz, J. A. Musick, and J. Wyneken, editors. The Biology of Sea Turtles Volume II. CRC Press, New York.

Jin, L., Gaus, C., & Escher, B. I. (2015). Bioanalytical approaches to understanding toxicological implications of mixtures of persistent organic pollutants in marine wildlife. Comprehensive analytical chemistry, 67, 57-84

Jin, L., Gaus, C., van Mourik, L., & Escher, B. I. (2013). Applicability of passive sampling to bioanalytical screening of bioaccumulative chemicals in marine wildlife. Environmental science & technology, 47 (14), 7982-7988.

Komoroske LM, Lewison RL, Seminoff JA, Deheyn DD, Dutton PH. (2011). Pollutants and the health of green sea turtles resident to an urbanized estuary in San Diego, CA. Chemosphere; 84, 5: 544-52

Labrada-Martagon V, Rodriguez PA, Mendez-Rodriguez LC, Zenteno-Savin T. (2011). Oxidative stress indicators and chemical contaminants in East Pacific green turtles (Chelonia mydas) inhabiting two foraging coastal lagoons in the Baja California peninsula. Comp Biochem Physiol C Toxicol Pharmacol; 154, 2: 65-75

McFadden KW, Gomez A, Sterling EJ, Naro-Maciel E. (2014) Potential impacts of historical disturbance on green turtle health in the unique & protected marine ecosystem of Palmyra Atoll (Central Pacific). Mar Pollut Bull., 89, 1-2: 160-7

Maniatis T, Fritsch E, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Woodbury, NY

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215

Owens, D. W., and G. J. Ruiz. (1980). New methods of obtaining blood and cerebrospinal fluid from marine turtles. Herpetologica 36:17-20.

Panther, J.G., Bennett, W.W., Welsh, D.T. and Teasdale, P.R., (2013). Simultaneous Measurement of Trace Metal and Oxyanion Concentrations in Water using Diffusive Gradients in Thin Films with a Chelex–Metsorb Mixed Binding Layer. Analytical chemistry, 86(1), pp.427-434.

Pella, J., & Masuda, M. (2001). Bayesian methods for analysis of stock mixtures from genetic characters. Fisheries Bulletin, 99, 151–167.

Rotander A, Karrman A, Toms LM, Kay M, Mueller JF, Gomez Ramos MJ. (2015). Novel fluorinated surfactants tentatively identified in firefighters using liquid chromatography quadrupole time-of-flight tandem mass spectrometry and a case-control approach. Environ Sci Technol., 49, 4: 2434-42

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Tang, J. Y., Aryal, R., Deletic, A., Gernjak, W., Glenn, E., McCarthy, D., & Escher, B. I. (2013). Toxicity characterization of urban stormwater with bioanalytical tools. Water research, 47(15), 5594-5606

Villa CA, Finlayson S, Limpus C, Gaus C. (2015). A multi-element screening method to identify metal targets for blood biomonitoring in green sea turtles (*Chelonia mydas*). Sci Total Environ.

Villa CA, Flint M, Gaus C, Bell I, Hof C, Limpus C. Trace Element (in review) Reference Intervals in Blood of Healthy Green Sea Turtles To Evaluate Exposure of Coastal Populations. Environmental Pollution.

Work, T. M., R. E. Raskin, G. H. Balazs, and S. D. Whittaker. (1998). Morphologic and cytochemical characteristics of blood cells from Hawaiian green turtles. American Journal of Veterinary Research 59:1252-1257.

Zhang, H. and Davison, W., (1995). Performance characteristics of diffusion gradients in thin films for the in situ measurement of trace metals in aqueous solution. Analytical chemistry, 67(19), pp.3391-3400.

The Rivers to Reef to Turtles project is a collaboration of the following project partners, supported by Banrock Station Environmental Trust

Why we make a difference

Reaching new audiences

We will create new ways to inspire and motivate a new generation of Australians and truly realise our collective power to make a difference to the world in which we live.

High Impact Initiatives

Over the next 5 years, we will accelerate our on-ground conservation and advocacy work, focusing on new priority areas where we have the greatest impact and influence.

Building a strong network

We will draw strength from WWF's 50 years of rich history, knowledge and experience, harnessing our network of people around the world.

Walking the talk

We will continue to commit to reducing our overall environmental footprint, with an ambitious vision to reduce energy consumption by 30% and emissions from travel by 50% by 2015.

Loyal supporters

WWF's supporters make an invaluable contribution to our conservation work. We couldn't do without their loyalty, generosity and personal involvement. We will expand the ways in which supporters can connect with WWF, giving them a greater choice of programs from which they can choose to protect our planet's future.

Transforming business

Through building influential relationships with business and industry, we will continue to create solutions to address the major threats to our natural environments.

ANEMONE, FIJI © CAT HOLLOWAY / WWF-CANON

WWF

Why we are here

To stop the degradation of the planet's natural environment and to build a future in which humans live in harmony with nature.

wwf.org.au

WWF-Australia National Office

Level 13, 235 Jones Street,
Ultimo NSW 2007
GPO Box 528
Sydney NSW 2001

Tel: 61 2 9281 5515
Freecall: 1800 032 551
Fax: 61 2 9281 1060
Email: enquiries@wwf.org.au