Integrated chemical exposure assessment of coastal green turtle foraging grounds on the Great Barrier Reef

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Highlights

- Case-control sampling investigated green turtle exposure to land-based pollutants
- Analysis of water and sediment showed catchment-specific pollutant profiles
- Low concentrations of pesticides, pharmaceuticals and industrial chemicals detected
- Turtles foraging in coastal areas are exposed to a diverse mixtures of chemicals

Abstract

The Great Barrier Reef receives run-off from 424,000 km^2 catchment area across coastal Queensland, incorporating diffuse agricultural run-off, and run-off point sources of land-based chemical pollutants from urban and industrial development. Marine biota, such as green turtles (*Chelonia mydas*), are exposed to these diverse chemical mixtures in their natural environments, and the long term effects on turtle and ecosystem health remain unknown. This study was part of a larger multi-disciplinary project characterising anthropogenic chemical exposures from the marine environment and turtle health. The aim of this study was to screen for a wide range of anthropogenic chemical pollutants present in the external and internal environment of green turtles, using a combination of traditional targeted chemical analyses, non-target suspect screening, and effect-based bioassay methods, while employing a case-control study design. A combination of passive (water) and grab (water, sediment) samples were investigated. Three known green turtle foraging sites were selected for sampling: two coastal 'case' sites influenced primarily by urban/industrial and agricultural activities, respectively; and a remote, offshore 'control' site. Water and sediment samples from each of the three sampling locations showed differences in chemical pollutant profiles that reflected the dominant land uses in the adjacent catchment. Targeted mass spectrometric analysis for a range of pesticides, industrial chemicals, pharmaceuticals and personal care products found the greatest detection frequency and highest concentrations in coastal samples, compared to the control. Non-target screening analysis of water showed clear differentiation in chemical profile of the urban/industrial site. *In-vitro* assays of sediment samples from the control site had lowest induction, compared to coastal locations, as expected. Here we present evidence that turtles foraging in coastal areas are exposed to a range of anthropogenic pollutants derived from the adjacent coastal catchment areas.

KEYWORDS: high resolution mass spectrometry (HRMS); chemical exposure; exposure assessment marine wildlife; green turtle; passive sampling

1. Introduction

The World Heritage Great Barrier Reef (GBR) Marine Park covers an area of 344,400 km^2 and spans 2,300 km along the Queensland coast of Eastern Australia. The GBR receives run-off from 35 river catchments that drain approximately 424,000 km^2 of coastal Queensland; 80% of this catchment area is currently used for agriculture (DSITIA 2012). Diffuse pesticide run-off from agricultural land has been identified as a threat to the GBR ecosystem (Kroon et al. 2013, GBRMPA 2014), and herbicides inhibiting photosynthetic function, such as photosystem II (PSII) herbicides, are recognised as priority pollutants due to their heavy use and demonstrated toxicity (Davis et al. 2013). These and other agrichemicals can remain in the marine environment at elevated concentrations for extended periods of time *(i.e.* several weeks) (Devlin and Schaffelke 2009, Grant et al. 2017). Urban and industrial development including ports and aquaculture can represent smaller point sources of land-based chemical pollutants (*e.g.* pharmaceuticals and personal care products; PPCPs).

Green turtles (*Chelonia mydas*) are one of the iconic species of the GBR, and are regarded as either endangered or vulnerable by both The World Conservation Union and the Australian Government (GBRMPA 2016a). Green turtles are long-lived marine reptiles that show strong fidelity to inshore foraging areas (few km^2) where they spend approximately two to eight years between breeding cycles, and feed primarily on seagrass and algae (Arthur et al. 2008, GBRMPA 2016a). Exposure to chemical contaminants occurs via consumption of contaminated water or food, and sediment-bound compounds are an important exposure source for benthic marine biota such as the green turtles (Gaus et al. 2001a, Gaus et al. 2004, Hermanussen et al. 2004). Once ingested or absorbed, contaminants may act on target sites at a molecular level to trigger adverse health effects, as recently demonstrated (Dogruer et al. 2018).

The contribution of agricultural runoff, urban and industrial development may contribute to declining water quality in the inshore marine environment, and in turn, may decrease the resilience of green turtle to other stressors, such as climate change. Considered sentinels for a healthy marine ecosystem, changes in green turtle population health may affect the entire ecosystem. Ensuring the continued health of this iconic species is of universal value to the biodiversity of the Reef itself and the communities that rely on it economically.

In 2014, the authors were part of a multidisciplinary team which aimed to determine the role, if any, of anthropogenic pollutants on adverse turtle health. In parallel, a turtle toxicology and health sampling program was undertaken with the goal to explore any correlations between identified

pollutants and turtle heath baseline parameters. Chemical monitoring activities within the GBR to date have focused on agricultural chemicals (Huggins et al. 2017, Grant et al. 2018). The aim of this study was to screen for a wide range of anthropogenic chemical pollutants present in the external (*i.e.* water, sediment) and internal (blood) environment of green turtles, using a combination of traditional targeted chemical analyses, non-target suspect screening, and effect-based methods employing a case-control study design.

2. Materials and Methods

2.1 Sample Collection

Water quality monitoring was conducted using both grab 'snap-shot' sampling and passive sampling techniques, where chemicals sorb from water to a collection membrane via passive diffusion. Grab water samples (1 L) were collected directly into high density polyethylene bottles (pre-rinsed with acetone and MilliQ water) at each site (n=9-12 per site), typically during passive sampler deployment or retrieval. The bottle was attached to a sampling pole, submerged to depth of 50cm, filled, capped, covered in foil to prevent photodegradation and frozen within 24 h of collection. Field blank samples (MilliQ water) were uncapped for the duration of sampling activities.

Two types of passive samplers were used:

(1) *Styrenedivinylbenzene Reverse Phase Sulfonated (SDB-RPS) Empore^{TM} Extraction Disks* (EDs; Phenomenex, Sydney, AUS). Polar passive samplers for hydrophilic organic chemicals with relatively low octanol-water partition coefficients ($\log K_{OW} < 3$) were deployed in the naked configuration *i.e.* without diffusion limiting polyether sulfone membranes (Stephens et al. 2009), and archived at -20°C following retrieval. For quantitative analysis a correction factor based on Shaw and Mueller (2009) was applied to account for naked deployment. For chemicals where a correction factor was not reported, the factor ratio for atrazine was used.

(2) *Polydimethylsiloxane* (PDMS; Purple Pig, Brisbane, AUS). Non-polar passive samplers were used for hydrophobic organic chemicals with relatively higher octanol-water partition coefficients ($\log K_{ow} > 3$). A minimum of two PDMS strips (25mm × 92cm × 500 µm) were deployed per housing (i.e. stainless steel cage), and combined during extraction and analysis. Passive flow monitors (PFMs) were deployed alongside EDs and PDMS as a method of *in situ* calibration to estimate site-specific flow conditions. The rate of loss of plaster from the PFM can be used to predict changes in the uptake of chemicals into a sampler dependent on flow and turbulence (O'Brien et al. 2011).

Sediment samples were collected and stored in acetone-rinsed 375 mL glass jars using gloved hands from the seabed surface (*i.e.* the top 10cm) at low tide and within the turtle foraging areas. Sediment samples were collected from three locations within each foraging site and pooled by weight prior to extraction and analysis. Jars were wrapped in aluminium foil to prevent photodegradation and frozen at -20°C within 24 h of collection.

2.2 Sampling sites

Three sampling locations were selected for the case-control comparison: two 'case' coastal locations influenced by adjacent catchment activities; and a remote, offshore location as a 'control'. A summary of the type and number of samples collected at each site are shown in Figure 1. The sites included:

1) Upstart Bay, a rural coastal area within the Burdekin region that receives agricultural run-off from sugar cane cultivation *via* the Burdekin Delta River;
2) Cleveland Bay, approximately 100 km north of Upstart Bay and 20 km South East of Townsville, home to ~20% of the GBR population (Gunn and Manning 2009). Land use activities include urban/residential land use, grazing, and small areas of manufacturing, industry and waste treatment; and
3) Howicks Group of Islands, a collection of remote, unpopulated islands approximately 100 km off-shore from the adjacent Cape York region.

Sampling activities were carried out between May and August of 2015, by members of the multidisciplinary team who were also conducting turtle health studies at the same time. Details of types of samples collected (grab water, passive samplers and sediment), dates and descriptions of the sampling sites are provided in the supplementary information (Tables S1-S3, Figure S1).

2.3 Sample preparation for chemical analysis and bioassay

2.3.1 Passive samplers (Empore Disks (EDs) and Polydimethylsiloxane (PDMS))

The methods for preparation, deployment, transportation and extraction of passive samplers have been extensively described previously (**Page et al. 2014**, **GBRMPA 2016b**, **O'Brien et al. 2016**), and further details are provided in the Supplementary Material and Figure S2.

2.3.2 Grab water samples

500 mL water was fortified with isotope-labelled internal standards (mixture of herbicides and PPCPs; Table S4), extracted using Strata-X 200mg 6cc cartridges (Phenomenex), concentrated under nitrogen, filtered (0.2 µm regenerated cellulose syringe filter; Phenomenex) and reconstituted in 0.5 mL 20%

methanol prior to analysis by LC-MS/MS. For EDs, PDMS and grab water samples, field and procedural blanks were processed in parallel.

2.3.3 Sediment

For chemical analysis sediment samples were pooled, homogenized, refrozen and freeze-dried. 20 g sediment was fortified with isotopically-labelled surrogate (50 ng 2,7-dichlorodibenzodioxin; Wellington Laboratories, Guelph, CAN), and extracted with hexane:dichloromethane (1:1, v/v) using accelerated solvent extraction (ASE, Thermo Scientific 350; Dionex, USA). Extracts were concentrated under nitrogen, purified with 3% deactivated silica/6% deactivated aluminium oxide (Sigma Aldrich, Sydney, AUS), eluted with 40 mL hexane:dichloromethane (1:1), concentrated on a rotary evaporator, and reconstituted in 200 µL hexane for analysis *via* GC-MS/MS.

2.3.4 Bioassays

Six g of freeze-dried sediment was extracted using pre-cleaned diatomaceous earth (approx. 1/3 of the cell`s volume) (Sigma-Aldrich) and hexane:acetone (1:1, v/v) using accelerated solvent extraction (ASE) and following the standardised US EPA Method 3545A (Li et al. 2013). The extraction efficiency of this ASE method was tested previously and ranges from 80-120% for chlorinated pesticides, semi-volatile organics and PCBs (Li et al. 2013). The extracts were concentrated under nitrogen stream, and reconstituted in 60 µL DMSO in 1.5 mL amber HPLC vials prior to *in vitro* assay. DMSO extracts were directly used for analysis on the in vitro bioassays with no further clean-up procedures. Furnaced DE (550°C for 24h) served as a process control for QA/QC.

2.4 Sample analysis

2.4.1 Mass spectrometric analysis

2.4.1.1 Polar analytes (target and non-target analysis)

Grab water samples, ED and PDMS extracts, and sediment samples were subjected to a range of mass spectrometric analyses (Figure 1). Briefly, EDs and grab water samples were analysed for polar compounds (herbicides and PPCPs) using targeted LC-MS/MS; screened against a commercial spectral library containing >3000 common pesticides, PPCPs and forensic compounds using high resolution and subjected to unknown (non-targeted) analysis *via* LC-QTOF-MS. For non-targeted analysis, full details of the analytical system and data processing methods are described in **Heffernan et al. (2017)**. A full list of target chemical analytes is provided in Tables S4-S5. Further details of the

analytical system(s), chromatography and mass spectrometry parameters are described in the Supplementary Material.

When processing target chemical results (EDs), blank subtraction was performed when field blank concentration was 5-20% measured concentration in unknown sample; if >20%, the result was discarded. Contamination of both field and laboratory blanks with several PPCPs including N,N-diethyl-meta-toluamide (DEET), caffeine, triclosan, paracetamol, salicyclic acid and ibuprofen, was evident in passive samplers and grab water samples. This was likely due to common use of products containing these compounds by either field or laboratory personnel. Thus, any results of these detected in deployed samplers have been excluded.

2.4.1.2 Non-polar analytes (target and non-target analysis)

Passive PDMS samplers and sediment samples were analysed for polychlorinated biphenyls (PCBs) and pesticides using GC-MS/MS; and polyaromatic hydrocarbons (PAHs) *via* high resolution GC-MS (Figure 1). Non-target screening of sediment and PDMS samples was conducted at the Norwegian Institute of Water Research (NIVA) via GC-MS/MS. Details of the analytical system are described in the Supplementary Material.

2.4.2 *In vitro* bioassays

Pooled sediment sample extracts were subjected to a battery of in vitro bioassays (AhR-CAFLUX, AREc32, NFκB-bla, VM7Luc4E2), each with a different mode of action (MOA; Ah receptor mediated xenobiotics, Nrf2-mediated oxidative stress, NFκBmediated response to inflammation, estrogen activity, respectively), as described previously (Bräunig et al. 2016; Dogruer et al. 2018). All cells, AhR-CAFLUX, AREc32, and VM7Luc4E2 bioassays in monolayers and for the NFκB bioassay in suspension were grown in 75 cm^2 flasks in 10 mL growth medium at 37˚C in a 5% CO_2 atmosphere. A detailed description of each assay is described in supplementary information. For the bioanalytical screening, an aliquot of the extract was added to the microtiter plate and reconstituted (diluted) in the respective assay medium. For this, DMSO extracts were directly used for analysis with no further clean-up procedures. The representative effect-concentrations for both the samples and reference compounds for each assay are described in the Supplemental Information (Table S8). The highest final concentration of DMSO was 1 % in the AhR-CAFLUX assay and 0.1 % in the other assays. Each experiment was run as 3-5 replicates on independent plates on different days. The experimental data were evaluated and statistically analysed using Prism 5.0 (GraphPad, San Diego, CA, USA). The statistical significance of differences between the three sites was assessed using ANOVA, whereby values of $p< 0.05$ were considered statistically significant.

3. Results and Discussion

3.1 Concentration of polar chemicals in water

A range of herbicides and PPCPs were detected at all sites using the ED passive samplers. Water concentrations for several compounds detected in EDs were estimated from available calibration data (*i.e.* sampling rates; Table 1). Additionally there were detections for chemicals for which no passive sampler calibration data is available, and these have simply been reported as 'detected' (Table 1). Overall, the estimated concentrations of target chemicals in the EDs was low (*i.e.* < 1 ng/L). The total number of chemicals detected in the EDs was highest in Cleveland Bay (10 herbicides and herbicide metabolites and 9 PPCPs), followed by Upstart Bay (9 herbicides and herbicide metabolites) and the Howicks Group of Islands (5 herbicides and herbicide metabolites) (Table 1). Catchment-specific chemical profiles were evident between the coastal sites, with agrichemicals detected at Upstart Bay (rural/ agricultural catchment) and agrichemicals and PPCPs detected at Cleveland Bay, (urban/ industrial catchment). At both coastal locations, the PSII herbicides atrazine, diuron and hexazinone were consistently detected in the highest concentrations (ranging between 0.13 to 0.68 ng/L). Atrazine and diuron were also detected sporadically at Howicks, although at very low concentrations (<0.1 ng/L). In Cleveland Bay, the PPCPs were primarily detected in the EDs located in closest proximity to the WWTP, suggesting effluent is a potential source of these chemicals. In Upstart Bay, two atrazine metabolites (desethyl atrazine and desisopropyl atrazine), together with ametryn-hydroxy (a metabolite of either atrazine or ametryn) and simazine were detected at very low concentrations at UB 2 only (< 0.05 ng/L). The carbamate insecticide methomyl was detected at Upstart Bay and none of the other locations

In the grab samples, concentrations of chemicals were also similarly low (< 2 ng/L). The number of chemicals detected in grabs at Howicks was again the lowest of all three sampling locations as expected (four herbicides and two PPCPs), however converse to the EDs, Upstart Bay had a slightly higher number of detections (10 herbicides and herbicide metabolites and one PPCP) than Cleveland Bay (seven herbicides and herbicide metabolites and one PPCP). Atrazine was again the most frequently detected chemical at both coastal locations (range 0.30 to 0.58 ng/L). Diuron was also frequently detected in 83 % of Upstart Bay samples, but less frequently (33 %) of samples from Cleveland Bay. Notably absent was hexazinone, which was not detected in any samples but was found in all EDs at both coastal locations. The herbicide metabolites desethyl atrazine and ametryn hydroxy were both frequently detected at Cleveland Bay (both in 67 % of samples) and also at Upstart Bay (33 % and 25 % of samples respectively), reflecting the ED results. Finally, the insecticide methomyl was

detected only at Upstart Bay (42 % of samples; mean concentration 0.27 ng/L), also consistent with its detection in the EDs.

The results show differences in the chemical profiles detected between the EDs and grab samples. Overall, fewer chemicals were detected in the grab samples than in the EDs with the PPCPs primarily detected in the EDs. Differences can reasonably occur as the two sampling methods are representative of different time periods (i.e. 'instant, point-in-time' for grab samples, and 'over several days' for EDs). In addition, they represent different volumes/ packets of water sampled (500 mL extracted for the grabs *versus* up to approximately 7 L for certain chemicals using the EDs). Due to the low concentrations present in the marine environment, the concentrating effect of the EDs allow accumulation of enough mass of chemical to exceed the detection limits of the analytical methods, that may not be overcome using grab samples. Additionally, the physico-chemical properties of chemicals may make them more or less suitable for uptake onto the EDs. Therefore employing a combination of these sampling techniques provides the broadest approach to capturing the widest range of anthropogenic pollutants.

Similarly, screening of grab water samples against a commercial spectral library containing >3000 common pesticides, PPCPs and forensic compounds generated few positive matches (Table 2); caffeine and paracetamol in Cleveland Bay, and amphetamine in Upstart Bay. Non-targeted analysis had limited discriminant statistical power due to low sample numbers (n=9, 3 per site). Methyl-, ethyl-, butyl- and propyl-paraben, commonly used in PPCPs, were all tentatively identified in one sample each from Upstart Bay and Howicks, with a relative concentration of seven orders of magnitude higher in the Upstart Bay sample. The parabens were not detected in field and procedural blanks, suggesting that the contamination was an isolated incident during field sampling (Data not shown).

3.2 Herbicide concentrations on the Great Barrier Reef

Diffuse pesticide run-off is a significant contributor to declining water quality on the GBR. Environmental monitoring and research activities have focused primarily on five priority PSII herbicides - ametryn, atrazine, diuron, hexazinone and tebuthiuron. As part of best farming practice, the use of alternative 'knockdown' herbicides (including 2,4-dichlorophenoxyacetic acid (2,4-D), acifluorfen, imazapic, imazethapyr, isoxaflutole, metribuzin, trifloxysulfuron-Na, metolachlor, trifluralin, pendimethalin) have been encouraged (Smith et al. 2015). These non-selective herbicides are being marketed as preferred tools to enable no-till fallows to be economically and efficiently

managed, theoretically reducing the risk of erosion and improving soil structure and water content. However, these too have been reported in passive samplers in marine environments (Garzon-Garcia et al. 2015) with metolachlor detected in EDs at both coastal locations in the current study (0.03 ng/L to 0.16 ng/L). Overall, the types and concentration of pollutants detected in water in this study are within the range observed in other marine monitoring activities and also consistent with the adjacent land uses, with the chemical profile of Upstart Bay dominated by agrichemicals, rather than PPCPs which are often associated with urban areas and water effluent. Herbicide concentrations in this study are lower than previously reported, but consistent with low reported PSII herbicide concentrations during periods of low rainfall and river discharge, which were the hydrological conditions under which these samples were collected. Some of the difference may be due to differences in passive sampler configuration and deployment time between relevant studies (Gallen et al. 2014). Despite efforts to further clean up the sample extracts, non-target analysis of ED extracts suffered from high levels of background interference and generated little useful data.

3.3 Estimated semi-polar and non-polar chemical concentrations in water and sediment

3.3.1 Targeted analysis of PDMS passive samplers and sediment

PDMS and pooled sediment extracts underwent targeted analysis for a range of PCBs, pesticides and PAHs (Table S6). In pooled sediment samples, six PAHs were detected at Upstart Bay (benzo[b + k]fluoranthene, benzo-[e]-pyrene, benzo-a-pyrene; benzo[ghi]perylene; indeno[1,2,3-cd]pyrene, benzo[a]anthracene) at a range of 1-3 pg/g sediment; and ten at Cleveland Bay (anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[bkj]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene), at a higher concentration range of 13-80 pg/g sediment. There were no reportable results from PDMS extracts. These very low concentrations are consistent with previous studies undertaken on coastal GBR sediments adjacent to the Burdekin region (Cavanagh et al. 1999, Müller et al. 1999, Davis et al. 2012).

3.3.2 Non-targeted screening of PDMS and sediment

PDMS and sediment extracts underwent non-target screening for GC-amenable compounds. By applying filtering criteria for detector response, NIST match score, and presence in procedural blank, the number of candidate spectral features was reduced to <100 per sample. Tentative identification of compounds with a confidence level of 3 (*i.e.* with evidence for possible structure(s), but insufficient evidence for one exact structure) are presented in Table 3. This included pesticides, herbicides, human and veterinary pharmaceuticals, fragrances, flavours, and industrial hydrocarbons. Additionally, hundreds of identified spectral features were suggestive of organic compounds including long chain

aliphatic and aromatic hydrocarbons, alcohols and ketones were identified, but their source and/or use was not identified (data not shown). The main source of hydrocarbons on GBR are spills, leaks and discharge from vessels, and industrial and urban activity (Kroon et al. 2015). It is also possible some of these compounds were synthesised by endogenous algae and phytoplankton. Upstart Bay showed markers of both agricultural and urban input into the marine environment (i.e. herbicides and PPCPs), and Cleveland Bay showed primarily urban markers (PPCPs), both consistent with the adjacent land use. Despite its relatively remote location, numerous pharmaceuticals were also identified at Howicks. Both hydrocarbons and PPCPs have been named as emerging chemicals of concern in the GBR (Kroon et al. 2015).

3.4 Correlating results from blood and water samples

To correlate internal and external exposure, results from non-target screening of EDs (2015 sampling campaign) and grab water samples were compared with previously reported results from whole turtle blood from the same sampling locations (Heffernan et al. 2017) (Table 2). Of these, 20:4 long chain fatty acid, was detected in the blood, passive and grab samples at Upstart Bay and Cleveland Bay; and the DNA adduct n-ethylguanine was detected in turtle blood and passive samplers from the same locations. Few PPCPs were identified in blood samples: allopurinol and milrinone in Cleveland Bay, and azaelic acid in both Cleveland Bay and Upstart Bay but these compounds were not detected in the corresponding water samples. Pesticides were identified in water samples from Cleveland Bay (atrazine and TEPP), and Upstart Bay (cyromazine), but not from Howicks. This is consistent with reported tentative identification of an insecticide metabolite (ethiofencarb sulfone) in blood samples from turtles in Cleveland Bay (Heffernan et al. 2017), providing evidence that (i) insecticides are used in the adjacent land in Cleveland Bay; and (ii) these insecticides are being metabolised by the resident turtle population.

Five pharmaceuticals were identified in turtle blood and water samples from Cleveland Bay, but no single compound was detected in both matrices (Table 2; excluding DEET and Salysilic acid). We expect most compounds to be metabolised and excreted by turtles. Thus, detecting short-lived parent chemicals in water and matching them to the corresponding metabolite in blood is challenging. One polybrominated compound was indentified in turtle blood from Cleveland Bay, but no structure or compound name was generated. It is possible that this brominated compound is of natural origin, with many natural halogenated chemicals identified previously in passive samplers on the GBR (Vetter et al. 2009).

3.5 Correlating data from sediment chemical analysis and bioassay results

Sediments can act as a sink for many chemicals in the aquatic environment. Incidental sediment ingestion may occur during seagrass foraging, with turtles potentially exposed to a diverse chemical mixture over a long period of time (Gaus et al. 2001b). Characterising such a diverse chemical mixture with traditional targeted mass spectrometry methods is expensive and time consuming. An effect-based approach using a battery of *in vitro* bioassays each with a different mode of action (MOA) can help mitigate this limitation. Bioassay results for pooled sediment samples relative to their bioanalytical equivalent concentrations (BEQs; TCDD (2,3,7,8- Tetrachloro-dibenzo-diozin), tBHQ (t-Butylhydroquone), TNF-alpha (Tumor necrosis factor- alpha), and E (17-beta estradiol) equivalent (EQ) values for AhR-CAFLUX, AREc32, NFκB-bla, and VM7Luc4E2; respectively) are presented in Figure 2 and supplementary information (Table S8).

For the AhR–CAFLUX assay, data was evaluated after exposure times of 24 and 72 hours to reduce the contribution of potentially labile AhR-active compounds present in the samples (e.g. PAHs). The impact of such labile compounds can be assessed by comparing the effect concentrations for both exposure times. A longer exposure time (72 hours) isolates the effects of more persistent compounds according to Ling et al (2015). For the AhR–CAFLUX assay evaluated after 24h exposure, Cleveland Bay showed the highest BEQ_{TCDD} induction (559 ng/kg sediment dry weight; dwt), followed by Howicks and Upstart Bay (283 and 202 ng/kg dwt, respectively). Results from Cleveland Bay are lower than previously reported concentrations in the nearby Brisbane River (927 ng/kg dwt), but higher than Oxley Creek (360 ng/kg dwt) and the Port of Brisbane (352 ng/kg dwt) (Li et al. 2013). Furthermore, Cleveland bay results were comparable to values found in Gladstone harbour (Bräunig et al. 2016). Interestingly, differences between remote Howicks and agricultural/industrial Upstart Bay areas were insignificant. This is despite a previous report of high concentrations of dioxin-like chemicals in soil and sediment in coastal areas of Queensland from extensive use of pesticides in the area (Holt et al. 2008). A longer exposure time isolates the effects of more persistent compounds in the AhR-CAFLUX assay. After 72h BEQs had decreased to 21, 26, and 128 ng/kg dwt for Howicks, Upstart and Cleveland Bay, respectively. This suggests that labile compounds (*e.g.* PAHs) play a more significant role in inducing cellular response than highly persistent compounds (*e.g.* organochlorine pesticides; OCPs).

As many AhR ligands also exhibit endocrine disrupting potential, we expected a similar response in the VM7Luc4E2 assay. Surprisingly, Upstart bay had the greatest induction with 44 ng EEQ/kg dwt, followed by Cleveland bay (26 ng/kg dwt) and Howicks (13 ng/kg dwt).), which suggest that endrocrine active compounds play a bigger role in Upstart bay in relation to the two other study sites. In our study BEQ_{tBHQ} concentration was 2.6, 5.6 and 15.7 mg/ kg dwt for Howicks, Upstart and Cleveland Bay,

respectively. The BEQ_{tBHQ} values from another Australian study ranged from 5.7 to 21.2 mg/kg wet weight in sediments associated with a broad range of land-uses (*e.g.* industry, urban, mining and agriculture) as well as reference sites (*e.g*. Hummock Hill Island) and are comparable with values from the present study (Bräunig et al. 2016). Furthermore, the values from the present study were all relatively low compared with the control site in Li et al. (2013) of 3.1 mg/kg dwt, suggesting low levels of chemicals capable of inducing oxidative stress in these sediment samples. Similarly, we apply the NF-κB-bla assay, an indicator of inflammatory response, for the first time to sediment samples. Howicks had the lowest induction with TNF-alpha equivalent concentration of 0.68 pg/kg dwt, followed by Cleveland and Upstart Bay (2.13 and 2.19 pg/kg dwt, respectively). Due to the low specificity of this assay, and the large number of potential chemical compounds that can illicit this response, we cannot narrow down likely candidates in these samples. However, the extraction method is validated (recoveries ranging from 80- 130 %) for several groups of compounds (organophosphorus and organochloride pesticides, chlorinated herbicides, PCBs, and PCDDs/PCDFs) all of which are possible candidates. Overall, Howicks sediments had low BEQ results in all bioassays, confirming its suitability as a control site. The results showed differences in the chemical mixture among the three turtle habitats. Howicks sediments had low BEQ results in all bioassays in comparison with the two other study sites (Cleveland Bay and Upstart Bay). Considering the feeding behaviour of marine sea turtles and the previously documented correlation of contaminant levels in sea turtles with sediments of their foraging ground, sediments represent an important exposure source to anthropogenic contaminants (Hermanussen et al. 2004, 2006).

3.6 Emerging anthropogenic pollutants of concern in the GBR

The majority of pollutant monitoring on the GBR has focused on five priority PSII herbicides, but research into other compounds is emerging. Information on PPCPs in the GBR is limited to data from two waste water treatment plants north of both Upstart and Cleveland Bay (O'Brien et al. 2014, Scott et al. 2014). Authors reported 26 pharmaceuticals in waste water effluent in the sub-µg/L range, including venlafaxine, hydrochlorathiazide, and citalopram. These three compounds were also detected in our study, typically < 1 ng/L, demonstrating the substantial dilution that occurs. More broadly, in a survey of 73 sites from 19 waterways across Queensland the PPCPs caffeine, paracetamol and salicylic acid were detected in 60% of samples, followed by carbamazepine (27 %) and triclosan (25 %) (Scott et al. 2014). Wastewater is one of the most significant sources of PPCPs pollution, and the risk is greatest around urban centres (Kroon et al. 2015). At least 50 WWTPs are operational within GBR catchment areas that discharge effluent into the environment. As the population increases

over the coming decades, chemicals associated with urban and industrial uses may become of greater concern to coastal environments and wildlife.

There are no recent or reliable data available for use of agricultural chemicals in GBR catchments. End-of-catchment pesticide loads monitoring demonstrates that usage is dynamic and can fluctuate yearly based on specific pest pressures, climatic conditions, regulatory action, use of resistant crop varieties or the development of herbicide resistance in weeds (Devlin et al. 2015). Due to this significant knowledge gap, the use of non-targeted mass spectrometry and effects-directed analyses are the most informative approach for characterising chemical exposure of marine wildlife.

4. Conclusions

This study describes an innovative and comprehensive approach to monitoring exposure and associated health effects of organic environmental pollutants on marine wildlife. A combination of effect-based and non-targeted chemical analysis screening tools allowed detection of a large number of chemicals that are not captured by existing monitoring programs which arguably underestimate the exposure risk to marine wildlife. The 'case-control' approach to sampling and comparative analysis confirmed spatial differences in the external exposures of resident coastal versus offshore green turtles. Overall low concentrations of pesticides, pharmaceuticals and personal care products associated with known uses in the adjacent catchments were detected in water, passive samplers and sediment. Despite these low concentrations, the cumulative effects of exposure on such long-lived species such as the green turtle are unknown.

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Integrated chemical exposure assessment of coastal green turtle foraging grounds on the Great Barrier Reef

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SUPPLEMENTARY INFORMATION

Detailed Materials and Methods

1. Sampling sites

Three sampling locations were selected for the case-control comparison: two 'case' coastal locations influenced by adjacent catchment activities; and a remote, offshore location as a 'control'. The sites included:

1) Upstart Bay, a rural coastal area within the Burdekin region of the GBR. Intensive sugar cane cultivation occurs in the Burdekin Delta which drains into the north of Upstart Bay. The Burdekin River has historically discharged the largest annual volumes of freshwater into Reef inshore areas (Devlin et al. 2012). The Don River Basin is located adjacent to the southern part of Upstart Bay and supports nature conservation, grazing and small areas of irrigated cropping and aquaculture (GBRMPA 2013). Only a small urban population is situated in proximity to the Upstart Bay sampling activities. Three sampling sites (UB1, UB2 and UB3) were visited for grab water and sediment sample collection. ED passive samplers were deployed at UB1 (ED and PDMS) and UB2 (replicate EDs) (Figure S1).
2) Cleveland Bay is approximately 100 km north of Upstart Bay, also within the Burdekin region and lies 20 km southeast of the city of Townsville, which is home to approximately 20% of the GBR population (Gunn and Manning 2009). Land use activities in close proximity to Cleveland Bay include urban/residential land use, grazing, other minimal use activities (*e.g.* golf courses, nature conservation), and small areas of manufacturing, industry and waste treatment. Storm water, urban run-off, and effluent discharged from industry and wastewater treatment plants are all potential sources of chemical compounds identified in water samples collected at this location. Three sampling sites (CB1, CB2 and CB3) were visited for grab sampling with a passive sampler deployed at CB2 (ED and PDMS) and additionally at CBWT (replicate EDs) near

the waste water treatment plant. Sediment samples were collected from CB2, CBWT and Cockle Bay (the location of a seagrass foraging area also within Cleveland Bay; Figure S1)

3) Howicks Group of Islands (HOW), a remote collection of unpopulated islands is located approximately 100 km off-shore from the adjacent Cape York region, which is the least developed of the entire GBR catchment (DSITI 2015). Three sampling sites (HOW1, HOW2 and HOW3) were visited, for grab water and sediment sample collection. Passive samplers (EDs) were deployed at all three sites and PDMS was deployed at HOW2.

Sampling activities were carried out between May and August of 2015, by members of the multidisciplinary team who were also conducting turtle health studies at the same time. Details of types of samples collected (grab water, passive samplers and sediment), dates and descriptions of the sampling sites) are provided (Tables S1-S3, Figure S2).

2. Sample Preparation, Transportation and Extraction Methods

The methods for preparation, transportation and extraction of passive samplers have been extensively described previously (Page et al. 2014, GBRMPA 2016, O'Brien et al. 2016). A brief description of each are as follows.

2.1 Passive samplers (EDs)

3M^{TM} Extraction Disks (SDB-RPS; Phenomenex) were conditioned in methanol (HPLC grade, Merck) for 2 minutes, followed by MilliQ water for a minimum of 5 minutes. The disks were loaded into the acetone rinsed Chemcatcher^{TM} housing (Figure S1).and covered with a solvent rinsed wire mesh. The housing was filled with MilliQ water and the transportation cap fitted to seal for transport. Assembled samplers were sealed in ziplock bags, stored at 4°C prior to deployment and transported with ice packs. Following deployment, the surface of the disk was wiped with a kimwipe to remove biofouling and excess water. The surface of the disk was spiked with a mixture of isotope-labelled internal standards (mixture of herbicides and PPCPs; Novachem, Collingwood, AUS); see Table S4 for full list). The disk was extracted first using 5 mL acetone (HPLC Grade; Merck) and then 5 mL methanol (HPLC Grade; Merck) in a solvent rinsed 15 mL centrifuge tube in an ultrasonic bath (5 mins each). The combined extracts were concentrated using evaporation under purified N_2, filtered (0.22 µm PFTE) and adjusted to a final volume of 0.5 mL (20% methanol and 80% MilliQ water solution) for LC-MS/MS analysis. Following target analysis, the sample extracts were subjected to a further clean up step, in an effort to remove background interference common for these sampler types during non-target analysis. Extracts were loaded onto Strata X 60 mg 3cc SPE cartridges (Phenomenex), dried under

vacuum (30 mins) and eluted with 2 x 2 mL methanol (HPLC grade; Merck). The eluates were concentrated under purified N_2 to a final volume of 0.5 mL (20% methanol and 80% MilliQ water solution). Field blanks and procedural blanks were processed in parallel.

2.2 Passive samplers (PDMS)

Prior to use, strips were pre-cleaned by dialysis with acetone (AR grade) (2 x 24 hr) and then hexane (AR grade)(2 x 24 hr) in solvent rinsed glass jars in batches on a benchtop shaker, refreshing the solvents every 24 hr. Cleaned strips were placed on alfoil and allowed to air dry. Individual strips were wound around stainless steel spikes within the deployment cage (acetone rinsed) and the cage lid secured on with wingnuts. The assembled cages were wrapped in alfoil, sealed in a ziplock bag, stored at 4^{o}C and transported with ice packs. Following deployment, biofouling was removed from each strip by scrubbing with water. Each strip was dried with kimwipes and spiked with a surrogate standard (2,7-Dichlorodibenzodioxin; 50 ng) (Wellington Laboratories, Guelph, CAN). Each strip was dialysed with 200 mL of hexane (HPLC grade) (2 x 24 hours), the extracts for two strips deployed at each location were combined and rotary evaporated, filtered (0.45 µm PTFE) and subjected to size exclusion gel permeation chromatography. Separation was achieved using a 19 mm by 150 mm guard column, followed by a 19 mm by 300 mm main column, packed with Envirogel [100 Å pore size, 15 µm particle size, Waters] as the stationary phase, and with dichloromethane (DCM) as the mobile phase. The flow rate was 4.5 mL/min and the sample fraction was collected between 15 to 24.2 min after sample injection onto the GPC column. The extracts were further evaporated under purified N_2, transferred to an insert and reduced to 200 µL final volume for GC-MS/MS analysis. Field blanks and procedural blanks were processed in parallel.

3. Detailed description of bioassays

3.1 AhR-CAFLUX assay

Cell growth and cell culture: The CAFLUX cell line H1G1.1C3 was originally a mouse hepatoma cell line (Hepa1c1c7), but is transfected with EGFP (enhanced green fluorescent protein (Nagy et al. 2002a, Nagy et al. 2002b, Denison and Nagy 2003) and was provided by Prof Dr M Denison (Environmental Toxicology, University of California Davis, USA). Cells were grown in monolayers in MEM-α (Minimum Essential Medium Eagle alpha modification, Gibco® Life Technologies, Australia) containing L-glutamine supplemented with 9% foetal bovine serum (FBS; Gibco® Life Technologies, Australia), 1% geneticin (G418; Gibco® Life Technologies, Australia) and 1% penicillin/streptomycin (Gibco® Life Technologies, Australia). Cells were maintained in 75 cm^2 flasks in 10 mL medium at 37°C in a 5% CO_2 atmosphere and passaged every 2-5 days when the cells were >80% confluent.

CAFLUX assay experiments: For the CAFLUX assay, cells were subcultured into 96 well black plates (Corning®) at a density of $3.0x10^4$ cells/well using MEM-α medium containing all supplements except G418, and were left to adhere for 24 hours. After 24 hours, cells were dosed with a serial dilution (2-fold) series of all individual extracts. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; Cambridge Isotope Laboratories Inc) was used as a reference compound while wells (cells) exposed to only medium were used as background value. Upon dosing, the well plates were kept in a 33°C in a 5% CO_2 atmosphere and were measured at different time points (t = 0 h, t = 24 h, t = 48 h, and t = 72 h) at 485/520 nm (excitation/emission) using a FLUOstar Omega plate reader (BMG Labtech) and MARS version 2.10 software.

Quality Assurance/Quality Control (QA/QC): The RLU (Relative Light Units) at the three different time points (t = 24 h, 48 h, and 72 h) were subtracted from the RLU values at t = 0, and the average medium value from each plate was subtracted from all other RLU values. All sample dilution series were compared to the maximum response of TCDD on the same plate. The EC_{50} of each sample was calculated using sigmoidal dose-response in GraphPadPrism 6.0.

3.2 AREc32 assay

Cell growth and cell culture: The human breast cancer cell line, MCF7, containing an ARE reporter plasmid attached to a reporter gene encoding for luciferase (Wang et al. 2006) was provided by Dr R Wolf (University of Dundee, UK). Cells were grown in monolayers in DMEM (Dulbecco's Modified Eagle Medium, Gibco® Life Technologies, Australia) containing sodium pyruvate, L-glutamine and high glucose was supplemented with 9% foetal bovine serum (FBS), 1% geneticin (G418) and 1% penicillin/streptomycin (all Gibco® Life Technologies, Australia). Cells were maintained in 75 cm^2 flasks in 10 mL medium at 37°C in a 5% CO_2 atmosphere and passaged every 2-5 days when the cells were >80% confluent.

AREc32 assay experiments: The experimental procedure was reported previously (Escher et al. 2012). Cells were subcultured into 96 well white plates (Corning®) at a density of $1.2x10^4$ cells/well using DMEM medium containing all supplements except for G418, and were left to adhere for 24 hours. After 24 hours, cells were dosed with a serial dilution (2-fold) series of all individual extracts. Tert-butylhydroquinone (tBHQ; Sigma-Aldrich, Australia) was used as a reference compound while wells (cells) exposed to only medium were used as background value. Cells were exposed for 24 hours (and kept in a 37°C and 5% CO_2 atmosphere) after which the luciferase response was quantified by 1) lysing

the cells for approx 20 min using 30 µL of a 20% 5x lysis buffer and 0.2% DTT (1 M; all Sigma-Aldrich) mixture in autoclaved Milli-Q water, and 2) adding 100 µL luciferase reagent containing 20% 5x substrate buffer, 3% DTT (1 M), 0.3 % CoA (100 mM), 0.5% ATP (all Sigma-Aldrich) and 4.6 % luciferin (100 mM; Promega VivoGlo) in autoclaved Milli-Q water. Luminescence was immediately measured after adding the luciferase reagent using a FLUOstar Omega plate reader (BMG Labtech) and MARS version 2.10 software.

Quality Assurance/Quality Control (QA/QC): The concentration of each sample was reported in relative enrichment factor (REF). Induction ratios (IR) were calculated by dividing the relative light units (RLU) of each well by the average RLU of the medium controls. Values above 5 and values that caused more than 10% cytotoxicity were excluded. The effect concentration (EC) was measured using a linear serial dilution with at least 8 different concentration to obtain a concentration-effect curve. The concentration that caused an induction ratio of 1.5 was named as the $EC_{IR1.5}$ and was calculated using a linear regression in GraphPadPrism 6.0.

3.3 NFκB-bla assay

Cell growth and cell culture: The NFκB-bla assay was based on THP1 human leukaemia cells, which are stably transfected with a β-lactamase reporter gene downstream of the NF-κB response element (Jin et al. 2015). This assay applies a fluorescence resonance energy transfer (FRET) method that generates a radiometric reporter response with minimal experimental noise for quantitative measurement of the pathway activation. The GeneBlAzer Beta-lactamase reporter technology is a highly accurate, sensitive and easy to use method to monitor cellular responses to chemicals or other stimuli (Zlokarnik et al. 1998).

The cell medium contained the esterified and lipophilic LiveBlAzer FRET-B/G CCF4-AM dye, which can enter the cells and can be hydrolysed by cytoplasmic esterase and gets converted to two fluoroprobes, coumarin and fluorescein (Jin et al. 2015)If the NF-κB pathway is inactive, the substrate molecule remains intact, and excitation of coumarin at 409 nm results in FRET to the fluorescing moiety, which emits a green fluorescence signal at 530 nm. If the NF-κB pathway is activated, which implies β-lactamase expression, the lactam ring of the substrate molecule is enzymatically split, which results in separating the fluorophores spatially and disruption FRET (Jin et al., 2015). In this case, the excitation of the coumarin at 409 nm produces a blue fluorescence signal at 460 nm. The blue:green ratio finally provides the normalized reporter response, which allows the quatitative measure of NF-κB activation.

The cell viability was also tested via measuring the cellulcar mitochondrial activity by means of the resazurin conversion using 50 µM final resazurin concentration.

NFκB-bla assay experiments: Cell cultivation was performed according to Yeh et al. (2014).The amount of cells on the black-coated clear bottom 384 well plates (Corning®) were adjusted to 6.25 x 10^5 cells/mL, and 32 µL of the cell suspension was added per well including unstimulated control wells and stimulated control wells. Cells were incubated for >6 hours. Samples and positive controls were serially diluted on two clear 96-well plates, than 8 µL was dosed on the cells. Cells were incubated for 5 hours at 37°C and 5% CO_2. TNF-α (Invitrogen) was used as reference compound.
For measuring cell responses, 8 µL of the LiveBLAzer mix and resazurin solution (Invitrogen) was added and the cells were incubated again for 2 hours at room temperature in the dark. The fluorescent measurement was done with the FLUOstar Omega plate reader (BMG Labtech) and MARS version 2.10 software.

Quality Assurance/Quality Control (QA/QC): The fluorescent emission after resazuring exposure for 2 hours was measured at 590 nm after excitation at 544 nm. The dye (CCF4) was excited at 409 nm and emission was measured at 460 (blue channel) and 530 nm (green channel). On the plate, 8 blank wells were included (assay medium without any cells), which were averaged to correct the fluorescence data. The induction ratio was evaluated by dividing the blue:green emission ratio of a sample by the average emission ratio of the solvent control, which indicates the NF-κB activation.

3.4 VM7Luc4E2 assay

Cell growth and cell culture: The commonly used cell line for estrogen-induced proliferation is the human breast cell line, MCF7. In this study, however, alternative cells were used which originate from ovarian tumors and which are also estrogen receptor (ER)-positive (Geisinger et al. 1989, Rogers and Denison 2000). This specific receptor mediated assay is based on the binding of an estrogen with the ER which, in turn, binds to an estrogen responsive element (ERE) in the cell nucleus.

The stably transfected cells were cultured in α-MEM and transferred 5 days prior to the experiments into DMEM estrogen stripped and phenol free medium (Gibco® Life Technologies, Australia) with 5% estrogen stripped FBS and 1% streptomycin/penicillin (all Gibco® Life Technologies, Australia). The amount of cells on the 96-well microtiter plate (Corning®) was adjusted to 3.0 x 10^5 cells/mL. The subsequent procedure followed that of the AREc32 assay described earlier.

VM7Luc4E2 assay experiments: At the start of the experiments, cells were left to adhere for 24 hours. After 24 hours, cells were dosed with a serial dilution (2-fold) series of all individual extracts. 17β-estradiol was used as a reference compound while wells (cells) exposed to only medium were used as background value. Cells were exposed for 24 hours (and kept in a 37°C and 5% CO_2 atmosphere) after which the luciferase response was quantified by 1) lysing the cells for approximately 20 min using 30 µL of a 20% 5x lysis buffer and 0.2% DTT (1 M) mixture in autoclaved Milli-Q water, and 2) adding 100 µL luciferase reagent containing 20% 5x substrate buffer, 3% DTT (1 M), 0.3 % CoA (100 mM), 0.5% ATP and 4.6 % luciferin (100 mM) in autoclaved Milli-Q water. Luminescence was immediately measured after adding the luciferase reagent using a FLUOstar Omega plate reader (BMG Labtech) and MARS version 2.10 software.

Quality Assurance/Quality Control (QA/QC): The concentration of each sample was reported in relative enrichment factor (REF). Induction ratios (IR) were calculated by dividing the relative light units (RLU) of each well by the average RLU of the medium controls. Values above 5 and values that caused more than 10% cytotoxicity were excluded. All sample dilution series were compared to the maximum response of 17β-estradiol on the same plate. The EC_{50} of each sample was calculated using sigmoidal dose-response in Graph Pad Prism 6.0.

3.5 Sample analysis

3.5.1 Mass spectrometry analysis

3.5.1.1 Polar analytes (target and non-target analysis)

ED passive samplers and water sample extracts were analysed for herbicides and PPCPs using targeted LC-MS/MS (Nexera ultra high-pressure liquid chromatography, Shimadu Corp, Kyoto, Japan; and 6500 QTRAP, SCIEX, Concord, CA) operating in positive and negative ionization mode. Chromatographic separation was achieved using a 2.6 µm, 50 × 2.0 mm biphenyl column (Phenomenex, Torrance, CA) maintained at 45˚C. Mobile phases were 1:99 methanol:water (v/v, A) and 95:5 methanol:water (v/v, B), both containing 0.1% acetic acid, with a linear gradient at 0.3 mL min^{-1} as follows: 0min, 5%B; 5.2 min, 100%B; 9.5min, 100%B; 13min, 5%B (Grant et al. 2017).

Non-target screening of water samples was conducted using a Nexera X2 UPLC (Shimadzu) coupled to a hybrid quadrupole time-of-light mass spectrometer (QTOF-MS; Triple-TOF 5600, Sciex), with an electrospray (ESI) interface operating in positive and negative ionization mode. Separation was

achieved using a reverse-phase Gemini-NX C18 column (3 µm × 2.0 mm × 50 mm, Phenomenex). Full details of the analytical system and data processing methods are described in Heffernan et al. (2017). A full list of target chemical analytes is provided in Tables S4-S5. When processing target chemical results (EDs), blank subtraction was performed when field blank concentration was 5-20% measured concentration in unknown sample; if >20%, the result was discarded. Contamination of both field and laboratory blanks with several PPCPs including N,N-diethyl-meta-toluamide (DEET), caffeine, triclosan, paracetamol, salicyclic acid and ibuprofen, was evident in passive samplers and grab water samples. This was likely due to common use of products containing these compounds by either field or laboratory personnel. Thus, any results of these detected in deployed samplers have been excluded.

3.5.1.2 Non-polar analytes (target and non-target analysis)

PDMS and sediment samples were analysed for polychlorinated biphenyls (PCBs) and pesticides using GC-MS/MS (GC ultra-TSQ triple quadrupole Quantum XLS, Thermo Fisher) in electron ionisation mode. Analytes were separated using a 0.18mm (i.d.) × 30m fused silica capillary column coated with a 5% diphenyl, 95% dimethyl polysiloxane (0.18 µm film thickness). The transfer line and source were both held at 280°C and the flow rate was maintained at 1.0mL min^{-1}. The GC oven temperature program 80°C (hold; two minutes), was ramped to 180°C at 20°C/min (hold; 0.5 minutes) and then increased to 300°C at 10°C/ min (hold; 10.5 minutes) (Grant et al. 2017).

Polyaromatic hydrocarbons (PAHs) were analysed using a Thermo Scientific DFS High Resolution GC/MS in splitless injection mode using an Agilent J & W DB-5MS column (30m × 0.25mm × 0.25µm). Analysis was conducted in MID mode at 10,000 resolution (10% valley definition). The inlet, transfer line and source were held at 250°C, 280°C and 280°C respectively and the flow rate was maintained at 1.0 mL min^{-1}. The GC oven temperature program commenced at 80°C (hold; 2 min), was ramped to 180°C at 20°C min^{-1} (hold; 0.5 min) and then increased to 300°C at 10°C min^{-1} (hold; 5 min).

Non-target screening of sediment and PDMS samples was conducted at the Norwegian Institute of Water Research (NIVA) using a Thermo Fisher Scientific Q Exactive GC Orbitrap MS, and a BD-5 column (30m × 0.25mm, 0.25µm, Agilent), with 1 µL injection volume in splitless mode with helium as carrier gas. The injection port, transfer line, and source were maintained at 280 °C. The oven program commented at 60 °C (hold; 2 min) and ramped to 310 °C at 5 °C min^{-1}, followed by 30 min bake out to avoid carry over. The TOFMS collected 2 spectra every second from 50-600 m/z, with resolution of ∼8000 at half width full range (i.e. 50 to 600 m/z). The detector was operated at 2850 V and a filament current of ∼ 1 mA.

Supplementary Tables

Table S1 Details of passive sampler deployments

Sampling Location	Site Description	Passive Sampler types	Date Deployed	Date Retrieved	Latitude	Longitude
CBWT	offshore of the WWTP	ED	29/05/2015	5/06/2015	19°16.778	147°52.186
CB 2	Middle	ED, PDMS	29/05/2015	5/06/2015	19°14.500	147°57.674
UB 1	Knobbys	ED, PDMS	14/06/2015	21/06/2015 (ED) 14/7/15 (PDMS)	19°47.344	147°45.404
UB 2	Rocky Ponds	ED	14/06/2015	21/06/2015	19°49.267	147°40.887
HOW 1	Coombe Reef	ED, PDMS	25/7/2014 (PDMS) 9/8/2014 (EDs)	16/08/2016	14°23.969"S;	144°55.702"E
HOW 2	Outer Reef	ED	9/08/2015	16/08/2016	14°22.197"S;	144°57.581"E
HOW 3	Ingram Island	ED	9/08/2015	16/08/2016	14°25.241"S;	144°52.469"E

WWTP = waste water treatment plant

Table S2 Details of sediment sample collection

Sampling Location	Sample composition	Date	Latitude	Longitude
Cleveland Bay (Pooled)	CB2	29/05/2015	19°14.500	146° 57.674
	Cockle Bay	1/06/2015	19°14.863	146° 59.615
	Offshore of the WWTP	29/05/2015	19°16.889	146° 52.075
	Offshore of the WWTP	29/05/2015	19°16.878	146° 51.966
	Offshore of the WWTP	29/05/2015	19°16.898	146° 51.971
	Offshore of the WWTP	29/05/2015	19°16.892	146° 52.085
Upstart Bay (Pooled)	(UB1) Knobbys	14/06/2015	19° 47.679	147°45.848
	(UB2) Rocky Ponds	15/06/2015	19° 49.585	147° 40.921
	(UB3) Wunjunga	14/06/2015	19° 45.001	147° 36.109
Howicks Gp (Pooled)	(HOW1) Coombe Reef	13/08/2015	14°24.279	144°55.628
			14°24.461	144°55.972
	(HOW2) Outer Reef	12/08/2015	14°21.905	144°57.511
			14°22.300	144°57.875
	(HOW 3) Ingram Island	14/08/2015	14°25.242	144°52.693
			14°25.417	144°53.033

Table S3 Details of grab sample (water) collection sites across the three study sites: Cleveland Bay, Upstart Bay, Howicks group.

Sampling Location	Site Description	Date	Latitude	Longitude
Cleveland Bay (CB1)	Site near creek	5/06/2015	19°15.969	146° 56.595
Cleveland Bay (CB1)	Site near creek	5/06/2015	19°15.002	146° 59.610
Cleveland Bay (CB2)	Middle	5/06/2015	19°17.024	146° 57.767
Cleveland Bay (CB2)	Middle	1/06/2015	19°14.533	146° 57.674
Cleveland Bay (CB3)	Site near beach	5/06/2015	19°16.084	146° 58.848
Cleveland Bay (CB3)	Site near beach	5/06/2015	19°13.964	146° 58.634
Upstart Bay (UB1)	Knobbys	21/06/2015	19°46.478	147°44.810
Upstart Bay (UB1)	Knobbys	21/06/2015	19°47.319	147°45.298
Upstart Bay (UB1)	Knobbys	19/06/2015	19°47.286	147°45.396
Upstart Bay (UB1)	Knobbys	19/06/2015	19°46.729	147°45.031
Upstart Bay (UB2)	Rocky Ponds	14/06/2015	19°49.267	147°40.887
Upstart Bay (UB2)	Rocky Ponds	14/06/2015	19°49.267	147°40.887
Upstart Bay (UB2)	Rocky Ponds	21/06/2015	19°49.284	147°40.767
Upstart Bay (UB2)	Rocky Ponds	21/06/2015	19°49.125	147°40.337
Upstart Bay (UB3)	Wunjunga	14/06/2015	19°45.336	147°36.491
Upstart Bay (UB3)	Wunjunga	14/06/2015	19°45.167	147°36.320
Upstart Bay (UB3)	Wunjunga	19/06/2015	19°45.343	147°36.678
Upstart Bay (UB3)	Wunjunga	19/06/2015	19°44.962	147°36.598
Howicks Gp (HOW1)	Coombe Reef	9/08/2016	14°20.950	144°55.691
Howicks Gp (HOW1)	Coombe Reef	9/08/2016	14°23.896	144°55.672
Howicks Gp (HOW1)	Coombe Reef	16/08/2016	14°23.961	144°55.687
Howicks Gp (HOW1)	Coombe Reef	16/08/2016	14°23.960	144°55.684
Howicks Gp (HOW2)	Outer Reef	9/08/2016	14°22.165	144°57.576
Howicks Gp (HOW2)	Outer Reef	9/08/2016	14°22.152	144°57.555
Howicks Gp (HOW2)	Outer Reef	16/08/2016	14°22.192	144°57.572
Howicks Gp (HOW2)	Outer Reef	16/08/2016	14°22.173	144°57.588
Howicks Gp (HOW3)	Ingram Island	9/08/2016	14°25.244	144°52.442
Howicks Gp (HOW3)	Ingram Island	9/08/2016	14°25.207	144°52.409
Howicks Gp (HOW3)	Ingram Island	16/08/2016	14°25.253	144°52.469
Howicks Gp (HOW3)	Ingram Island	16/08/2016	14°25.240	144°52.454

Table S4 Components of isotopically-labelled internal standard (1 ppm) spiked onto passive samplers (EDs) and grab samples prior to extraction

Chemical
2,4 Dichlorophenoxy acetic acid (ring-13C6)
Caffeine-13C3
Codeine-D3
Gabapentin-D10
Venlafaxine-D6 HCl
Carbamazepine D10
Atenolol-D7
Acesulfame-D4
Atrazine-D5
Simazine-D10
Diuron-D6
Temazepam-D5
Fluoxetine-D6

Table S5 List of targeted chemicals included in the liquid chromatography tandem mass spectrometry analysis for water and ED passive samplers.

LC-MS/MS Target Analytes	
2,4 DB	Hexazinone
24 D	Hydrochlorthiazide
245T	Ibuprofen
3,4 DiCl Aniline	Imazapic
Acesulfame	Imazethapyr
Ametryn	Imidacloprid
Ametryn hydroxy	Iopromide
Asulam	Malathion
Atenolol	MCPA
Atorvastatin	Mecoprop
Atrazine	Methiocarb
Bromacil	Methomyl
Bromoxynil	Metolachlor
Caffeine	Metribuzin
Carbamazepine	Metsulfuron-Methyl
Carbofuran	Naproxen
Chlorpyriphos	Paracetamol
Citalopram	Paraxanthine
Clopyralid	Pendimethalin
Codeine	Picloram
DCPMU	Prometryn
DCPU	Propazine
DEET	Propiconazole
Desethyl Atrazine	Propoxur
Desisopropyl Atrazine	Salicylic acid
Desmethyl Citalopram	Sildenafil
DesmethylDiazepam	Simazine
Diazinon	Simazine hydroxy
Dicamba	Tadalafil
Dichlorvos	Tebuconazole
Diuron	Tebuthiuron
Fenamiphos	Temazepam
Fluazifop	Terbuthylazine
Flumeturon	Terbuthylazine des ethyl
Fluoxetine	Terbutryn
Fluroxypyr	Tramadol
Furosemide	Triclopyr
Gabapentin	Triclosan
Haloxyfop	Venlafaxine

Table S6 Target chemicals analysed in an initial screen of PDMS and sediments using gas chromatography tandem mass spectrometry (GC-MS/MS).

PAHs	**Pesticides**	**PCBs**
acenaphthene	Pentachlorobenzene	PCB-52
acenaphthylene	a-HCH	PCB-81
fluorene	b-HCH	PCB-77
phenanthrene	r-HCH (lindane)	PCB-101
anthracene	d-HCH	PCB-123 + 118
fluoranthene	HCB	PCB-114
pyrene	Heptachlor	PCB-105
benzo[a]anthracene	Chlordane	PCB-126
chrysene	DDE (o,p + p,p)	PCB-153
benzo[b + k]fluoranthene	DDT (o,p + p,p)	PCB-138
benzo (e) pyrene	DDE (o,p + p,p)	PCB-167
benzo (a) pyrene	Mirex	PCB-156
perylene	Permethrin	PCB-157
benzo[ghi]perylene		PCB-169
indeno[1,2,3-cd]pyrene		PCB-180
dibenzo[a,h]anthracene		PCB-189

Table S7 Annual freshwater discharge volumes (mega litres) from Great Barrier Reef rivers nearby to Cleveland Bay and Upstart Bay. Yellow indicates 1.5 – 2 times the long term median, orange indicates 2 to 3 times the long term median, red indicates >3 times the long term median; water years are from 1 Oct to 30 Sept. (Grant et al. 2017) Data courtesy of Eduardo da Silva (James Cook University).

River	Black	Ross/Bohle	Burdekin	Don
Long Term median	4.56E+04	2.12E+04	5.31E+06	5.12E+04
2001 - 2002	4.04E+04	4.75E+04	4.49E+06	3.86E+04
2002 - 2003	1.04E+04	7.85E+03	2.09E+06	4.37E+04
2003 - 2004	4.54E+04	5.60E+04	1.52E+06	5.46E+04
2004 - 2005	2.77E+04	2.17E+04	4.33E+06	9.74E+04
2005 - 2006	5.36E+04	4.28E+04	2.20E+06	4.12E+04
2006 - 2007	1.39E+05	1.35E+05	9.77E+06	1.65E+05
2007 - 2008	1.81E+05	1.61E+05	2.75E+07	4.62E+05
2008 - 2009	2.99E+05	2.31E+05	2.94E+07	2.45E+05
2009 - 2010	1.49E+05	1.45E+05	7.95E+06	1.44E+05
2010 - 2011	3.47E+05	2.43E+05	3.48E+07	8.48E+05
2011 - 2012	1.82E+05	1.54E+05	1.56E+07	2.17E+05
2012 - 2013	4.60E+04	3.22E+04	3.42E+06	1.56E+05
2013 - 2014	1.02E+05	1.37E+05	1.46E+06	8.76E+04
2014 - 2015	4.31E+03		8.81E+05	4.63E+04
yellow	1.5 to 2-times LT median			
orange	2 to 3-times LT median			
red	more than 3 times LT median			

Values were obtained from DNRM (https://www.dnrm.qld.gov.au/water/water-monitoring-and-data/portal); Values are in Megalitters per water year (i.e., 1-Oct to 30-Sep) for each river gauge station; All data from the Ross gauge station, which ceased in 2007-08-01 with no substitute in the same river, was replaced by Bohle gauge station.; Long-term median was calculated from water year 1970-1971 to 1999-2000.

Table S8 Bioanalytical screening of sediments in *in vitro* bioassays (H= Howicks, C= Cleveland Bay, U= Upstart Bay, LOD= limit of detection)

In vitro bioassay	Site	EC50/IR1.5	BEQ	STD	CV	Reference compound	EC50/IR1.5	STD	CV	LOD
	H	0.5163	25.**0935**	6.6003	26%					
AhR- CAFLUX (ng/kg sed.$_{72h}$)	C	0.1089	118.1491	25.0221	21%	TCDD (ng/L)	12.4377	1.7609	14.16 %	1.2723
	U	0.4313	29.2784	4.6065	16%					
	H	0.1630	2.6759	0.9054	34%					
AREc32 (mg/kg sed.)	C	0.0269	16.1664	4.6282	29%	t-BHQ (mg/L)	0.4112	0.0924	22.46 %	1.0703
	U	0.0628	6.7143	1.4767	22%					
	H	0.0510	0.6805	0.2610	38%					
NF- κB-bla (pg/kg sed.)	C	0.0139	2.1325	0.0818	4%	TNF-alpha (pg/L)	29.6900	2.8598	9.63 %	0.0609
	U	0.0135	2.1948	0.0571	3%					
	H	0.5721	13.5094	1.4010	10%					
VM7Luc4E2 (ng/kg sed.)	C	0.2915	26.3408	1.0625	4%	17ß-estradiol (ng/L)	7.6693	3.5701	46.55 %	0.2826
	U	0.1748	44.3717	5.8406	13%					

Supplementary Figures

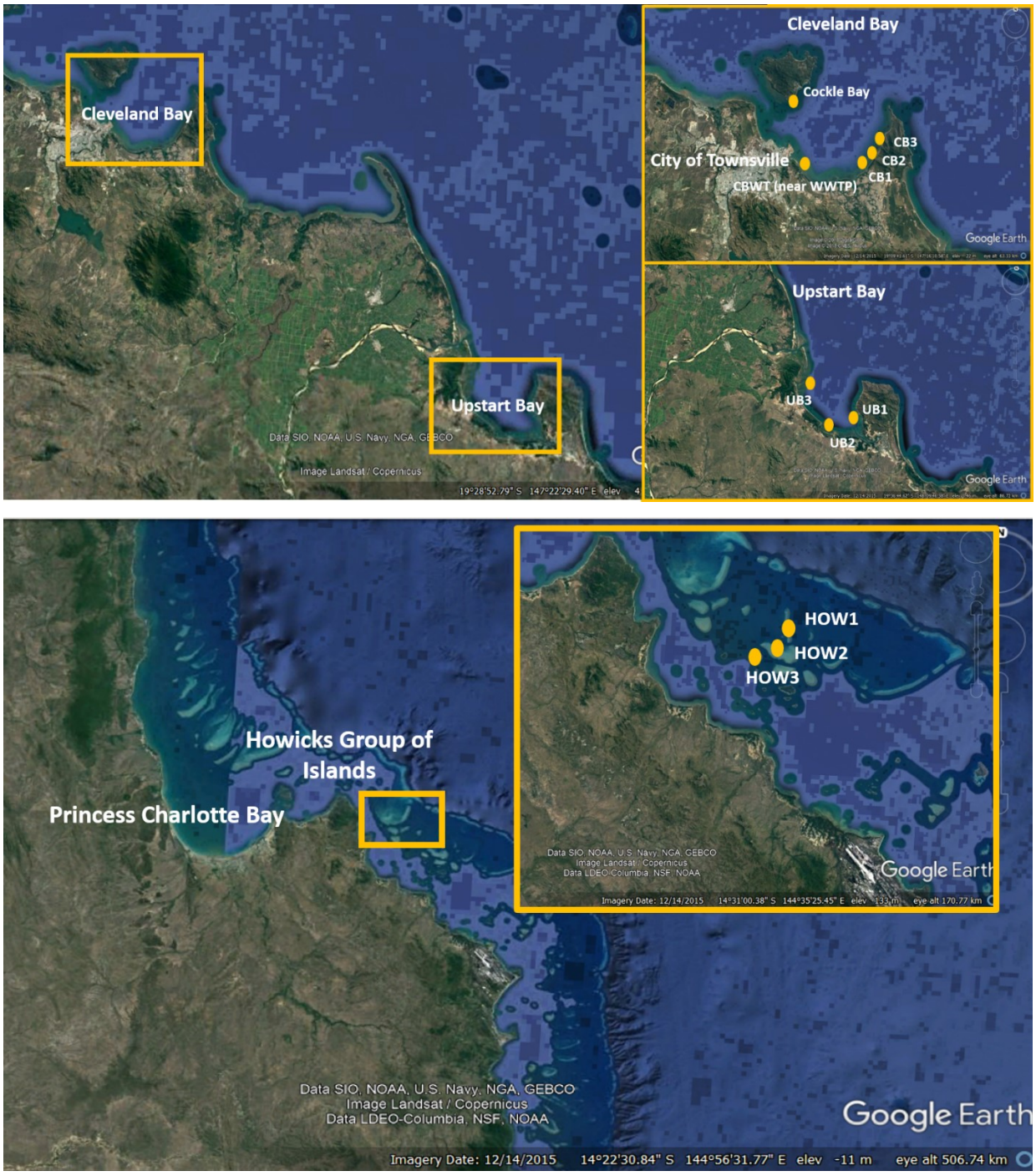

Figure S1 Map of sampling locations. Coastal locations (top) and control site (bottom)

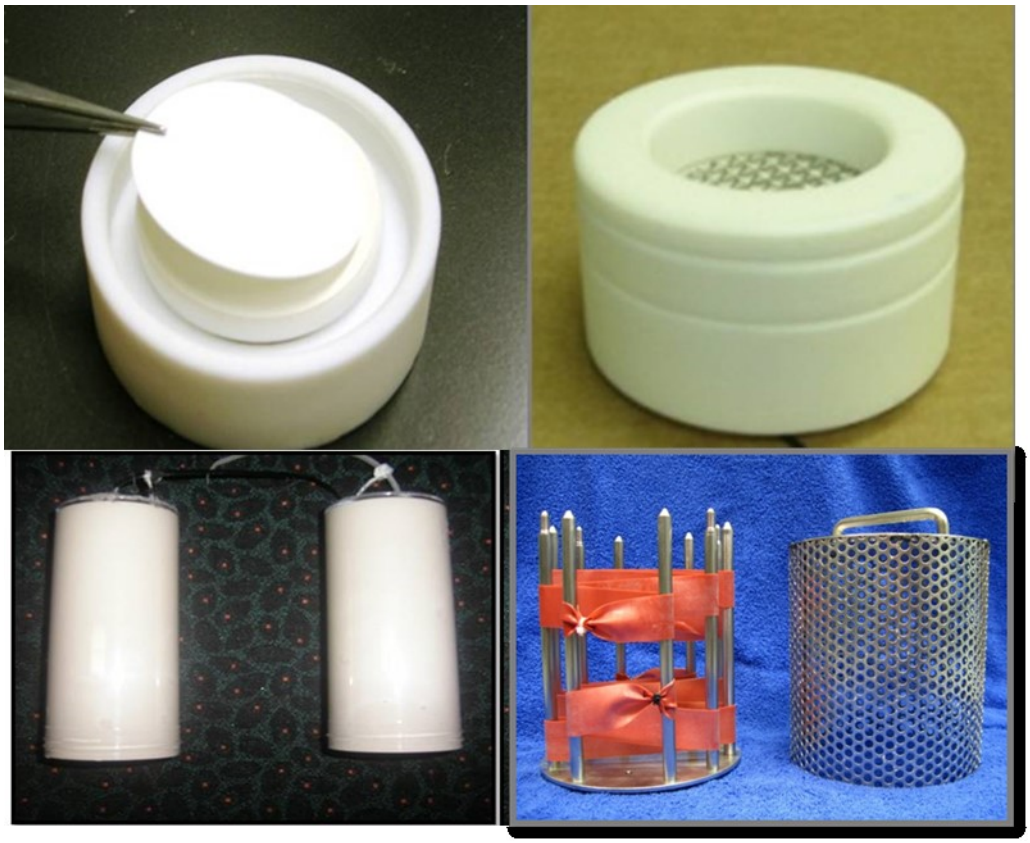

Figure S2 Assembly of Empore Disks (EDs) into Chemcatcher passive sampler housing (top left and right), Passive Flow monitors (PFMs) co-deployed with passive samplers (bottom left) and PDMS passive sampling strips loaded into marine cages (bottom right)

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