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Pollution signature for temperate reef biodiversity is short and simple

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ABSTRACT

Pollution increasingly impacts healthy functioning of marine ecosystems globally. Here we quantify concentrations of major pollutant types (heavy metals/sewage/petrochemicals/plastics) as accumulated within marine sediments on and/or immediately adjacent to shallow reefs for 42 sites spanning coastal population centres across south-eastern Australia. Gradients in pollutants were revealed, but few pollutants co-varied, while increasing wave exposure ostensibly diluted concentrations of all pollutants except microplastics. Examination of reef biodiversity indicators revealed that maximum size of fauna and flora, a key life-history parameter summarised by the *Community shortness index*, plus declining functional and species richness, were the most sensitive bioindicators of pollutants – for which heavy metals and nutrient-enrichment were most pervasive. Results indicate that assemblages of biogenic habitat formers and associated fauna collapse from “long and complicated” to “short and simplified” configurations in response to increasing pollution, and this community signature may form an effective bioindicator to track human-driven degradation.

1. Introduction

The legacy of unregulated historical pollution in combination with accelerating production of human waste and synthetic compounds poses an accumulating threat to the health of ecosystems globally (Islam and Tanaka, 2004; Vörösmarty et al., 2010; Halpern et al., 2008, 2009; Crain et al., 2009). In developing nations, unregulated pollution remains a contemporary issue, while for developed countries legacies remain (e.g. Knott et al., 2009) and/or new regulatory needs are evolving in response to increasing pollution threats and/or “viral” shifts in social-consciousness (e.g. cosmetic micro-bead plastics). Representing ultimate sinks for pollution, contemporary pollutants are continually superimposed upon past legacies in the marine environment (e.g. Halpern et al., 2009; Crain et al., 2009). However, marine pollution is generally “out-of-sight, out-of-mind” compared to pollution in either terrestrial or freshwater environments.

The general lack of visibility of marine pollution in subtidal marine environments means that mitigation for solely aesthetic reasons is seemingly unlikely. Furthermore, pollution concerns appear more likely to escalate if toxins become evident within seafood or occur at popular swimming destinations, or become highly visible via interactions (distressing/smothering/entanglement/ingestion) involving charismatic marine megafauna such as seabirds, turtles, seals or whales (e.g. Page et al., 2004; Wilcox et al., 2015). Beyond these highly visible and

confronting signs, the broader impacts of marine pollution on non-seafood species, less charismatic fauna or the dynamics of marine populations, communities and broader functioning of marine ecosystems is comparatively less understood (Chapman, 2002; but see Peterson et al., 2003; Lotze et al., 2011).

Shallow reef communities are among the most visible of all subtidal marine ecosystems and also harbour the greatest concentrations of biodiversity in the ocean (Roberts et al., 2002). Furthermore, reefs in estuaries and embayments have been suggested to be amongst the habitats of greatest value to society (Costanza et al., 1997; Bennett et al., 2016). The flora and fauna on subtidal reefs are readily assessable using visual underwater census techniques in less turbid areas, and are frequently surveyed by scientific and/or citizen science divers, with sometimes longer-term > 10 year time series for particular reefs (Babcock et al., 2010; Edgar and Stuart-Smith, 2014). Particular components of subtidal reef communities are known to exhibit variable sensitivity to human impacts, with specific functional components of the reef community, beyond individual species (e.g. Stuart-Smith et al., 2013), also appearing susceptible to pollution (e.g. McLean et al., 1991; Costanzo et al., 2001; Gaston and Suthers, 2004; Airoldi et al., 2008; Lotze et al., 2011; Strain et al., 2014; Oh et al., 2015; Stuart-Smith et al., 2015, 2017).

While environmental monitoring of pollution typically focusses on the presence of chemical compounds and laboratory based toxicology

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studies, longer term population and ecologically relevant effects are less well understood (e.g. Lam and Gray, 2001; Chapman, 2002). Although management of pollution is guided by knowing levels of pollutants in the system, a seemingly powerful driver of change in management practices and public behaviour is the visible impact of pollution on biodiversity. Such a capacity to track biological signatures of impact could be useful for environmental monitoring (e.g. State-of-the-Environment reporting) and ultimately mitigation (Stuart-Smith et al., 2017). The impact of pollution on subtidal reef communities in temperate Australia has been broadly assessed (e.g. Edgar and Barrett, 2000; Edgar et al., 2003; Stuart-Smith et al., 2015; Oh et al., 2015; Kriegisch et al., 2016). However, examination of structural changes of the entire reef ecosystem (including faunal and floral components) alongside assessments of gradients in specific pollutants, as measured directly at reef sites, has not previously been undertaken.

Here we provide new insights into community-level pollution responses, and thus useful bioindicators of pollution, by examining co-located data on fishes, invertebrates and macroalgae with measurements of a suite of heavy metal, sewage and plastic pollutants taken from the benthos adjacent to 42 reef monitoring sites spanning the capital cities and marine environmental gradients in south-eastern Australia. Our aim was to explore system wide changes in reef community structure across pollution gradients common among regions, and independent of biogeographical influences. We utilised a community-trait based approach, instead of focussing on species-specific patterns, to inform the applicability of general and visually-detectable signatures of pollution-associated change for reef communities.

2. Methods

2.1. Field sites

Sampling of pollutants and reef communities was undertaken in coastal estuaries and embayments influenced by the major urban populations of south-eastern Australia, including the four state capital cities: Sydney (New South Wales), Melbourne (Victoria), Adelaide (South Australia) and Hobart (Tasmania). These cities have major ports and industry, and regions of substantial heavy metal pollution as a legacy from both historical industrial pollution and contemporary, but ostensibly reduced, inputs of heavy metals, organic enrichment and other pollutants from storm water runoff and effluent discharges from sub-catchments dominated by urban and agricultural land use (Birch, 2000; Gorman et al., 2009; Knott et al., 2009; Townsend and Seen, 2012). In an attempt to achieve a gradient of pollution levels, additional sampling was undertaken at sites in less densely populated areas along adjacent coastlines, where lower levels of pollutants were expected. Study sites were required to have low turbidity (i.e. > 5 m visibility) to ensure surveys of reef life using visual census was effective (see below), thus sites high within estuaries were not sampled as part of this study.

2.2. Pollutants

Concentrations of heavy metals (Antimony, Arsenic, Cadmium, Chromium, Copper, Cobalt, Lead, Manganese, Nickel, Selenium, Silver, Vanadium, Zinc and Mercury), organic (total nitrogen, nitrogen $\delta^{15}\text{N}$ isotope ratio [hereafter termed $\delta^{15}\text{N}$], total organic content), petrochemicals, and plastic pollution were measured on and within sediments adjacent to 42 south-eastern Australian rocky reef sites (NSW, $n = 12$; SA, $n = 6$; Vic, $n = 8$; Tas, $n = 16$). Within each south-eastern Australian state, sites were distributed across contrasting polluted and relatively pristine sub-locations of Sydney Harbour, Jervis Bay and Eden in NSW; from adjacent to the city of Melbourne towards The Heads in Port Phillip Bay, Victoria; from Port Adelaide south along the Adelaide metropolitan coast in South Australia; and from the Derwent Estuary south to the D'Entrecasteaux Channel plus more pristine sites in eastern Tasmania (Fig. 1).

Study sites were spread as evenly as practically possible across pollution gradients with a minimum separation distance of 2 km within a region. Measurements of pollutants at each site involved sampling duplicate sub-sites spread 50 m apart, which were averaged for each site for all pollutants except for micro-plastics, for which extraction and enumeration was highly time consuming and thus only a single sample per site was processed within the time-frame of the study.

At each site, subtidal marine sediment was collected from depths of 5 to 13 m using a vessel-deployed Van Veen sediment grab (30 cm by 30 cm gape) during September to November 2015, with laboratory determination of pollutant levels occurring from Oct 2015 to Dec 2016. Labile pollutants (e.g. nutrients and petro-chemical compounds) were held on ice then frozen and assessed within 2 weeks of collection, while non-labile material such as micro-plastic concentrations were processed within 12 months of sample collection.

Heavy metal and organic pollution samples (i.e. total organic carbon) were analysed by ALS Environmental Pty Ltd. Australia following all standard operating procedures avoiding contamination, e.g. the use of sterile gloves (<http://www.alsenviro.com>; 277-289 Woodpark Rd., Smithfield, NSW, 2164). Heavy metal concentrations were analysed for both total metals in sediments extracted by ICP-AES (ALS method code: EG005-SD), plus the bio-active fraction extracted by weak acid 1 M HCl extractable Mercury by FIMS (ALS analysis code: EG035-SDH); notably the bio-active fraction represented 38% of the total metals extractable ($R^2 = 0.97$). Analysis of nitrogen and $\delta^{15}\text{N}$ enrichment, indicating urban sources of N (after Costanzo et al., 2001, 2005), was performed by Environmental Isotopes Pty Ltd., again following standard protocols (<http://www.isotopic.com.au/>). Micro-plastics were extracted from marine sediments using density separation by NaI and centrifuging with all plastics within the size range of 38 μm to 4 mm collected onto filter paper and enumerated under dissecting microscope (see Ling et al., 2017). Counts distinguished plastic particles from filaments such as polyesters shed from clothing made from synthetic fabrics (see detailed microplastic methodology in Ling et al., 2017). Exposure of samples to other sources of plastics was minimised and blanks run at increasing exposure times to potential sources of airborne microplastic filament contamination (i.e. 1, 3 and 6 h exposures) revealed a contamination rate of 1.02 filaments hr^{-1} ($n = 9$). This contamination rate was considered negligible as samples were exposed for < 30 min and an increase of a single filament per hour, represented only a 0.46% increase in the average microfilament count per sample.

In order to obtain signals of pollutants directly from reefs where fish, invertebrates and macroalgal data were collected, divers also sampled fine sediment layers trapped within algal turfs by suctioning with 50 ml syringes. Comparison of heavy metal pollution measurements for turf-trapped sediments on reefs and conventional Van Veen grabs of sediment from adjacent sandy/silty habitats (within 300 m of the reef site) were highly correlated (Pearson Correlation Coefficient of 0.77). Heavy metal concentration in the turf-sediment matrix on reefs, summed for all heavy metal types, was therefore used for statistical analyses as this was the most direct measure of conditions experienced by the reef community. By contrast, isotopic signals of organic pollutants, petro-chemical surrogates and micro-plastics required larger volumes of sediment than was readily obtainable from the reef surface, consequently soft-sediment habitats adjacent to reef sites were sampled by Van Veen grabs for these purposes. All pollutant/environmental data are available via: <http://metadata.imas.utas.edu.au/geonetwork/srv/en/metadata.show?uuid=11075fdf-e53e-4d8c-8999-0b239a742243>.

2.3. Reef communities

Reef fish and invertebrate abundances, and percent cover of biogenic habitat-forming species (e.g. macroalgae, sponges, bivalves), were sampled at all 42 south-eastern Australian sub-tidal reef sites adjacent to pollutant sampling sites, using underwater visual census.

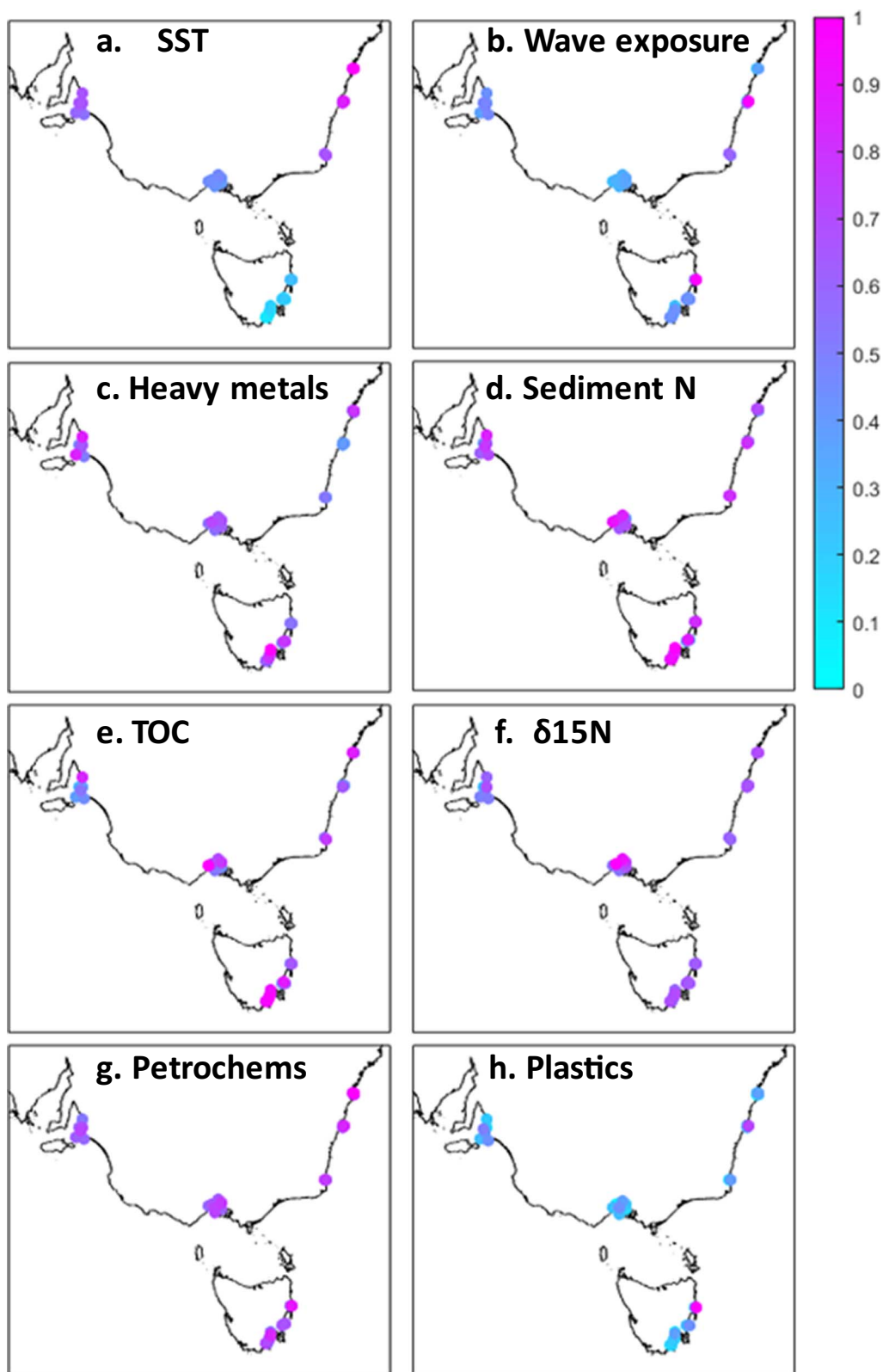


Fig. 1. Heat maps of non-pollutant variables: Sea Surface Temperature (a.) and Wave exposure (b); and pollutant variables: Heavy metals (c.), Sediment Nitrogen (d.), Total Organic Carbon (e.), $\delta^{15}N$ (f.), Petrochemicals (g.), Plastics (h.) included in multiple regression modelling of candidate biological indicators of pollution. For optimal display, all variables have been re-scaled from 0 to 1 with maximum values discernible for overlaid points.

Visual census is effective for water visibility > 5 m given that less than this reduces the effectiveness of underwater visual census of mobile fishes. Visual census data was obtained from the Long-term Marine Protected Area and Reef Life Survey (RLS) monitoring programs (Edgar

and Stuart-Smith, 2014; Stuart-Smith et al., 2015; Reef Life Survey, 2015) following Stuart-Smith et al. (2017) and <http://reeflifesurvey.com/>. Where particular reef sites were sampled on multiple occasions, data were standardised as mean densities per 50 m transect across all

Table 1

Pearson correlation coefficients between levels of different pollutant types and environmental variables, as measured for south-eastern Australian reef sites. Asterisks indicate correlated variables at coefficients > 0.65.

| | Metals | Petro-chemicals | d15N | TOC | Sed_N | Sed_P | Microplastics | Pop500 | PS.63 | Secchi | Turf_depth | SST | Exposure |
|-----------------|--------|-----------------|--------|--------|--------|--------|---------------|--------|--------|--------|------------|-------|----------|
| Metals | 1.000 | | | | | | | | | | | | |
| Petro_chemicals | -0.426 | 1.000 | | | | | | | | | | | |
| d15N | -0.087 | 0.147 | 1.000 | | | | | | | | | | |
| TOC | 0.259 | -0.311 | -0.001 | 1.000 | | | | | | | | | |
| Sed_N | 0.641 | -0.588 | -0.073 | 0.577 | 1.000 | | | | | | | | |
| Sed_P | 0.381 | -0.372 | -0.086 | 0.524 | 0.665 | 1.000 | | | | | | | |
| Microplastics | -0.176 | -0.093 | -0.218 | -0.347 | -0.126 | -0.361 | 1.000 | | | | | | |
| Pop500 | -0.252 | 0.446 | 0.368 | -0.120 | -0.371 | -0.426 | -0.032 | 1.000 | | | | | |
| PS.63 | 0.442 | -0.408 | 0.073 | 0.720* | 0.593 | 0.547 | -0.233 | -0.064 | 1.000 | | | | |
| Secchi | -0.335 | 0.085 | -0.047 | -0.224 | -0.172 | -0.054 | 0.274 | -0.032 | -0.312 | 1.000 | | | |
| Turf_depth | 0.107 | -0.057 | 0.682* | 0.019 | 0.074 | -0.014 | -0.154 | 0.460 | 0.265 | -0.322 | 1.000 | | |
| SST | -0.274 | 0.525 | -0.072 | -0.299 | -0.449 | -0.472 | 0.098 | 0.629 | -0.379 | -0.202 | 0.025 | 1.000 | |
| Exposure | -0.307 | 0.123 | -0.065 | -0.308 | -0.090 | -0.234 | 0.598 | -0.284 | -0.294 | 0.313 | -0.271 | 0.005 | 1.000 |

sampling occasions. Comparisons between aggregated and the most recent community-level data for each site revealed broad qualitative similarity, and many of the pollutants examined have a legacy of impact (e.g. Knott et al., 2009) regardless of potential reductions of inputs over recent years due to improved environmental management. Thus, including all available data for all sampling periods was considered appropriate, providing time-integrated measures of reef biodiversity.

The *maximum body size* (i.e. *Lmax* from www.fishbase.org for fishes, and Edgar (2001) for maximum sizes of invertebrates and sessile habitat-forming species; many also available at www.reeflifesurvey.com) was obtained for each species and used to calculate a *Community shortness index*. This was the mean of the maximum body sizes of all fishes, mobile invertebrate and biogenic habitat formers at each site, weighted by their abundance (fishes and mobile invertebrates) or percent cover (sessile species). Additional traits for reef fishes were examined comprising *Trophic level*, *Trophic breadth*, *Trophic group*, *Substrate type*, *Complexity*, *Time*, *Gregariousness*, and *Vulnerability* (see Stuart-Smith et al., 2013 for full derivations). For benthic biogenic habitats, additional to *maximum body size*, trait tables were constructed from available data and semi-quantitative scores for the traits: *Colonisation potential* (1/(months to dominance on open substratum)), *Storey* (encrusting (1), prostrate (2), understory (3), overstorey (4), canopy (5)), *Structural complexity* (simple (1), moderate (2), complex (3)), *Structural flexibility* (low (1), medium (2), high (3)), *Trophic level* (autotrophic (1), heterotrophic (2), auto/heterotrophic (3)), *Palatability* (unpalatable (1), palatable (2), highly palatable (3)), see Appendix I. Functional traits were examined singularly and as community-weighted means (by abundance, biomass or percent cover), and also combined to calculate functional diversity (see below for calculations, following Stuart-Smith et al., 2013).

2.4. Environmental covariates

Environmental covariates measured at each site included sea surface temperature (long-term mean annual SST values from 2002 to 2009 from the Bio-ORACLE data set; Tyberghein et al., 2012), wave exposure (after Hill et al., 2010; which estimated proximal distance to land masses and thus a proxy of wave exposure based on wind fetch), and the sediment size composition for each sediment sample by determining the volume of sediment within each of the sieve size categories of 4, 2, 1, 0.5, 0.25, 0.125, < 0.063 mm; with the finest particle size-class, i.e. < 0.063 mm, used as a covariate for further analysis. Turbidity was also measured using Secchi disc and sediment accumulation on the reef surface was measured in terms of percentage cover (Reef Life Survey methodology, see above) and depth (measured at 5 positions defined as

a quincunx within a 0.25 m⁻² area at each reef station sampled for pollutants). In defining the local human environment, a human-population index was derived for each sampling site based on an attenuation from major population centres (described in Stuart-Smith et al., 2015, and derived from the glp00g gridded world population density dataset grid size of ~1 km; available at: <http://sedac.ciesin.columbia.edu/data/collection/gpw-v3/sets/browse>).

2.5. Statistical analyses

Reef community metrics were examined across pollutant gradients using multiple linear regression to identify the most informative biological indicators of pollution. The list of candidate indicators included abundance (N) and the diversity indices of species richness (total species S), Shannon diversity (*H'*, i.e. Log base e), and number of effective species (*N1*, i.e. exp. (*H'*)) for reef community components and whole communities (inclusive of fish, invertebrate and sessile biogenic habitat components; see Appendix II for full list of candidate community metrics investigated). Diversity indices were generated using the “Diverse” suite available in *Primer V6.0*. Functional diversity of fishes, invertebrates, and benthic habitats was examined using the FD package available in R (R Core Team, 2015), see <https://cran.r-project.org/web/packages/FD/FD.pdf>.

Initially, the relationships between each candidate bioindicator and the different pollutant types were examined by cross-correlation, with exclusion of highly-correlated pollutant/environmental variables (i.e. $R^2 > 0.65$ across the 42 sites; Table 1) from subsequent analyses, whereby only the most informative or cost effective variables were retained in the model. Sediment nitrogen and sediment phosphorous were correlated, therefore only sediment nitrogen, which is often reported to impact marine communities, was retained in models. The volume of particle sizes < 0.063 mm was correlated with TOC, and so particle size was dropped but TOC, which can be directly influenced by sewage pollution, was retained; human population index was also excluded as it was correlated with sea surface temperature (Table 1). Due to known relationships of fishes, invertebrates and habitat types with sea temperature at large scales, and with wave exposure at local scales (see Stuart-Smith et al., 2015), both average sea surface temperature and wave exposure (derived via wave fetch modelling, after Hill et al., 2010) were initially included in multiple regression models (see Fig. 1a & b). Sea surface temperature was strongly correlated with “region” in the full analysis, so region was excluded from the full model, as sea temperature describes important additional variation relevant to ecological and biogeographic patterns. To consider the effect of region, however, model results were subsequently explored for consistency

Table 2

Model results and ranking of pollution indicators for rocky reef communities as calculated from RLS and LTMPA surveys spanning 42 sites in temperate south-eastern Australia where pollutant levels were co-measured. (a.) Pollutant indicators are listed by contribution to parsimonious model fit and consistency in regional trends across NSW, Vic, SA and Tasmania; note that a total of 42 candidate biological indicators were regressed against measured pollutant levels (see Appendix 1 for full results and data transformation used). Final rank is shown for the top ten pollution indicators that demonstrated greatest R^2 contribution to model fit plus also showed consistent trends across the 4 south-eastern Australian locations NSW, Vic, SA, Tas. (b.) Top-ranked indicators for the pollutant variables of $\delta 15N$, TOC and Petrochemicals (which ranked well outside the top-10) explained low variability in reef community patterns relative to heavy metals and/or nitrogen. All diversity/functional diversity values were calculated as community-weighted means, with the mean index value of members of the community weighted by abundance ('B' indicates biomass weighting instead of abundance). Note the saturated model includes the independent variables SST, Exposure, Metals, $\delta 15N$, TOC, Sed_N, Petrochemicals, Microplastics; with parsimonious terms identified using "backward" stepwise regression. Further note that pollutant types showed little inter correlation (max. Pearson correlation coefficients < 0.65 or > -0.65; see Table 1). All primary pollutants had negative effects on the biological indicator of interest, except where highlighted with an asterisk, in which case the primary pollutant had a positive effect on the indicator.

| Indicator | Saturated model fit R^2 | Saturated model P | Parsimonious model terms (<i>italics</i> = negative; normal font = positive; bold = significant effect) | Parsimonious model fit R^2 | Parsimonious model P | Primary pollutant | Primary pollutant R^2 contribution | Regional consistency in primary pollutant trends | Rank |
|---|---------------------------|---------------------|--|------------------------------|------------------------|-------------------|--------------------------------------|--|------|
| Habitat functional richness | 0.37 | 0.035 | SST + Sed_N | 0.29 | 0.001 | Sed_N | 0.26 | Yes (4/4) | 1 |
| Turf cover | 0.53 | 0.001 | SST + Exposure + $\delta 15N$ + Sed_N + Microplastics | 0.49 | 0.000 | Sed_N* | 0.26 | No (3/4) | |
| Canopy seaweed cover | 0.37 | 0.037 | SST + Exposure + Sed_N | 0.34 | 0.001 | Sed_N | 0.25 | No (3/4) | |
| Habitat richness | 0.50 | 0.002 | SST + Metals + $\delta 15N$ | 0.44 | 0.000 | Metals | 0.24 | No (2/4) | |
| Community shortness index (1/summed sqrt Lmax) ^a | 0.39 | 0.022 | Exposure + Metals | 0.33 | 0.000 | Metals* | 0.23 | Yes (4/4) | 2 |
| Fish functional richness | 0.29 | 0.155 | Metals | 0.22 | 0.002 | Metals | 0.22 | No (3/4) | |
| Fish length | 0.61 | 0.000 | SST + Exposure + TOC + Sed_N | 0.58 | 0.000 | Sed_N | 0.21 | No (3/4) | |
| Invertebrate Lmax | 0.25 | 0.240 | Metals | 0.20 | 0.003 | Metals | 0.20 | Yes (4/4) | 3 |
| Invertebrate diversity | 0.40 | 0.020 | SST + Exposure + $\delta 15N$ + Sed_N | 0.33 | 0.004 | Sed_N | 0.17 | Yes (4/4) | 4 |
| H ⁺ | | | Exposure + Metals | 0.31 | 0.001 | Metals | 0.16 | Yes (4/4) | 5 |
| Whole community richness | 0.36 | 0.040 | Exposure + Metals | 0.36 | 0.001 | Metals | 0.15 | Yes (4/4) | 6 |
| Invertebrate & fish Lmax | 0.37 | 0.035 | SST + Exposure + Metals | 0.47 | 0.000 | Sed_N | 0.14 | No (3/4) | |
| Fish biomass | 0.47 | 0.004 | Exposure + $\delta 15N$ + Sed_N + Microplastics | 0.24 | 0.014 | Sed_N | 0.14 | Yes (4/4) | 7 |
| Invert. & cryp. fish diversity H ⁺ | 0.35 | 0.055 | Exposure + Metals + Sed_N | 0.32 | 0.014 | Sed_N | 0.13 | Yes (4/4) | 8 |
| Invert. & cryp. fish no. effective species | 0.36 | 0.041 | SST + Exposure + Metals + $\delta 15N$ + Sed_N | 0.52 | 0.000 | Sed_N | 0.13 | No (2/4) | |
| Fucoid seaweed cover | 0.52 | 0.001 | SST + Exposure + $\delta 15N$ + Sed_N | 0.39 | 0.000 | Metals | 0.13 | Yes (4/4) | 9 |
| Invertebrate richness | 0.42 | 0.011 | SST + Exposure + Metals | 0.24 | 0.005 | Metals | 0.13 | Yes (4/4) | 10 |
| Laminarian kelp cover | 0.25 | 0.231 | Metals + TOC | 0.43 | 0.002 | $\delta 15N$ * | 0.09 | No (3/4) | |
| Invertebrate & cryptic fish abundance | 0.43 | 0.010 | SST + Metals + $\delta 15N$ + Sed_N + Petrochem + Microplastics | 0.38 | 0.000 | TOC* | 0.09 | No (2/4) | |
| Fish trophic level (B) | 0.42 | 0.011 | SST + Exposure + TOC | 0.41 | 0.000 | Petrochem* | 0.05 | Yes (4/4) | |
| Fish trophic level | 0.47 | 0.003 | SST + Exposure + Metals + Petrochem | 0.20 | 0.012 | Micro-plastics | 0.05 | No (3/4) | |
| Large fish index (30 cm) | 0.28 | 0.165 | Exposure + Microplastics | | | | | | |

^a (1/(Community L_{max})) where Community L_{max} = fish L_{max} + Invertebrate L_{max} + biogenic habitat L_{max}.

across regions, with regionally consistent models indicating general patterns of response at the scale of southeastern Australia. Models assessing candidate bioindicators of pollutants were of the following general form:

Candidate bioindicator
 $(y_i) = \beta_0 + \beta_1 \times \text{SST} + \beta_2 \times \text{Exposure} + \beta_3 \times \text{Metals} + \beta_4 \times \delta^{15}\text{N} + \beta_5 \times \text{TOC} + \beta_6 \times \text{Sed}_N + \beta_7 \times \text{Petrochemicals} + \beta_8 \times \text{Microplastics} + \epsilon_i$ where y_i is a reef community metric (e.g. fish abundance, invertebrate richness, habitat diversity etc), β_0 is a vector of the predictor variable, SST is Sea Surface Temperature in °C (which can account for large proportions of variability in faunal biodiversity patterns and was thus included first, e.g. Stuart-Smith et al., 2015), Exposure is wave exposure (after Hill et al., 2010; again a key environmental variable known to account for reef biodiversity patterns, Stuart-Smith et al., 2015); Metals is total heavy metals (inclusive of those stated above which were aggregated due to high correlation between some metals and to also provide generality and to reduce the number of independent variables to be estimated in model), $\delta^{15}\text{N}$ is 15 Nitrogen (an isotopic form of Nitrogen enriched by anthropogenic N synthesis), Sed_N is total sediment nitrogen (inclusive of natural and anthropogenic sources), TOC is Total Organic Carbon (inclusive of natural and anthropogenic sources); Petrochemicals is the concentration of petro-chemical surrogates of ethylene dichloride, Toluene-d8, 4-Bromofluorobenzene; Microplastics is total micro-plastic concentration in marine sediment immediately adjacent to reef, and ϵ_i is the error term. For spatial maps of modelled non-pollutant and pollutant variables across the south-eastern Australian sampling sites see Fig. 1.

Sampling over such a large scale inevitably required logistical tradeoffs between level of replication (i.e. number of sites surveyed) and degree of clumping of sites, thus was not ideal from a statistical perspective (Oksanen, 2001). Our design focused on maximising the extent of replication within the four locations – the capital city estuaries – that were identified a priori to possess the strongest pollution gradients within the study region (and also conditions amenable to underwater visual survey techniques). Consequently, data are partially nested, with multiple sites concentrated approximately equidistantly along the four major estuaries. Dealing with associated autocorrelation requires a tradeoff between the probability of Type I error (i.e. a relationship is erroneously identified) and Type II error (i.e. a real relationship is overlooked) (Diniz-Filho et al., 2003).

As detailed above, we initially include region (i.e. Australian state) in the model; however, this factor was highly correlated with SST, consequently only the latter was used. SST contributes as a regional blocking factor in models (i.e. if strong regional patterns exist, then this will be expressed as a strong influence of SST). Given that our study represents the first major analysis across this region and is largely exploratory, we focused on minimising Type II error at the cost of elevated probability of Type I error by using a statistical design with sites considered randomly distributed. We then assessed consistency in identified relationships for the four regions. Despite not explicitly addressing spatial autocorrelation in our model, we consider that the likelihood of Type I error is low given that probability values associated with full model fit were generally very low (all but three < 0.005; maximum 0.014), and because of the secondary test of consistency between regions. Moreover, other than SST, environmental and pollutant metrics were well interspersed across the full survey domain (Fig. 1).

All correlation and regression analyses were undertaken using R (R Development Core Team 2015) in RStudio (RStudio Team 2015); specifically, the R package “Relaimpo” (<https://cran.r-project.org/web/packages/relaimpo/relaimpo.pdf>) was used to partition the contribution of each predictor variable to the overall R^2 of each model fit. Furthermore, the stepwise statistical regression routine was subsequently used to identify predictor variables defining the most parsimonious models, with Relaimpo then used to examine the contribution of predictor variables to the most parsimonious models.

3. Results

3.1. Variable pollutant trends across urban to pristine sites

Variable gradients in seafloor pollutant concentrations were revealed from urban reef sites to those in less densely populated areas of south-eastern Australia (Fig. 1b). While gradients in heavy metal concentrations broadly followed expectations of high levels at sites experiencing the legacy of industrial outfalls (exceeding acceptable levels in many cases, i.e. trigger values of ANZECC, 2000; see downloadable pollution data set above), and lower levels for more remote areas, different pollutant types only weakly co-varied. That is, unexpectedly, no two pollutants revealed correlation coefficients > 0.65, other than nitrogen and phosphorous (Table 1).

3.2. Relative impact of pollutants on reef communities

Analysis of the suite of candidate bioindicators for impacts of pollution on south-east Australian rocky reefs revealed heavy metals to have the greatest and most consistent community-level impacts relative to all other pollutants, with heavy metals accounting for 6 of the top 10 best fit, parsimonious and regionally consistent multiple regression models (Table 2a). Overall, potential bioindicators were negatively associated with increasing heavy metal concentrations, with heavy metals implicated as having deleterious biological effects in 21 of 26 parsimonious models (Appendix II).

Following heavy metals, the pollutant next most associated with negative community-level changes was total nitrogen, which contributed the remainder of the top 10 models. Across all multiple regression models, nitrogen was negatively associated with 12 potential indicators (Appendix II). The importance of pollutant variables $\delta^{15}\text{N}$, microplastics, petrochem surrogates, and TOC decreased relative to heavy metals and total nitrogen. These apparently less influential pollutants appeared to have relatively weak effects, each adding only small apparent contributions to the variability of candidate bioindicator models. Additionally, at their current concentrations, these less deleterious pollutants had as many positive effects as negative effects on indicators across the different locations (Table 2b; see also Appendix II).

Summed across all indicators, the non-pollutant variable of wave exposure showed greatest importance to multiple regression models by contributing to 29 of 42 parsimonious models, while sea surface temperature contributed to 23 of 42 models (Appendix II). Intuitively countering the effects of pollution, wave exposure had an apparent ameliorating effect on 25 of 29 candidate bioindicators (Appendix II).

3.3. Top-ranked community-level pollutant indicators

The top-ranked indicator of any pollutant type was the community-weighted metric *Habitat functional richness* (i.e. the richness of functional traits present among biogenic habitat-forming species weighted by percent cover contributions at the site-level), which best predicted increasing nitrogen concentration (Table 2a; Fig. 2a.i). Decline in *Habitat functional richness* with increasing nitrogen concentrations was consistent across reefs for all south-east Australian regions, i.e. this response demonstrated regional consistency (Fig. 2b.i).

The best-performing indicator of heavy metal pollution, and second ranked indicator of any pollutant overall, was the *Community shortness index*, whereby reef communities became progressively dominated by shorter mobile and sessile species as heavy metal concentrations increased (Table 2a; Fig. 2a.ii). The reduction of community body/colony size with increasing heavy metal concentrations was regionally consistent across reefs for all south-east Australian states (Fig. 2b.ii).

The third ranked community-level indicator of pollution was *Invertebrate diversity* (i.e. the Shannon diversity of mobile reef invertebrates, H'), which declined and was most sensitive to increasing nitrogen concentration present on the benthos (Table 2a; Fig. 2a.iii).

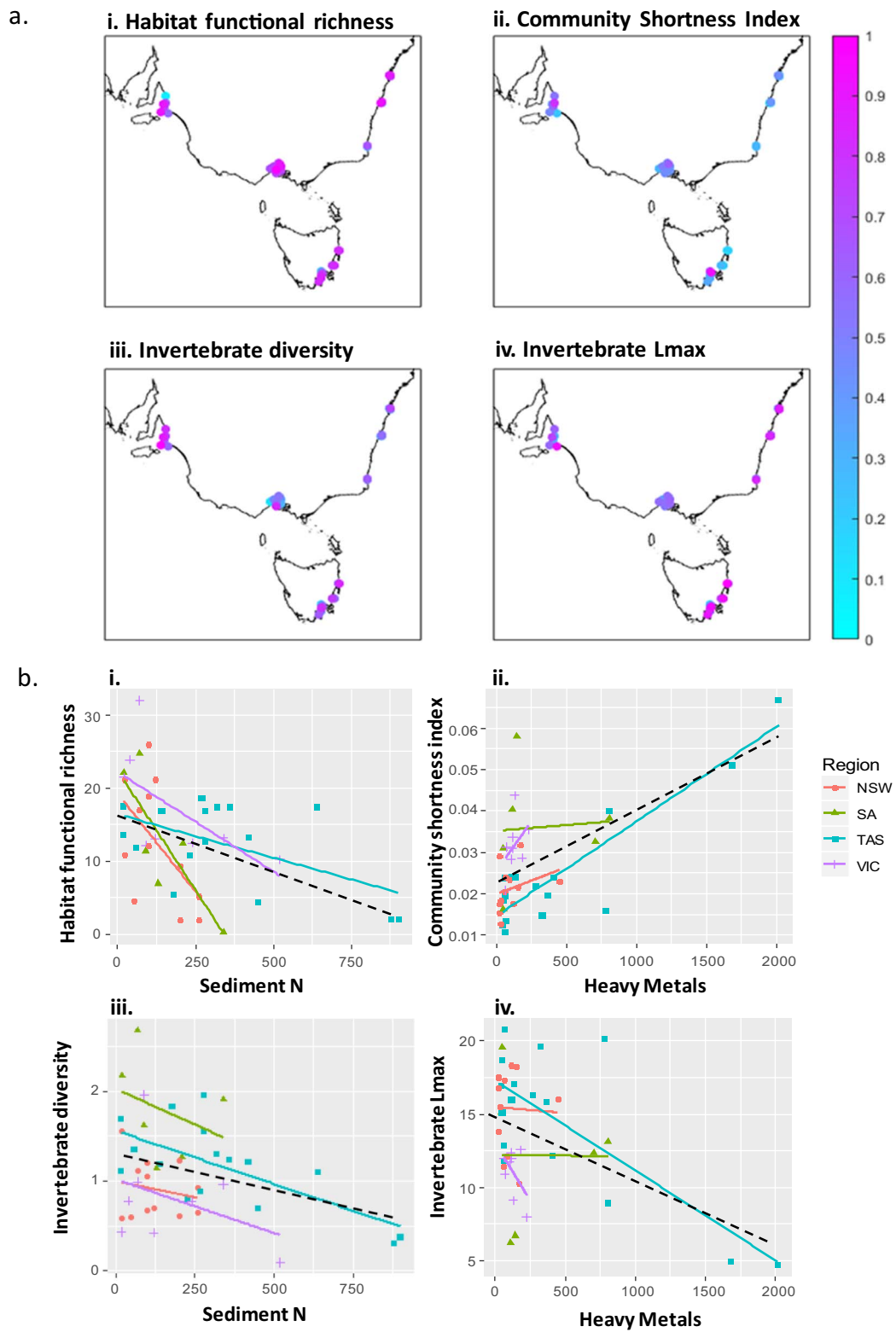


Fig. 2. (a) Spatial maps of best performing pollution indicators: i. Benthic habitat functional richness; ii. Community shortness index; iii. Invertebrate diversity; iv. Invertebrate Lmax; all re-scaled from 0 to 1 with maximum values discernible for overlaid points. (b) Trends in pollution indicators across increasing pollutant concentrations (mg/kg) for south-eastern Australia overall (dashed line) and by location (coloured lines, as per legend), i-iv as per (a). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Decline in *Invertebrate diversity* with increasing nitrogen concentration was regionally consistent across south-eastern Australia (Fig. 2b.iii).

The fourth ranked (and regionally consistent) community-level indicator of pollution was *Invertebrate max size* (i.e. the community-weighted mean of the maximum of each species of mobile reef invertebrate), which declined and was most sensitive to increasing heavy metal concentrations (Table 2a; Fig. 2a&b.iv). Other top-ten ranked community indicators of pollution were *Whole community richness* (5th), *Invertebrate & fish max size* (6th), *Invertebrate & cryptic fish diversity H* (7th) and *Invertebrate & cryptic fish no. effective species* (8th), *Invertebrate richness* (9th), and *Laminarian kelp cover* (10th) (Table 2a).

4. Discussion

4.1. Variable gradients in pollutant concentrations across regions

Here our results show distinct pollutant-specific trends, as opposed to consistent generalizable (overall) industrial-to-open coasts pollution gradients. Specific patterns were observed in relation to heavy metal, sewage and plastic pollution at 42 reef monitoring sites spanning the major cities and marine environmental gradients in south-eastern Australia. Our sampling clearly revealed that the distributions of the intensity of the six major pollutant types (i.e. heavy metals, nitrogen, $\delta^{15}\text{N}$, Total Organic Carbon, petrochemical surrogates, and microplastics) were not highly correlated across the 42 study sites (Table 1). Only heavy metals and nitrogen, and nitrogen and total organic carbon, revealed correlation coefficients above 0.5, while petrochemicals displayed negative but again weak correlation with heavy metals and nitrogen (Table 1). Thus, this result identifies pollutants as originating from different local sources and/or have different mechanisms of retention on the benthos (see also Ling et al., 2017), and are likely to differ in the distribution of impacts.

4.2. Pollutant-specific responses of reef communities

Using a traits-based approach, our examination of system wide changes in reef ecological communities across pollution gradients revealed that no single community-level indicator showed a general response to pollution. Rather, indicators correlating most strongly with pollution were also specific to particular pollutant types. Heavy metal pollution was associated with the most pervasive changes to reef community structure (see also Stuart-Smith et al., 2015). The benthic concentration of nitrogen, which is derived from both natural and anthropogenic sources (e.g. Costanzo et al., 2001, Costanzo et al., 2005; Gorman et al., 2009), was associated with the next clearest overall responses. The anthropogenic contributions to total N, as estimated by the concentrations of isotopic ratio of N15 to N14 were, however, less strongly associated with declines in benthic diversity than natural variability in nitrogen (Table 2).

Greater wave exposure apparently reduced of the levels and impacts of pollution observed on reef communities (a significant contributor for 25 of 29 candidate bioindicators), consistent with flushing of pollutants at reef sites through increased water movement and greater exposure to wind generated waves. Wave exposure increases with distance from sheltered harbours, where flushing times are higher and where pollutants (e.g. heavy metals) are dispersed and occur at lower concentrations on the benthos (Fig. 1). However, this trend was not found to be generalizable for all pollutants, in particular microplastics. Differences in the interaction between hydrological/sediment-matrix properties and water motion appears important for delivery/deposition/retention of particular pollutants on the benthos, but high concentrations of microplastics on the benthos were present at even low human density locations (see also Ling et al., 2017).

4.3. Best performing bioindicators of pollution

The strongest broad-scale indicators for the impacts of different types of pollution on reef biodiversity appeared to be those associated with species

composition shift towards an assemblages dominated by smaller species and a reduction in functional richness of biogenic habitats, which best predicted increasing heavy metal and nitrogen levels, respectively. Indeed, 6 of the top 10 indicators were related to functional or species richness and 3 of the top 10 related to species' maximum sizes (Table 2). This result is consistent with pollution driven reductions in large habitat formers/benthic diversity (Airoldi et al., 2008; Johnston and Roberts, 2009; Clark et al., 2015) and reduction in species diversity for fishes (McKinley and Johnston, 2010) and for seagrass beds (Deegan et al., 2002). Our results support previous findings that "general" increases in pollution, where heavy metals and nutrient enrichment form important components of this, lead to "short and simplified" communities, whereby constituent species are fewer and dominated by r-strategists that do not reach large body sizes (e.g. "flattening" reviewed by Airoldi et al., 2008). By including biogenic habitat types in this study (i.e. contained within the *Community shortness index* and *Habitat functional richness*), we have further shown this trend of shortening and simplifying to extend through the entire reef ecosystem, from important benthic habitat formers such as kelps to invertebrates and fishes.

Relatively pristine reefs, on the other hand, are expected to be "long and complicated", containing more fish, invertebrate and biogenic habitat forming species that grow to larger sizes (out of the species pool present in a given biogeographical region). Examples of this situation include diverse and tall kelp forests or diverse and structurally complex coral reefs dominated by massive, plate and branching corals. Notably, once established, the trophic functioning of such "long and complicated" reefs can also feedback to maintain long and diverse communities. For example, large predatory-capable lobsters (Ling and Johnson, 2012; Redd et al., 2014; Ling et al., 2015) and fishes (Shears and Babcock, 2002) are capable of exerting top-down control of barrens-forming sea urchins, promoting resilience of tall and diverse kelp bed communities (Johnson et al., 2013).

4.4. Tracking reef bioindicators of pollution

Management of pollution impacts at the local scale ideally involves tracking a suite of reef community indicators through time, with more detailed investigation of any major changes in indicators to identify causal mechanisms. Attribution of cause is most likely when baselines exist prior to impact, and external reef communities, not incurring the same pressure, remain relatively unchanged and act as effective control sites, thereby achieving a Before-After-Control-Impact design (e.g. Roberts et al., 2007; Barrett et al., 2009). Where BACI assessments of pollution events are not available, as is generally the case, detection of change in characteristic bioindicators from monitoring data should be used to inform possible impacts rather than providing a basis for definitive cause and effect. For example, a shortening of the reef community could also occur due to size-selective harvest of larger organisms from the reef community, whereby larger longer-lived species also locally decline in abundance due to increased fishing (e.g. Barrett et al., 2009, as opposed to pollutant induced mortality, emigration or habitat-related change). Notably, fishing can also lead to impoverishment of fish communities (e.g. Worm et al., 2005) and decreases in *Lmax* (e.g. Shin et al., 2005). Therefore, while community shortening through time may suggest "specificity" of a particular pressure, further contextual information of all potential pressures acting or absent from particular reefs would be required to clearly identify the likely driver of change and possible thresholds involved (e.g. Kriegisch et al., 2016). Furthermore, routine analysis of trends in a suite of indicators is also likely to be much more informative than examining one indicator in isolation (Stuart-Smith et al., 2017), which may manifest in a similar or subtly different manner to other properties of the community.

5. Conclusion

Sampling of marine pollutants and associated reef communities across pollution gradients spanning major cities and comparatively pristine coasts in southeastern Australia revealed shifts in reef

community structure associated with increasing pollutant concentrations. Our analysis further indicated that issues of marine pollution should be addressed on a pollutant-by-pollutant basis, as few pollutants covaried and community-level responses were pollutant-specific. Increasing heavy metal and nitrogen concentrations were found to generate the strongest community impacts, as indicated by decreasing size of the reef community and declining functional diversity of the benthos. Thus, while marine pollution impacts largely occur out-of-sight, the “impoverished” and “short” community signatures of pollution are visually detectable for reef ecosystems. Regular census programs aimed at detecting community-level change on reefs through time should provide a sentinel of pollution-driven degradation, allowing the identification of point source pollution before impacts spread across larger spatial scales.

Author contributions

S. D. Ling, A. Davey, S. E. Gaylard, P.L. Davies & G. J. Edgar designed sampling; S. D. Ling, A. Davey and S. E. Reeves sampled pollutants; G. J. Edgar, R. D. Stuart-Smith, S. D. Ling, S. E. Reeves and others performed reef life sampling; S. D. Ling, R. D. Stuart-Smith, G. J. Edgar wrote the draft

manuscript; all authors contributed to revised manuscript. G. J. Edgar proposed and was awarded with funding for the research.

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Appendix A

Appendix I

Temperate reef biogenic habitat traits used to examine benthic functional diversity metrics. Trait tables were constructed from available data (*after Edgar, 2001*) and semi-quantitative scores for the traits: *Colonisation potential* (1/(months to dominance on open substratum)); *Storey* (encrusting (1), prostrate (2), understorey (3), overstorey (4), canopy (5)); *Structural complexity* (simple (1), moderate (2), complex (3)); *Height max.* (m); *Structural flexibility* (low (1), medium (2), high (3)); *Trophic level* (autotrophic (1), heterotrophic (2), auto/heterotrophic (3)); *Palatability* (unpalatable (1), palatable (2), highly palatable (3)).

| Biogenic habitat | Colonisation potential | Storey | Structural complexity | Height max. (m) | Structural flexibility | Trophic level | Palatability |
|--|------------------------|--------|-----------------------|-----------------|------------------------|---------------|--------------|
| Anemones & Zoanthids | 0.08 | 3 | 1 | 0.05000 | 2 | 2 | 1 |
| Ascidians | 0.17 | 3 | 1 | 0.25000 | 2 | 2 | 2 |
| Barnacles | 0.17 | 3 | 1 | 0.08000 | 1 | 2 | 1 |
| Small to medium foliose brown algae | 0.06 | 3 | 3 | 0.20000 | 4 | 1 | 3 |
| Bryozoan | 0.17 | 1 | 2 | 0.30000 | 1 | 2 | 1 |
| Caulerpa | 0.17 | 3 | 3 | 0.65000 | 3 | 1 | 1 |
| Crustose coralline algae | 0.08 | 1 | 1 | 0.15000 | 1 | 1 | 2 |
| Encrusting corals | 0.04 | 1 | 1 | 0.30000 | 1 | 3 | 1 |
| Encrusting leathery algae | 0.08 | 1 | 1 | 0.20000 | 2 | 1 | 1 |
| Filamentous epiphytic algae | 4.00 | 1 | 1 | 0.05000 | 2 | 1 | 3 |
| Filamentous rock-attached algae | 4.00 | 2 | 1 | 0.02000 | 2 | 1 | 3 |
| Large brown furoid kelps | 0.06 | 5 | 3 | 2.00000 | 4 | 1 | 2 |
| Geniculate coralline algae | 0.08 | 2 | 1 | 0.05000 | 1 | 1 | 1 |
| Green calcified algae | 0.17 | 3 | 2 | 0.25000 | 2 | 1 | 1 |
| Foliose green algae | 0.33 | 3 | 3 | 0.50000 | 3 | 1 | 2 |
| Hydrocoral | 0.04 | 3 | 2 | 0.09000 | 2 | 2 | 1 |
| Hydroids | 0.04 | 3 | 2 | 0.10000 | 2 | 2 | 1 |
| Large brown Laminarian kelps (+ Durvillaea) | 0.06 | 5 | 3 | 2.00000 | 4 | 1 | 3 |
| Polychaete tubes | 0.08 | 3 | 1 | 0.10000 | 1 | 2 | 1 |
| Foliose red algae | 0.33 | 3 | 3 | 0.30000 | 2 | 1 | 2 |
| Seagrass | 0.17 | 4 | 2 | 0.45000 | 4 | 1 | 2 |
| Diatom/algae/cyanobacterial fuzz/slime on bare rock | 8.00 | 2 | 1 | 0.00005 | 2 | 1 | 3 |
| Sessile mollusc | 0.08 | 3 | 1 | 0.50000 | 1 | 2 | 3 |
| Soft corals and gorgonians | 0.10 | 3 | 2 | 1.50000 | 2 | 3 | 1 |
| Sponges (encrusting) | 0.04 | 3 | 1 | 0.05000 | 1 | 2 | 1 |
| Sponges (erect) | 0.01 | 3 | 2 | 1.00000 | 2 | 2 | 1 |
| Turfing algae (< 2 cm high algal/sediment mat on rock) | 4.00 | 2 | 1 | 0.02000 | 2 | 1 | 3 |

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