# Ubiquity of microplastics in coastal seafloor sediments

Ling, S.D*, Sinclair, M., Levi, C.J., Reeves, S., Edgar, G.J.

Institute for Marine & Antarctic Studies, University of Tasmania, Hobart 7001

*Corresponding author

**Author contributions**: S.D. Ling & G. J. Edgar designed, proposed and acquired funding for the research; S. Reeves and S.D. Ling performed field sampling and designed microplastic extraction technique; S. Reeves, M. Sinclair & C. Levi enumerated microplastics; S.D. Ling & M. Sinclair wrote the manuscript.

**Keywords**: Plastic, marine debris, pollution, heavy metals, sewage, benthic ecosystems.

Paper type "*Research Report*"

## Abstract

Microplastic pollution occurs in marine environments globally however estimates of seafloor concentrations are rare. Here we apply a novel method to quantify size-graded (0.038-4.0 mm diam.) concentrations of plastics in marine sediments from 42 coastal sites spanning pollution gradients across south-eastern Australia. Acid digestion/ density separation revealed 9,552 individual microplastics from 2.84 litres of sediment across all samples; equating to a regional average of 3.4 microplastics.ml$^{-1}$ sediment. Microplastics occurred as filament and particle forms, constituting 84% and 16% respectively. Positive correlations between microplastic filaments and wave exposure, and microplastic particles and finer sediments, suggest hydrological/sediment-matrix properties are important for deposition/ retention. Contrary to expectations, positive relationships between microplastics and other pollutants (heavy metals/ sewage), and negative relationships with biota on neighbouring reefs, were not evident; rather microplastics were ubiquitous across sampling sites. Conversely, positive associations with some fauna (i.e. benthic invertebrate species richness) suggests high potential for microplastic ingestion.

## Introduction

Marine environments comprise the ultimate destination for many pollutants including waste plastics, which are now recognised as a global environmental problem. While marine plastic pollution was first identified in the 1970s (Frias et al., 2016 and Carpenter and Smith, 1972), meaningful social and scientific concerns were not raised until the early 21st century (e.g. Moore et al., 2001; Frias et al., 2016). The increasing accumulation of plastics throughout the world's oceans is concurrent with its increased production and functionality, with ~250-300 million tonnes of plastic produced per year since 2006 (Castillo et al., 2016). Currently, plastics are the most abundant category of marine litter (Frias et al., 2016), found everywhere from the deep ocean basins to the Arctic (Costa and Barletta, 2015). Waste mismanagement has facilitated an association between increased plastic production and increased concentrations of plastics in the oceans; with industrial discharge, general litter and terrestrial run off the main sources of marine plastic debris (Ng and Obbard, 2006).

Overall, the occurrence and distribution of marine plastic litter has been well documented (Derraik, 2002; Eriksen et al., 2014; Van Sebille et al., 2015), with the negative effects of plastic debris on the marine environment described extensively by both scientific and social communities (Costa and Barletta, 2015 and Claessens et al., 2011). While the largest pieces of plastic debris and their interaction with mega-fauna such as seabirds, turtles and cetaceans have historically received the most scientific and public attention, recent focus has shifted towards the prevalence and environmental effects of so called 'microplastics' (Clark et al., 2016). Microplastics (defined by NOAA (2016) as those plastic particles $\leq$5 mm diameter) are commonly derived from the fragmentation of larger plastic particles over time (secondary plastic; Costs and Barletta, 2015) but can also be directly manufactured (primary plastic), as is the case with many cosmetic products such as "micro-beads" (Clark et al., 2016). On a global scale, marine plastic particles are becoming smaller and more widespread, primarily due to the fragmentation by physical abrasion and photo-degradation of existing plastics into smaller, more mobile fragments (Barnes et al. 2009).

As scientific focus shifts from large plastic debris to microplastics, an increasing number of studies have assessed potential impacts of microplastics on marine fauna.

While the direct consumption of, and entanglement with, larger plastics by marine life has been well documented, consumption of microplastics has also been demonstrated (Clark et al., 2016). Inspection of the gut contents of many marine species, including sea birds, pelagic fishes and estuarine crustaceans, reveal that microplastic ingestion is commonplace throughout marine ecosystems (Clark et al., 2016). In addition, biomagnification of these ingested microplastics can potentially impact higher trophic levels (Fossi et al., 2012).

While the potential harmful effects of marine microplastic pollution have recently received greater consideration, much of the attention has focused on the prevalence of microplastics in pelagic waters (Frias et al., 2016; Eriksen et al., 2014). This research has mainly focussed on the occurrence of plastics floating at the top of the water column, largely ignoring those denser materials that make their way to the seafloor below (Frias et al., 2016). To gain an accurate and meaningful assessment of plastic prevalence, seabed plastic accumulation must also be accounted for. This is particularly important given that 70% of marine litter globally is projected to sink and remain in marine sediments (Frias et al., 2016). Despite the presumed prevalence of non-buoyant plastics in the marine ecosystem, plastic accumulation on the seafloor remains largely unquantified.

Here we apply a novel approach to determine concentrations, forms and sizes of plastics in subtidal marine sediments from 42 sites spanning urban population centres across the south-eastern Australian coastline. We examine these patterns with respect to potential drivers, environmental variables including other pollutants, and benthic biodiversity. Specifically, we ask whether patterns of microplastic concentrations in seafloor sediments vary consistently with local human population density or other pollutants (such as heavy metals/ sewage & run-off indicators), plus examine how benthic biodiversity correlates with plastic pollution – a critical first step in gauging possible patterns of negative impacts of increasing plastic pollution on sessile marine species and ecosystems.

## 2. Methods

### 2.1 Sampling of marine sediments for microplastics

With a focus on examining a strong gradient in pollution, we sampled marine sediments for microplastics from across the major urban centres, and thus point sources of pollution, in south-eastern Australian states (Fig. 1). Within each state, sites were distributed across contrasting polluted and relatively pristine locations. That is, 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 relatively pristine sites in eastern Tasmania. Each of the large, capital cities have major ports and industry, and substantial known pollution (e.g. heavy metals). This includes historical 'legacy' industrial pollution as well as presumably high contemporary inputs of heavy metals, petrochemicals, organic enrichment and plastics from storm water runoff and effluent discharges from urbanised / agricultural dominated sub-catchments (Birch, 2000; Johnston and Keough, 2002; Townsend and Seen, 2012; Stuart-Smith et al 2015).

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. The sample for microplastic extraction was then taken by 'coring' the retrieved sediment with a 70 ml sample tube pushed into the surface sediment layer to an effective maximum depth of 7 cm into the benthos. Samples were then frozen for storage and thawed prior to extraction of plastics as outlined below.

#### *2.1.1 Microplastic extraction*

Extraction of microplastics from sediment samples was achieved by modifying existing methods (Claessens et al., 2013; Masura et al., 2015; Nuelle et al., 2014) in combination with a novel size-graded approach. Notably, these methods have been validated by implanting a known number of microplastics within samples, which was then compared to the eventual number of microplastics extracted from the sample (Claessens et al., 2013; Masura et al., 2015).

#### *2.1.2 Removal of biological material and size fractionation*

Digestion using Wet Peroxide Oxidation was achieved using 20 mL aqueous 0.05 M iron oxide [Fe(II)] solution and 20 mL 30 % hydrogen peroxide mixed with the whole

~70 ml sediment sample within a 600 mL beaker. This was allowed to sit on the bench for 5 minutes and then heated to 75 $^{o}$C on a magnetic stirring hotplate for 45 minutes, at which point all biological material was visibly bleached. The digested sample was then poured and washed through a stack of stainless steel mesh sieves; containing sieve sizes of 4 mm, 1 mm, 0.50 mm, 0.250 mm, 0.125 mm, 0.063 mm and 0.038 mm.

### *2.1.3 Density separation of microplastics*

Density separation utilises differences in the density of items to discern their differential properties. Following biological digestion, we used high-density NaI solution (density of 1.6-1.8 g.ml) for density separation of plastics from non-plastic particles. NaI is an efficient broad-spectrum means of extracting plastics ranging 0.91 – 0.97 g.ml for Polyethylene, 0.94 g.ml Polypropylene, 1.05 g.ml Polystyrene, 1.14 – 1.56 g.ml Polyvinyl Chloride, and 1.32 – 1.41 g.ml Polyethylene terephthalate (Van Cauwenberghe et al. 2015). Beyond this broad-spectrum extraction of plastics using NaI, individual polymer types were not identified (e.g. with FTIR) given the size of samples and large amount of individual plastic material recovered.

Sediment collected within each size-graded sieve was placed into a 50 ml centrifuge tube which was then topped up with NaI solution ensuring a minimum of 30 ml of this solution overlaid the sediment sample. The centrifuge tube was manually shaken for 20 seconds and placed in a centrifuge for 5 minutes at 3,500 revolutions per minute. A second centrifuge tube of equal mass, containing NaI solution only, was placed in the centrifuge opposite the sample tube to achieve rotor balance. After centrifuging, the resultant supernatant (typically 10-15 mL) was then filtered into a Büchner vacuum apparatus fitted with a 1.2 $\mu$m polycarbonate membrane filter and the NaI reclaimed. The tube was refilled with NaI solution until it again equalled the mass of the balancing tube in the centrifuge. These steps were repeated a total of three times to ensure all microplastics from each sample was extracted on to the membrane filter which was then sealed within a petri dish using parafilm.

### *2.1.4 Microscopic enumeration of microplastics*

Microplastics extracted onto filters were enumerated under a well-illuminated dissecting microscope. Each filter paper was marked with sorting lines with a fine

probe to define a series of transects enabling systematic assessment. Microplastics were categorised as either particulate or filamentous forms (Fig. 2a), and due to large numbers of recovered plastics, identification of polymer types for individual plastic items (e.g. Polyethylene, Polypropylene, Polystyrene, Polyvinyl Chloride, Polyethylene terephthalate) was not attempted. Individual plastics are easily identified under the microscope due to their obvious bright colouration (typically blue/purple, red, grey/white; Fig 1a). Furthermore, all biological material which may otherwise be confused as plastics, lost colour due to the peroxide digestion and was brittle and disintegrated upon probing.

**2.2 Other pollutants**

Other non-plastic pollutants were also quantified from the same sediment grabs as assessed for microplastics. Non-plastic pollutants assayed included total heavy metals (i.e. sum of Antimony, Arsenic, Cadmium, Chromium, Copper, Cobalt, Lead, Manganese, Nickel, Selenium, Silver, Vanadium, Zinc, Mercury concentrations), Total Organic Carbon (TOC), Nitrogen (N), Phosphorus (P), and petrochemical surrogates (i.e. Ethylene Dichloride, Toluene-d8, 4-Bromofluorobenzene) quantified by *ALS Environmental Pty Ltd* Australia (http://www.alsenviro.com; 277-289 Woodpark Rd, Smithfield, NSW, 2164); and Nitrogen 15 isotope ratios (an indicator of anthropogenic sources of N, e.g. Costanzo et al 2005) were assayed by *Environmental Isotopes Pty Ltd* (http://www.isotopic.com.au/).

Both plastic and non-plastic pollutants were sampled across all sites during Sep-Dec 2015, with laboratory determination undertaken from Oct 2015 to Dec 2016. Labile pollutants (e.g. heavy metals, nutrients and petro-chemical surrogates) were assessed within 2 weeks of collection, while non-labile material such as microplastic concentrations, were subsequently processed within 12 months of sample collection.

**2.3 Environmental covariates**

Defining the local human environment, a human-population index was derived for each sampling site using the comparative index of Stuart-Smith et al 2015 (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). Other environmental covariates included turbidity (using Secchi disc depth

measurements taken as the mean depth of duplicate drops measured to nearest 0.25 metres at time of sediment collection), Sea Surface Temperature (Long-term mean annual SST values from 2002–2009 from the Bio-ORACLE data set, Tyberghein, L. 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 as used to determine size range of microplastics (i.e. 4, 2, 1, 0.500, 0.250, 0.125, 0.063 mm).

### 2.4 Biological "*Reef Community*" data

Shallow reef habitats harbour the greatest concentrations of biodiversity in the sea (Roberts et al. 2002), and are often the components of the marine environment with which humans interact with and value most (Stuart-Smith et al. 2017). Given coastal and estuarine reef habitats also overlap with the locations of major human pressures, such as pollution (Edgar et al. 2005, Crain et al. 2009; Stuart-Smith et al. 2015), we correlated patterns in reef life communities with microplastic concentrations. Reef biota, i.e. fish and invertebrate abundances, as well as percent cover of biogenic habitat forming species (e.g. macroalgae), were sampled at all 42 south-eastern Australian sub-tidal reef sites using underwater visual census. 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 (by either monitoring program), data were standardised as densities per individual transect.

Biological data including abundance and biomass of fishes, abundance of invertebrates, and cover of biogenic habitat formers on the reef surface (assessed as % cover of the benthos, including macro-algae and sessile invertebrates) was extracted from the LTMPA and RLS data sets.

### 2.5 Statistical analyses

All statistical analyses were performed using *Primer V6.0*, with a three-way *Permutational Multivariate Analysis of Variance* (PERMANOVA) performed to compare "*Region*" (i.e. NSW, Vic, SA, Tas), microplastic "*Form*" (filament vs.

particle), and "*Size*" (i.e. 0.50 mm, 0.250 mm, 0.125 mm, 0.063 mm and 0.038 mm) on microplastic concentrations. A non-parametric approach was used to analyse the univariate response of microplastic concentration due to its robustness for analysing unbalanced designs (i.e. in this case an uneven sample number across regions). Regional patterns in the microplastic 'community' (defined as the assemblage of plastic species spanning two plastic forms "*particles* and *filaments*" by seven different size-categories) was also analysed with PERMANOVA.

Relationships between concentrations of microplastic particles and filaments, reef community structure, and measured and derived environmental variables (including other non-plastic pollutants) were analysed by Pearson Correlation Coefficients and by Distance based Linear Models (DISTLMs). Patterns were additionally assessed by ordinating samples using distance based redundancy analysis (dbRDA) with the PERMANOVA+ extension in Primer (Anderson et al., 2008). DISTLMs were based on Bray–Curtis dissimilarity for multivariate community data and Euclidean distance for univariate measures. Optimal models were ranked based on step-wise procedure using adjusted $R^2$ as the selection criterion, with p-values calculated using 9999 permutations.

## 3. Results

***Variability in microplastic abundance by region, form and size***

Filament and particle forms of microplastics were both detected in all 42 sediment samples collected across south-eastern Australia (Fig. 1). In total, 9,552 microplastic pieces, inclusive of both filaments and particles (comprising 84% and 16% of all plastics respectively), were extracted from a total of 2.84 litres of marine sediments. For this region, the average concentration of microplastics across all forms was 3.4 microplastics per ml of marine sediment.

Ordered by rank, the South Australian samples contained the highest average abundance of total microplastics per ml sediment (mean=4.11, range 1.87-6.36; n=6), followed by NSW (mean=3.40, range 0.59-9.29; n=12) and then Victoria (3.17, 0.71-5.49; n=16), while Tasmania had the lowest mean, but the individual sample with the

highest overall concentration (3.15, range 0.93-12.53; n=8). Conversely, the sample containing the lowest concentration of microplastics was from NSW (0.59 microplastics.ml$^{-1}$ sediment). PERMANOVA revealed significant effects of "*Region*", "*Form*" and "*Size*" on total microplastic concentration. However, interactions between these factors were evident, with the effect of "*Region*" being dependent on microplastic "*Form"* and the effect of microplastic "*Form*" dependent on microplastic "*Size"* (Table 1). That is, concentrations of the different plastic forms were different across regions and, while plastic particle concentrations were consistent across regions, filament concentrations differed; NSW and Victoria had significantly higher concentrations of plastic filaments compared to SA and Tasmania, with higher (NSW/ Vic) and lower (SA/ Tas) regions not different from each other (Fig. 2b). The microplastic "*Form*" by "*Size*" effect relates to significantly more microplastic filaments than particles occurring at size-classes less than 0.25 mm diameter (Fig. 2b).

***Variability in the microplastic 'community' by region***

Considering microplastic forms (particles and filaments) and size-classes as separate classes, the overall microplastic 'community' did not differ statistically between the different regions examined in south-eastern Australia (1-Way PERMANOVA, "*Region*", $df_{3,41}$, *Pseudo-F*= 0.85, *P*=0.64).

***Environmental and other pollutant predictors of microplastic concentration and microplastic 'community'***

Concentrations of microplastic particles and filament forms, and total microplastics combined, were generally uncorrelated with variability in other pollutants and only weakly predicted by environmental metrics (Table 2). Significant effects for microplastic filaments included a highly significant positive correlation with wave exposure and a negative, but weak correlation, with phosphorus (Table 2i). For microplastic particles, a significant positive correlation was observed with fine 0.063 mm sediments (Table 2ii). When both filaments and particles were combined to examine patterns in total microplastics, the correlation with wave exposure was again highly significant (driven by the filament component), and negative correlations, albeit very weak, for petrochemicals and human population size were also detected (Table 2iii). Correlating the whole microplastic 'community' against other pollutant and environmental variables revealed positive correlations of the plastic community

structure with organic enrichment (delta 15 Nitrogen and Total Organic Carbon), and a negative correlation with the relatively coarse 1 mm sediment particle size (Table 2iv).

***Relationships between reef biota and microplastic communities***

Reef biota showed little correlation with specific metrics of biodiversity (Table 3). For univariate descriptors of the reef community, only invertebrate richness showed significant positive correlation with microplastic particles in the 0.25 mmm size-class (Table 3iv). The concentration of microplastic particles was significantly positively correlated with the structure of the fish community (Table 3vi).

## 4. Discussion

Recent studies on marine plastic pollution have largely focused on the increasing prevalence of microplastics in pelagic and beach environments. By 2012, 40 separate studies had quantified the abundance of microplastics on sandy beaches (Hidalgo-Ruz et al., 2012 and Frias et al., 2016), with primary focus on recently-deposited marine plastic debris at the high tide line (Frias et al., 2016). This body of information has largely overlooked the abundance and cumulative loading of microplastics in the marine environment as a whole.

Enumeration of 9,552 microplastics from 42 sub-tidal marine sediment samples spanning New South Wales, Victoria, South Australia and Tasmania revealed differences in the prevalent form of microplastics between region and size, but with plastic filaments between 0.038 mm and 0.250 mm the dominant categories of microplastic in all four regions. Overall, plastic filaments dominated the microplastic counts, with 84% of all extracted microplastics identified as filaments. In the larger size-classes of microplastics examined, i.e. >0.5 mm in diameter, no statistical differences were found between abundance of plastic filaments and particles.

Microplastic concentration was only weakly correlated with physical environmental variables, indicating their ubiquity in subtidal sediments. The notable exception to this was a significant and relatively strong positive correlation between microplastic

filaments and increasing wave exposure (Table 2). An increasing concentration of microplastic filaments suggests hydrodynamics play an important role in enhancing delivery of microplastic filaments to the seafloor and/ or perhaps increasing fragmentation of larger filaments to smaller, more-numerous filaments. However, a full explanation of causality associated with this correlation requires a dedicated hydrodynamic study. This should encompass differing seafloor sediment types given that, for example, the concentration of plastic particles was highest in fine sediments. Furthermore, while not assessed here, plastic compounds of different density (polyethylene, polypropylene, polystyrene, polyvinyl chloride, and polyethylene terephthalate plastics) may respond differently to environmental and pollution variables, while also generating different biological consequences.

Microplastics concentrations, regardless of form or size, showed weak correlation with other pollutants. Thus, our survey indicates that the sources and/ or deposition and/ or mobility of microplastics are very different to other pollutants. All forms of pollution have different sources, which may vary depending on local human population centres and environments.

As microplastic filaments are often produced by household washing machines, and particles transported with general litter and by industrial discharge, we expected a strong correlation between microplastic abundance and human population density. Positive relationships with human population have been found in a number of prior studies (e.g. Browne, 2007, Depledge et al., 2013 and Barnes, 2005), with Barnes (2005) concluding that 90% of variation in anthropogenic plastic debris could be explained by human population density. Regardless, we observed no such correlation, with high concentrations observed in remote areas far from urban centres. For example, the highest microplastic concentration measured during our study (12.0 microplastic filaments per ml sediment) was from Bicheno, a small Tasmanian township where an active fishing fleet resides. Microplastics at this location were dominated by what appeared to be plastic rope fibres, a material used heavily in maritime activities. Thus, our results contrast with others, including Browne (2007), who found significantly higher abundances of microplastics in estuarine sediments at waste disposal sites.

Our results showed plastic filaments to often dominate microplastic counts in the smaller sediment size-classes. In this respect, our results are similar to Browne (2007), who found that the transportation and eventual settlement of microplastics is related to their size. Browne (2007) concluded that the density of individual microplastic pieces was a major contributor to their distribution, as it affects transportability and eventual settlement into the natural sediment. Thus, the dominance and ubiquity of microplastic filaments appears due to their low weight and potential for long-distance oceanic transportation than for heavier plastic particles.

Potential negative impacts of microplastics on marine biodiversity were difficult to infer given that few correlations between microplastics and reef life were evident in our data (Table 5). The only significant correlation between metrics of reef life was a positive, rather than negative, correlation between invertebrate richness and microplastic particles in the 0.25 mmm size-class. This lack of observed negative effect of microplastics on reef life indicates that microplastics may not yet be having an immediate impact on reef populations. Conversely, a positive correlation between increasing invertebrate richness and microplastic concentration suggests potentially high rates of ingestion of plastics by a large range of marine invertebrates. While correlations between microplastics appeared weak at the level of the whole community, experiments exploring the interaction between the smallest microplastics and primary invertebrate and fish consumers is warranted given the potential for bioaccumulation of marine microplastics. Sites identified here to contain high levels of microplastics also provide an important starting point for assessing rates of microplastic ingestion by invertebrate and fish fauna as ingestion is predicted to increase with increasing local concentrations of microplastics.

## Conclusion

Microplastics occur in marine environments globally, however measurements of concentrations in seafloor sediments are generally lacking. Our sampling revealed a ubiquitous distribution of microplastics in coastal marine sediments across south-eastern Australia. Microplastic concentrations also occurred independently of other pollutants including heavy metals and sewage, which highlights a need to address the presence of microplastics in marine environments as a specific issue. Our findings

also highlight a need to identify causes of small-scale variability in microplastics, their different forms and chemical compound types under varying hydrodynamic regimes and marine sediment types. But moreover because the biological consequences of this pollution remain unknown, faunal ingestion rates and impacts of marine microplastics at the individual, population, and community levels need to be assessed through broader field surveys and mechanistic studies involving laboratory and, ultimately, field settings.

## Acknowledgments

Adam Davey assisted with sample collection. We would also like to thank all contributors to the Reef Life Survey and Temperate Australian Marine Protected Areas monitoring programs. The manuscript was improved following helpful comments from Denise Hardesty. Field surveys and analyses were facilitated by the Australian Research Council and the Marine Biodiversity Hub, a collaborative partnership supported through the Australian Government's National Environmental Science Program, and the Australian Research Council.

## References

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, **62,** 1596–1605. doi:10.1016/J.Marpolbul.2011.05.030

Barnes, D. K. (2005) Remote islands reveal rapid rise of Southern Hemisphere sea debris. *Directions In Science*, **5,** 915–921

Barnes, D. K. A., Galgani, F., Thompson, R. C. And Barlaz, M. (2009) Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions Of The Royal Society B: Biological Sciences*, **364,** 1985–1998. doi:10.1098/Rstb.2008.0205

Boerger, C. M., Lattin, G.L., Moore, S.L. and Moore C.J. (2010) Plastic ingestion by planktivorous fishes in the North Pacific central gyre. *Marine Pollution Bulletin*, **60,** 2275–2278

Browne, M. A. (2007) Environmental and biological consequences of microplastic within marine habitats. PhD thesis, University Of Plymouth, England, UK

Carpenter, E. J. and Smith, K.L. (1972) Plastics on the Sargasso Sea surface. *Science*, **175,** 1240-1241. Doi:10.1126/Science.175.4027.1240

Costanzo, S. D., Udy, J., Longstaff, B., & Jones, A. (2005). Using nitrogen stable isotope ratios (δ 15 N) of macroalgae to determine the effectiveness of sewage upgrades: changes in the extent of sewage plumes over four years in Moreton Bay, Australia. *Marine Pollution Bulletin*, **51**, 212-217

Castillo, A. B., Al-Maslamani, I. and Obbard, J. P. (2016) Prevalence of microplastics in the marine waters of Qatar. *Marine Pollution Bulletin*, **111,** 260–267 doi:10.1016/J.Marpolbul.2016.06.108

Claessens, M., De Meester, S., Van Landuyt, L., De Clerck, K. and Janssen, C. R. (2011) Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Marine Pollution Bulletin*, **62,** 2199–2204 doi:10.1016/J.Marpolbul.2011.06.030

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M. B. and Janssen, C. R. (2013) New techniques for the detection of microplastics in sediments and field collected organisms. *Marine Pollution Bulletin*, **70,** 227–233 doi:10.1016/J.Marpolbul.2013.03.009

Clark, J. R., Cole, M., Lindeque, P. K., Fileman, E., Blackford, J., Lewis, C., Lenton, T. M. and Galloway, T. S. (2016) Marine microplastic debris: a targeted plan for understanding and quantifying interactions with marine life. *Frontiers In Ecology And The Environment*, **14,** 317–324 doi:10.1002/Fee.1297

Cole, M., Lindeque, P., Halsband, C. and Galloway, T. (2011) Microplastics as contaminants in the marine environment: a review. *Marine Pollution Bulletin*, **62,** 2588–2597

Cole, M., Lindeque, P., Fileman, E., Halsband, C. and Goodhead, R.M. (2013) Microplastic ingestion by zooplankton. *Environmental Science & Technology*, **47,** 6646–6655

Costa, M. F. and Barletta, M. (2015) Microplastics in coastal and marine environments of the western tropical and sub-tropical Atlantic Ocean. *Environmental Science: Processes Impacts*, **17**, 1868–1879 doi:10.1039/C5em00158g

Depledge, M. H., Galgani, F., Panti, C., Caliani, I., Casini, S. and Fossi, M. C. (2013) Plastic litter in the sea. *Marine Environmental Research*, **92**, 279–281 doi:10.1016/J.Marenvres.2013.10.002

Derraik, J.G.B. (2002) The pollution of the marine environment by plastic debris: a review. *Marine Pollution Bulletin,* **44,** 842–852

Edgar, G. J. and Stuart-Smith, R. D. (2014) Systematic global assessment of reef fish communities by the reef life survey program. *Scientific Data,* **1,** 1–8 doi:10.1038/Sdata.2014.7

Eriksen, M., Lebreton, L. C. M., Carson, H. S., Thiel, M., Moore, C. J. and Borerro, J. C. (2014) Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PLOS ONE,* **9** (12), 1-15 doi:10.1371/Journal.Pone.0111913.

Fossi, M. C., Panti, C., Guerranti, C., Coppola, D., Giannetti, M., Marsili, L., & Minutoli, R. (2012). Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (Balaenoptera physalus). *Marine Pollution Bulletin,* **64**, 2374-2379.

Frias, J. P. G. L., Gago, J., Otero, V., And Sobral, P. (2016) Microplastics in coastal sediments from southern Portuguese shelf waters. *Marine Environmental Research,* **114,** 24–30 doi:10.1016/J.Marenvres.2015.12.006

Ivar Do Sul, J. A. and Costa, M. (2014) The present and future of microplastic pollution in the marine environment. *Environmental Pollution,* **185,** 352–364 doi:0.1016/J.Envpol.2013.10.036

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C. and Thiel, M., (2012) Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental Science Technol*ogy, **46,** 3060-3075 doi:10.1021/ Es203150.

Hill, N. A., Pepper, A. R., Puotinen, M. L., Hughes, M. G., Edgar, G. J., Barrett, N. S., Stuart-Smith R. D., Leaper , R. (2010) Quantifying wave exposure in shallow temperate reef systems: applicability of fetch models for predicting algal biodiversity. *Marine Ecology Progress Series* **417**, 83-95

Lenz, R., Enders, K. and Nielsen, T. G. (2016) Microplastic exposure studies should be environmentally realistic. *Proceedings Of The National Academy Of Sciences,* **113,** 4121–4122 doi:10.1073/Pnas.1606615113

Masura, J., Baker, J. E., Foster, G., Arthur, C. and Herring, C. (2015) Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. *National Oceanic And Atmospheric Administration: Marine Debris Program*

Moore, C.J., Moore, S. L., Leecaster, M. K. and Weisberg, S. B. (2001) A comparison of plastic and plankton in the north Pacific central gyre. *Marine Pollution Bulletin,* **42** (12), 1297-1300 doi:10.1016/S0025-326x(01)00114-X

Ng, K. L. and Obbard, J. P. (2006) Prevalence of microplastics in Singapore's coastal marine environment. *Marine Pollution Bulletin,* **52,** 761–767 doi:10.1016/J.Marpolbul.2005.11.017

NOAA (2016) What are microplastics? *National Ocean Service*. Available At: http://Oceanservice.Noaa.Gov/Facts/Microplastics.Html [Verified 5 September 2016]

Nuelle, M. T., Dekiff, J. H., Remy, D. and Fries, E. (2014) A new analytical approach for monitoring microplastics in marine sediments. *Environmental Pollution,* **184,** 161–169 doi:10.1016/J.Envpol.2013.07.027

Reef Life Survey (RLS) (2015) Standardised survey procedures for monitoring rocky & coral reef ecological communities. *Reef life survey*. Available At:

http://Reeflifesurvey.Com/Wp-Content/Uploads/2015/07/New-Methods-Manual_150815.Pdf [Verified 21 October 2016]

Reisser, J., Shaw, J., Wilcox, C., Hardesty, B. D., Proietti, M., Thums, M. and Pattiaratchi, C. (2013) Marine plastic pollution in waters around Australia: characteristics, concentrations, and pathways. *PLOS ONE,* **8,** 1–11 doi:10.1371/Journal.Pone.0080466

Stuart-Smith, R. D., Edgar, G. J., Stuart-Smith, J. F., Barrett, N. S., Fowles, A. E., Hill, N. A., Cooper, A. T., Myers, A. P., Oh, E. S., Pocklington, J. B. and Thomson, R. J. (2015) Loss of native rocky reef biodiversity in Australian metropolitan embayments. *Marine Pollution Bulletin,* **95,** 324–332 doi:10.1016/J.Marpolbul.2015.03.023

Tyberghein, L. Verbruggen, H., Pauly, K., Troupin, C., Mineur, F. and De Clerck, O (2012) Bio-ORACLE: a global environmental dataset for marine species distribution modelling. *Glob. Ecol. Biogeogr.* 21, 272–281

Van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., Van Franeker, J.A., Eriksen, M., Siegel, D., Galgani, F. and Law, K.L. (2015) A global inventory of small floating plastic debris. *Environmental Research Letters*, 10(12), p.124006.

## Tables and Figures

**Table 1.** 3-way PERMANOVA results comparing microplastic concentration using fixed factors: region, form and size-class. Significant effects (α=0.05) are highlighted by asterisks.

| Source | df | SS | MS | Pseudo-F | Unique perms | P(perm) |
|---|---|---|---|---|---|---|
| Region | 3 | 1.47 | 0.49 | 3.97 | 9960 | 0.0074* |
| Form | 1 | 14.00 | 14.00 | 113.18 | 9838 | 0.0001* |
| Size | 6 | 11.82 | 1.97 | 15.92 | 9952 | 0.0001* |
| Region x Form | 3 | 2.17 | 0.72 | 5.84 | 9951 | 0.0005* |
| Region x Size | 18 | 1.54 | 0.09 | 0.69 | 9935 | 0.82 |
| Form x Size | 6 | 6.24 | 1.04 | 8.41 | 9944 | 0.0001* |
| Region x Form x Size | 18 | 1.63 | 0.09 | 0.73 | 9925 | 0.77 |
| Residuals | 532 | 65.80 | 0.12 | | | |

**Table 2.** DISTLM results for the responses of microplastic filaments (i.), microplastic particles (ii.), and total microplastics concentration (iii.), plus the 'whole' microplastic 'community' (iv.) relative to environmental variables. Numbers represent the % variation explained when added to DISTLM models in step-wise procedure, with significance denoted by * at $p < 0.05$ and ** at $p < 0.01$, as determined using 9999 permutations. Cells without values denote situations where a trialled variable was not added to the model in the step-wise procedure. Total variation of the model is the sum of variability for each included term (as indicated by dbRDA for 'communities'). Numbers in parentheses indicate Pearson Correlation Coefficients (Cor.) between microplastics and each individual variable included in the model.

| | **Microplastics** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***i. Filaments*** | | ***ii. Particles*** | | ***iii. Total*** | | ***iv. Community*** | |
| ***Environmental variables*** | ***%*** | **(Cor.)** | ***%*** | **(Cor.)** | ***%*** | **(Cor.)** | ***%*** | **(Cor.)** |
| Petrochemicals | 4.80 | (-0.09) | 1.84 | (-0.03) | 7.09* | (-0.09) | | |
| Heavy Metals | | | 0.85 | (-0.13) | | | | |
| d15N | 4.86 | (-0.26) | 1.57 | ( 0.09) | 3.00 | (-0.22) | 4.86* | ( 0.53) |
| Total Organic Carbon | | | 1.19 | (-0.15) | 1.46 | (-0.35) | 8.47** | ( 0.50) |
| Nitrogen | | | 0.83 | (-0.02) | | | | |
| Phosphorus | 5.19* | (-0.33) | 8.04 | (-0.17) | 6.23 | (-0.37) | 3.96 | ( 0.42) |
| Human Population Index 500 | 3.50 | (-0.08) | 1.81 | ( 0.20) | 5.37* | (-0.01) | | |
| Secchi depth (visibility) | | | 1.73 | ( 0.05) | | | | |
| Sea surface temperature | | | 0.72 | ( 0.11) | | | | |
| Wave exposure | 38.55** | ( 0.62) | 0.01 | (-0.01) | 34.20** | ( 0.58) | | |
| Sediment particle size 4mm | | | 0.06 | ( 0.11) | | | | |
| Sediment particle size 2mm | | | 4.28 | ( 0.13) | | | 3.60 | ( 0.05) |
| Sediment particle size 1mm | | | 0.85 | (-0.01) | 1.37 | ( 0.33) | 5.69* | (-0.38) |
| Sediment particle size 0.5mm | | | 0.45 | (-0.14) | | | | |
| Sediment particle size | | | 5.79 | (-0.01) | | | 2.59 | ( 0.20) |
| Sediment particle size 0.125mm | | | 1.13 | ( 0.01) | | | | |
| Sediment particle size 0.063mm | | | 15.05* | ( 0.38) | | | 3.23 | ( 0.34) |
| *Total variation* | 56.89 | | 46.22 | | 58.73 | | 32.40 | |

**Table 3.** DISTLM results for multivariate structure of reef biotic community relative to microplastic community. Numbers represent the % variation explained when added to DISTLM models in step-wise procedure, with significance denoted by * at $p < 0.05$ and ** at $p < 0.01$, as determined using 9999 permutations. Cells without values denote situations where a trialled variable was not added to the model in the step-wise procedure. Total variation of the model is the sum of variability for each included term; for communities (v. & vi.), total variation refers to that defined by dbRDA1. Numbers in parentheses indicate Pearson Correlation Coefficients (Cor.) between microplastics and each individual variable included in the model.

| | **Reef biota** | | | | | |
|---|---|---|---|---|---|---|
| | ***i. Fish abundance*** | ***ii. Fish richness*** | ***iii. Invertebrate abundance*** | ***iv. Invertebrate richness*** | ***v. Fish community*** | ***vi. Invertebrate community*** |
| **Microplastic variables** | **% (Cor.)** | **% (Cor.)** | **% (Cor.)** | **% (Cor.)** | ***% (Cor.)*** | ***% (Cor.)*** |
| Microplastics total | | | | | | |
| Microplastic filaments | 7.65 (0.00) | | | | 1.66 (-0.09) | |
| Microplastic particles | | | 3.83 (-0.21) | | 3.27* ( 0.07) | 2.05 ( 0.20) |
| Filaments 0.038 mm | 10.51 (0.17) | | | | 2.63 ( 0.11) | 2.82 (-0.12) |
| Filaments 0.063 mm | 1.44 (-0.05) | | | | | |
| Filaments 0.125 mm | 0.05 (-0.01) | 2.86 (-0.01) | | 3.12 ( 0.13) | 2.73 ( 0.30) | 2.99 ( 0.25) |
| Filaments 0.25 mm | 0.07 (-0.05) | | | | 2.70 (-0.53) | 3.59 ( 0.78) |
| Filaments 0.5 mm | 3.58 (-0.05) | 4.77 ( 0.21) | | | | |
| Filaments 1 mm | 1.92 (-0.11) | | | | | |
| Filaments 4 mm | 0.83 (-0.03) | 4.94 (-0.06) | | 2.58 ( 0.18) | 2.48 ( 0.79) | |
| Particles 0.038 mm | 0.24 ( 0.08) | | | 2.68 (-0.15) | 2.48 ( 0.04) | 2.88 (-0.39) |
| Particles 0.063 mm | 7.23 (-0.07) | | 2.47 (-0.01) | | | |
| Particles 0.125 mm | 5.04 (-0.12) | 8.26 ( 0.29) | | | | |
| Particles 0.25 mm | 0.04 ( 0.05) | | 3.97 ( 0.13) | 11.33* ( 0.34) | | |
| Particles 0.5 mm | 1.41 ( 0.03) | | | | | |
| Particles 1 mm | 0.03 ( 0.02) | | 3.12 | | | |
| Particles 4 mm | 3.34 ( 0.19) | | 5.88 | 2.41 | | 3.08 (-0.41) |
| Total variation | 35.75 | 20.83 | 15.44 | 22.12 | 13.01 | 15.36 |

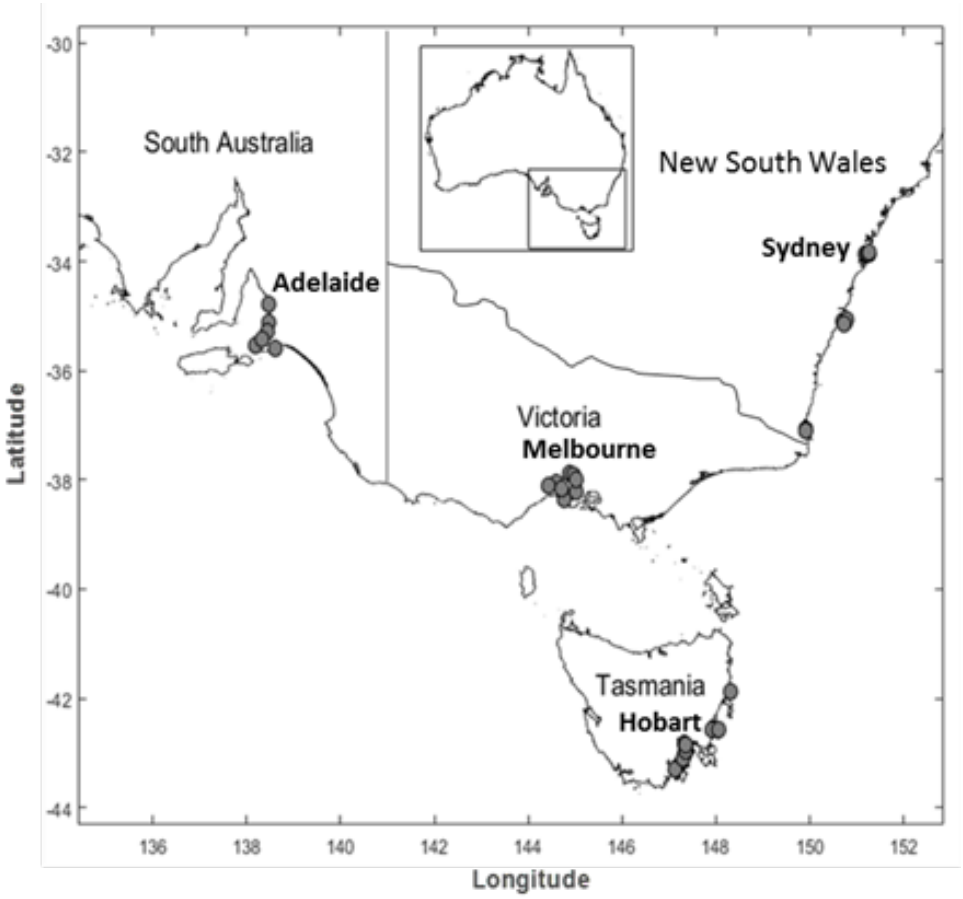


**Figure 1.** Map of south-eastern Australia showing sites where marine sediments were sampled. Samples were obtained near human population centres (shown in bold) and at relatively pristine sites in New South Wales (n=12 sites), South Australia (n=6 sites), Victoria (n=8 sites) and Tasmania (n=16 sites).

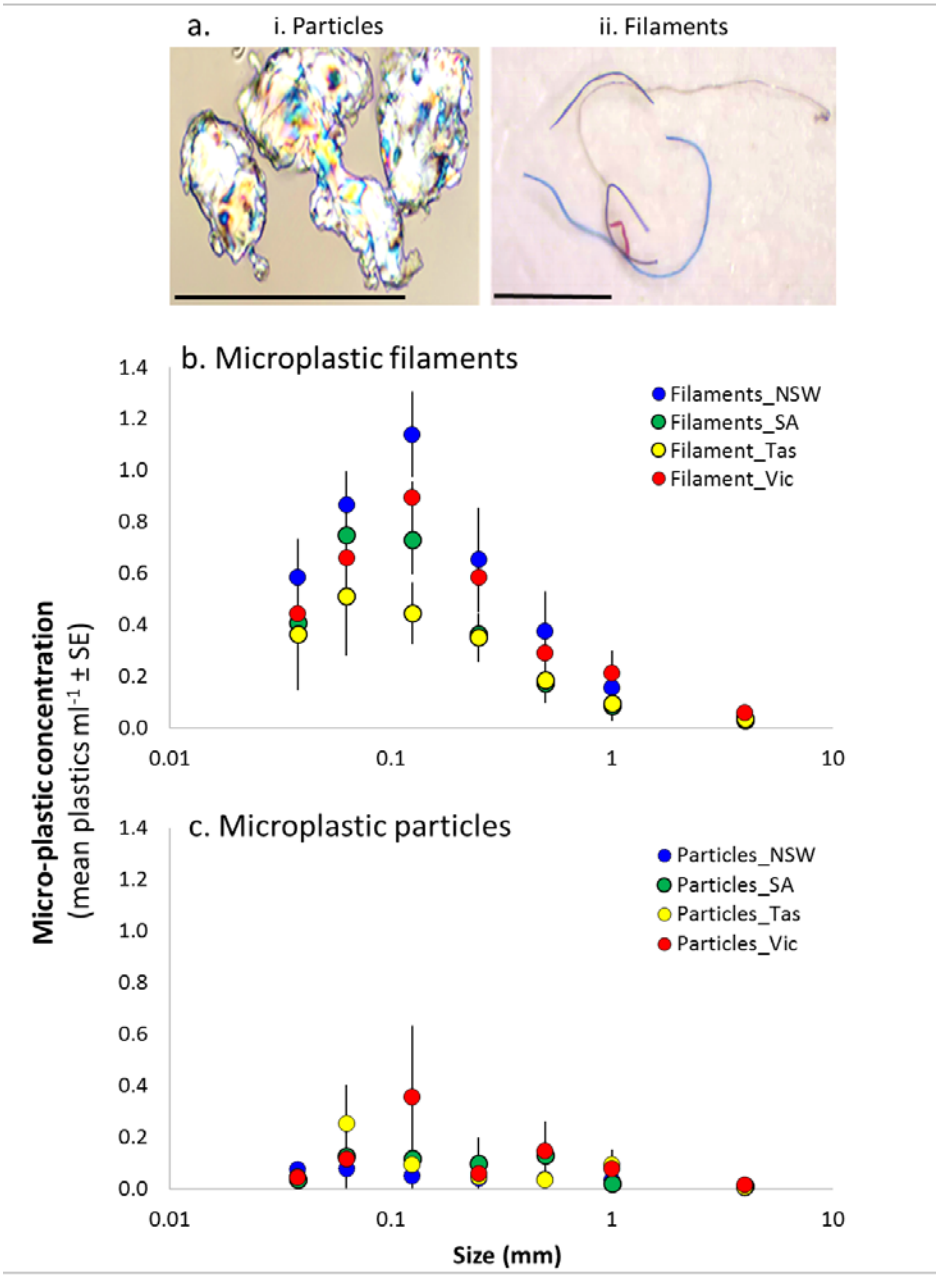


**Figure 2.** (a) Example microplastic particles (i) and filaments (ii) extracted from south-east Australian marine sediments, scale bar 0.5 mm. (b) Average concentrations of microplastic filaments and (c) partciles in marine sediments by region within each of the 7 different size categories; NSW= New South Wales, SA= South Australia, Vic= Victoria, and Tas= Tasmania.

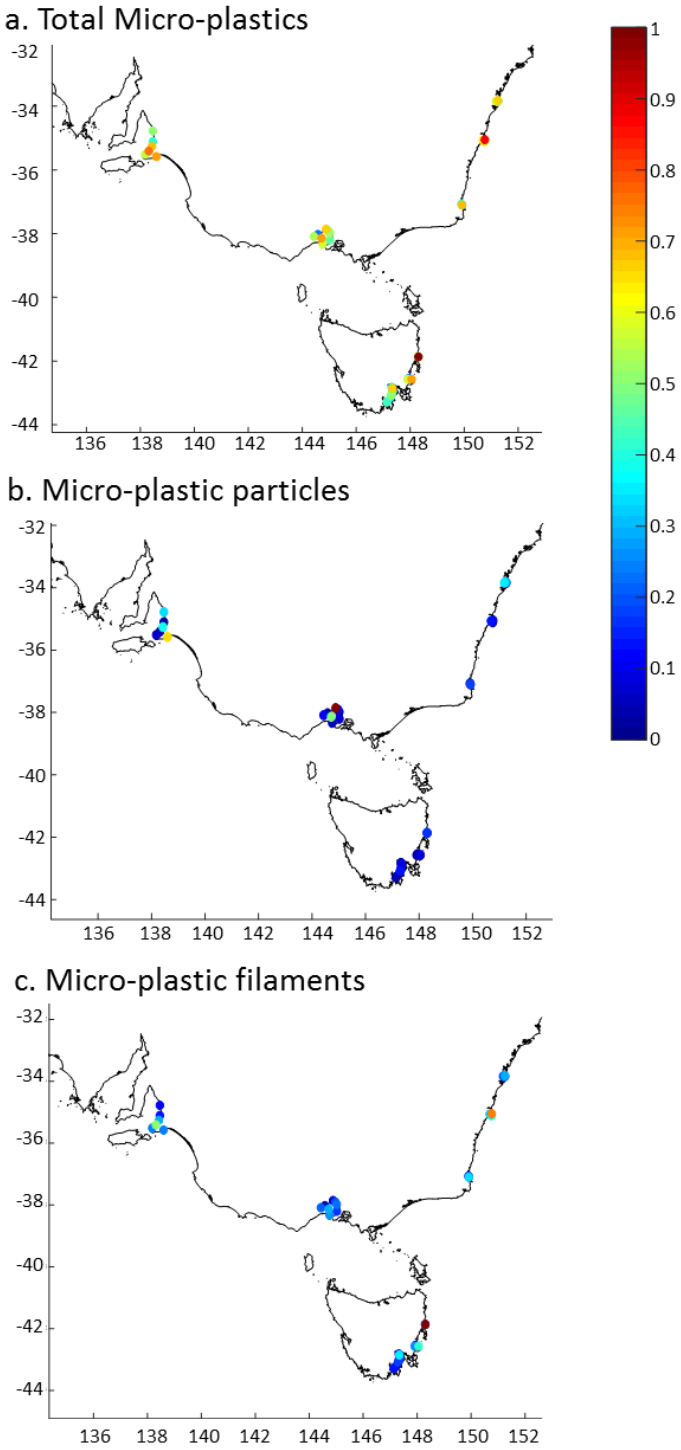


**Figure 3.** Heat map of spatial variability in relative concentrations of total microplastics (a), microplastic particles (b), and microplastic filaments (c) across south-eastern Australia. Concentrations are re-scaled from 0 to 1.