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Zooplankton and microplastics between New Zealand and the Ross Sea 2023–26, and the effects of sub-sampling of Continuous Plankton Recorder data

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PLAIN LANGUAGE SUMMARY

Zooplankton are small, drifting animals living in the ocean that are a crucial link in marine food webs and actively transport carbon to the deep sea. We have been sampling zooplankton in cold Subantarctic and Southern Ocean waters south of New Zealand since 2008, and the information helps us to understand how climate change is affecting marine ecosystems and to anticipate future change. Our study also monitors microplastics in the ocean, which have been increasing over the last 15 years. The samples of zooplankton and plastic are collected by an instrument called the Continuous Plankton Recorder (CPR) which is towed by a Sanford Ltd fishing vessel, the Fishing Vessel *San Aotea II*, as it travels between Timaru and the toothfish fishery grounds in the Ross Sea, Antarctica every year. This project extended the time series of observations by another two years and also investigated if subsampling could help us continue the work in a more affordable way. Our study found that subsampling the data 2:1 (that is, only analysing every-other sample of the continuous silk) makes the analysis cheaper and faster, only reduces the data quality by a small amount and does not affect our ability to understand change in zooplankton communities or our ability to monitor microplastic pollution.

EXECUTIVE SUMMARY

Pinkerton, M.H.[1]; Halfter, S.[1]; Robinson, K.V. [1] (2026). Zooplankton and microplastics between New Zealand and the Ross Sea 2023–26, and the effects of sub-sampling of Continuous Plankton Recorder data.

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Zooplankton – small, drifting marine animals in the water column – are crucial parts of marine ecosystems and affect how climate variability affects higher-level consumers such as fish, marine mammals and seabirds. Variations in zooplankton abundances over time can also impact carbon dioxide levels in the atmosphere by changing the amount of carbon transported to the seabed. As part of the international Southern Ocean Continuous Plankton Recorder (SO-CPR) project, New Zealand has been sampling zooplankton in the Subantarctic and Southern Ocean waters between South Island and the Ross Sea, Antarctica, since 2008. Zooplankton and plastics samples are collected using an instrument called the Continuous Plankton Recorder (CPR) which is towed by a Sanford Ltd fishing vessel, *San Aotea II*, as it travels between Timaru and the toothfish fishery in the Ross Sea every year. The information allows us to understand how climate change is affecting marine ecosystems. Previous modelling suggests that the environmental suitability for zooplankton generally has increased in the southern NZ EEZ and across the Southern Ocean on average over the last 20 years but has decreased in the Ross Sea. The same study also monitors microplastics in the ocean, which have been increasing over the 15 years of observation.

This project investigated to what extent subsampling in the laboratory analysis (that is, not analysing every sample collected) can help reduce the cost of maintaining the time series in the future. We compared the results of analysing all CPR data collected so far in the Southern Ocean with results based on analysing only part of the existing data. The general conclusions on 2:1 subsampling (analysing every second sample) were positive: *a posteriori* subsampling at 2:1 had relatively modest effects on key results, namely the estimates of: (1) absolute zooplankton abundances; (2) trends (long-term rates of change), and (3) biogeographic models (where the relationships between zooplankton abundances and oceanographic conditions are used to extrapolate measurements in time and space). Consequently, we recommend 2:1 subsampled analysis for CPR samples in the future.

This study also extended the time-series of CPR sampling for an additional two years (2024/25 and 2025/26 seasons) and also completed laboratory analysis of two years of samples using the 2:1 subsampling protocol (2023/24 and 2024/25 seasons). We have kept all samples which were not analysed which will be useful if a fine-scale spatial analysis is required in the future, and also to provide a resource for training, evaluation and cross-comparison if required.

Changing the SO-CPR analysis protocols for all international contributors will need agreement from all the major partners and we have already reached out to colleagues in Australia and Japan on our results. We received positive feedback and a jointly authored paper on the effects of subsampling has been accepted for presentation at the 2026 SCAR Open Science Conference in Oslo in August 2026.

The SO-CPR survey has proved effective at observing zooplankton communities and microplastic in the surface ocean but is under pressure from the squeeze on research budgets in New Zealand and internationally. Alternative methods of zooplankton and microplastic analysis (e.g. genetic, and AI-based image identification) are under development but are not yet operational. In the meantime, subsampling will help to make this valuable time-series observation more affordable and will potentially allow data collection to expand into crucial unsampled areas of the Southern Ocean by reducing the cost of laboratory analysis by nearly half.

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[1] Earth Sciences New Zealand

1. INTRODUCTION

Zooplankton are a diverse array of small animals that live predominantly in the upper layers of the ocean (shallower than about 1000 m) and are a key trophic link between microbial primary production and consumers such as fish, cephalopods, seabirds, and marine mammals (Atkinson 1998; Dubischar & Bathmann 1997; Bathmann et al. 2000; Pinkerton & Bradford-Grieve, 2014). Through excretion, egestion, and death, zooplankton also facilitate the transfer of organic matter to the deep ocean, supporting benthic biodiversity and helping remove carbon dioxide from the atmosphere (Accornero & Gowing 2003; Steinberg & Landry, 2017). Additionally, zooplankton can be useful indicators of the state of the marine environment (Atkinson 1998; Smetacek et al. 2004; Mackas & Beaugrand 2010) and can act as early warning indicators of critical shifts in ecosystem function (Beaugrand 2005; Batten et al. 2016). Zooplankton spatial analyses are also useful in the context of bio-regionalisation and spatial management including in the process of designing and evaluating Marine Protected Areas (Raymond & Constable, 2006; Sharp et al. 2007; Pinkerton et al. 2023).

The Continuous Plankton Recorder (CPR) is a high-speed plankton sampler designed to be towed behind ships of opportunity where it collects samples that are subsequently analysed under the microscope in a laboratory to identify and count zooplankton and microplastic particles using a standard methodology (Warner & Hays 1994; Hosie et al. 2003). CPRs have been used in the North Sea and Northeast Atlantic Ocean for over 75 years, and more recently the Southern Ocean Continuous Plankton Recorder (SO-CPR) survey was established in 1991 (Hosie et al. 2003). New Zealand became involved in the SO-CPR project in February 2002, when a CPR was deployed from the RV *Tangaroa*, and subsequent New Zealand research voyages continue to opportunistically deploy the CPR in the EEZ and Southern Ocean. In a collaboration with Sanford and NIWA funded by Fisheries New Zealand, the FV *San Aotea II* first collected zooplankton using the CPR in December 2008 while participating in the Ross Sea region fishery for Antarctic toothfish (*Dissostichus mawsoni*). The *San Aotea II* has subsequently gathered CPR data through the three previous phases of this project, beginning in 2008/09 and continuing to 2022/23, and New Zealand became the second largest contributor of data to the SO-CPR (providing 26.3% of all data), after Australia and just ahead of Japan (Pinkerton et al. 2024).

The previous analysis and report (Pinkerton et al. 2024) found that:

1. The Ross Sea region and the connected Pacific Sector of the Southern Ocean are unusual. Zooplankton are generally more abundant in the Ross Sea sector than in the rest of the Southern Ocean, by 28.6% for total zooplankton abundance. The broad environmental conditions (such as temperature, sea-ice) are similar in the Ross Sea sector and the rest of the Southern Ocean, so the higher zooplankton abundance is likely to be a result of higher primary productivity and more efficient transfer of energy through the lower food-web.

2. Southern Ocean zooplankton are changing. There was a decreasing trend in total zooplankton abundance in the Ross Sea sector between 2008 and 2023, with an average rate for zooplankton of -1.9% of the mean per year. Trajectories of change differed between taxonomic groups of zooplankton, with some decreasing and some increasing. In contrast, modelled environmental suitability for zooplankton generally increased in the Southern Ocean (+0.3% per year for zooplankton environmental suitability) meaning that ongoing zooplankton monitoring cannot yet be replaced by remote sensing methods.

3. Plastics are increasing. The mean number of plastic pieces, almost exclusively microfibres, in near-surface seawater in the Ross Sea sector increased 11-fold between late 2009 and 2023, to an average of about 29 pieces per m^3, and plastic was present in virtually every sample examined between 2018 and 2023. We found that plastic had a statistically significant and negative association with the abundance of many zooplankton groups. It is not known if this negative association between plastic and zooplankton is causal.

With the potential for increasing effects of climate change, marine heatwaves and microplastics on marine ecosystems, there is a desire to maintain New Zealand's contribution to the SO-CPR survey. However, funding was not available for full laboratory analysis of CPR samples in the present project or to support another 5-years of sampling. Because of the expense of the laboratory analysis, and suspected high autocorrelation between adjacent CPR samples, the present project had the objectives of continuing the 15-year sampling time series, and exploring whether it is possible to analyse only part of the CPR samples without compromising data quality (Table 1).

Subsampling would be a departure from standard SO-CPR sample analysis methodology and protocols (Hosie et al. 2003) but we note that the North Atlantic CPR survey has analysed only alternate ("odd-numbered") samples since 1946 (Reid et al. 2003), and the Australian EEZ CPR study analyses every 4th sample. If subsampling can be used to reduce cost but maintain data quality, this would make it more affordable to maintain the important SO-CPR time-series.

Table 1: Project objectives for ZBD2024-01.

Objective	Content
Overall Objective	To map changes in the quantitative distribution of epipelagic plankton (including copepods and euphausiid (krill) life stages) and to track the abundance and variability of marine microplastics, in the ocean between New Zealand (southern EEZ) and the Ross Sea (Antarctica).
Objective 1	To continue and extend the 15-year time-series sampling of zooplankton and microplastic sampling by conducting 2 annual return transits (4 transits in total) with the Continuous Plankton Recorder (CPR) between Aotearoa New Zealand and the Ross Sea (Antarctica) during 2 austral summers (2024/25 and 2025/26) in collaboration with Sanford Limited. Transport samples to Aotearoa New Zealand and store appropriately.
Objective 2	Using the 15-year New Zealand dataset, and the full international dataset from the SCAR Southern Ocean-CPR survey (SO-CPR), carry out retrospective analysis of the impact of subsampling on data analysis and conclusions. In particular, consider the effect of subsampling on analyses to: i Characterise latitudinal and temporal changes in the zooplankton populations in New Zealand's EEZ and transit to the Ross Sea (including long-term trends, and similarities/differences between zooplankton in the Ross Sea region and elsewhere in the Southern Ocean). ii Complete coupled analysis of zooplankton with environmental data (including phytoplankton) to investigate causes of variation in zooplankton in the Ross Sea region and elsewhere in the Southern Ocean. iii Quantify and track changes in the abundance and type of marine microplastics in the ocean between New Zealand (southern EEZ) and the Ross Sea. Discuss these results with Aotearoa New Zealand stakeholders and the international SO-CPR leadership. Present these results to the international CPR science community.
Objective 3	Conduct laboratory analysis of two years of samples collected by the CPR (samples collected in 2023/24 and 2024/25), using methods generally consistent with the SO-CPR but also subsampled, taking into account results from Objective 2. Contribute the data collected to the SO-CPR.
Objective 4	Broader outcomes

2. METHODS

2.1 The Continuous Plankton Recorder

The CPR is towed at approximately 10 m water depth behind a ship at full transit speed and collects zooplankton and microplastics by filtering waters through an internal roll of silk that is preserved in a formalin mesh (Figure 1, Figure 2). Each CPR sample represents 5 nautical miles (n.mile) of towed distance (equivalent to about 1.5 m^3 of filtered water. A CPR "tow" (lasting ~3 days, Figure 2) is a discrete deployment of the instrument and based on the data in Pinkerton et al. (2024), the median tow length is 165 n.mile and the longest usually possible is 450 n.mile (equivalent to a 2-day tow at 12 knots). Subsequent laboratory analysis provides measurements of the abundance of more than 100 groups of zooplankton and numbers and characteristics of microplastic particles for each 5 n.mile CPR sample (Figure 3, Figure 4, Figure 5). Details of the instrument, operation and laboratory analysis are described in the previous AEBR associated with precursors of this project (Pinkerton et al. 2024) and in the primary literature (Hosie et al. 2003; Pinkerton et al. 2020).

Nyegaard & Senga (2024) reported on the operation of the CPR during the Ross Sea 2023–24 (trip 162, FV *San Aotea II*) and highlighted issues where the silk became jammed in the CPR cassette which stopped proper sampling. In response, we carried out engineering work on the CPR body in August 2024, including adding spacing blocks to the base of the body to stop the cassettes moving laterally, and trimming the body port-side panel to avoid it from catching on the cassette. As is usual, we also conducted a pre-season CPR refresher training for the Sanford CPR handler (Kazuto Senga), which took place at Earth Sciences NZ campus in Wellington on 30 September 2024. In October 2025 we replaced the gears on the three cassettes with new units, inspected, cleaned and lubricated the units.

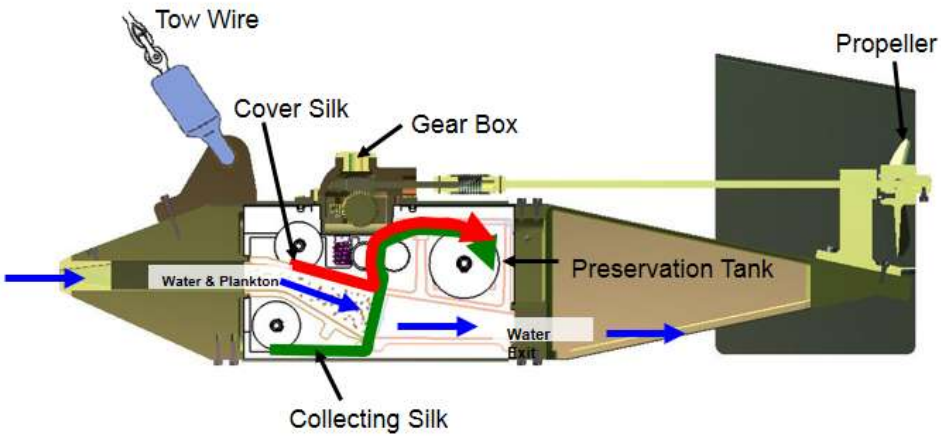


Figure 1: Continuous Plankton Recorder (CPR) schematic.

Figure 2: Continuous Plankton Recorder (CPR) retrieval and redeployment operation at sea. Photos courtesy of the FV *San Aotea II* crew.

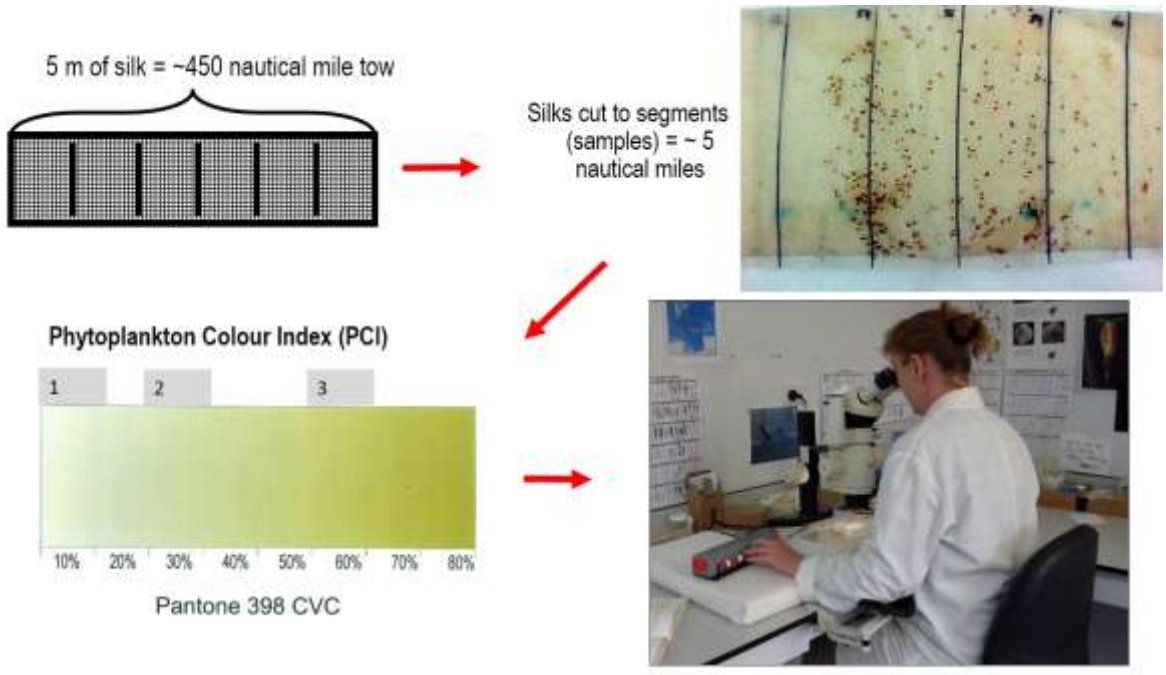


Figure 3: Preparation of CPR silk and analysis in the laboratory.

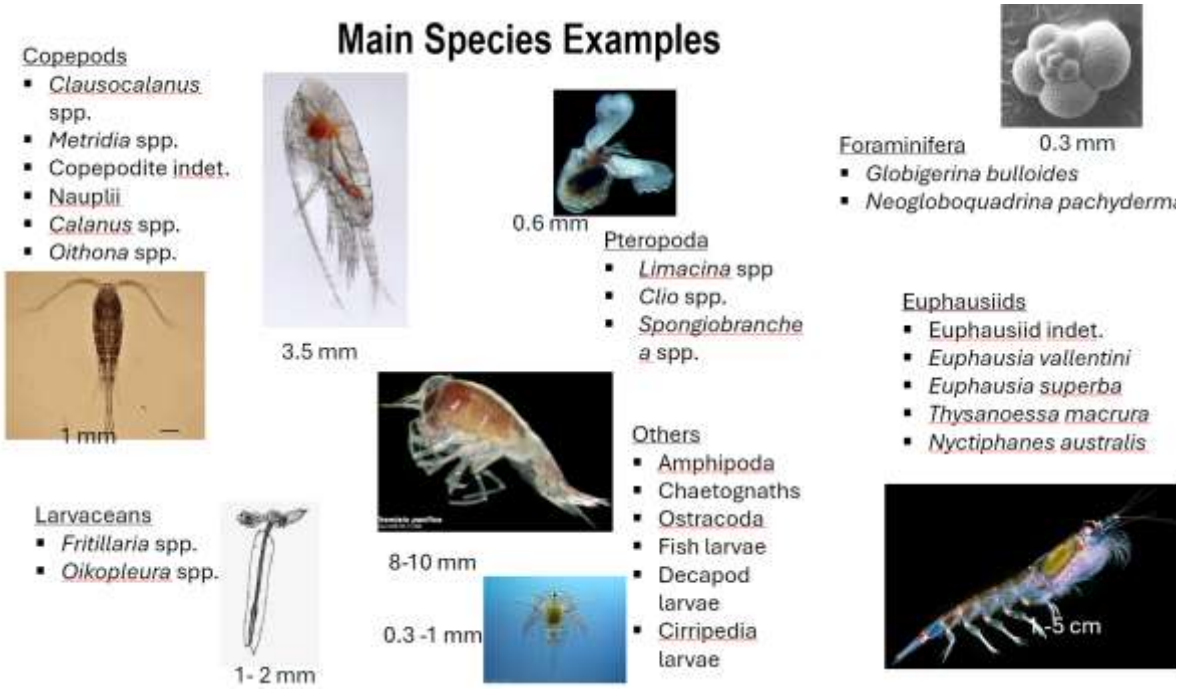


Figure 4: The main zooplankton groups that are identified in the laboratory during analysis.

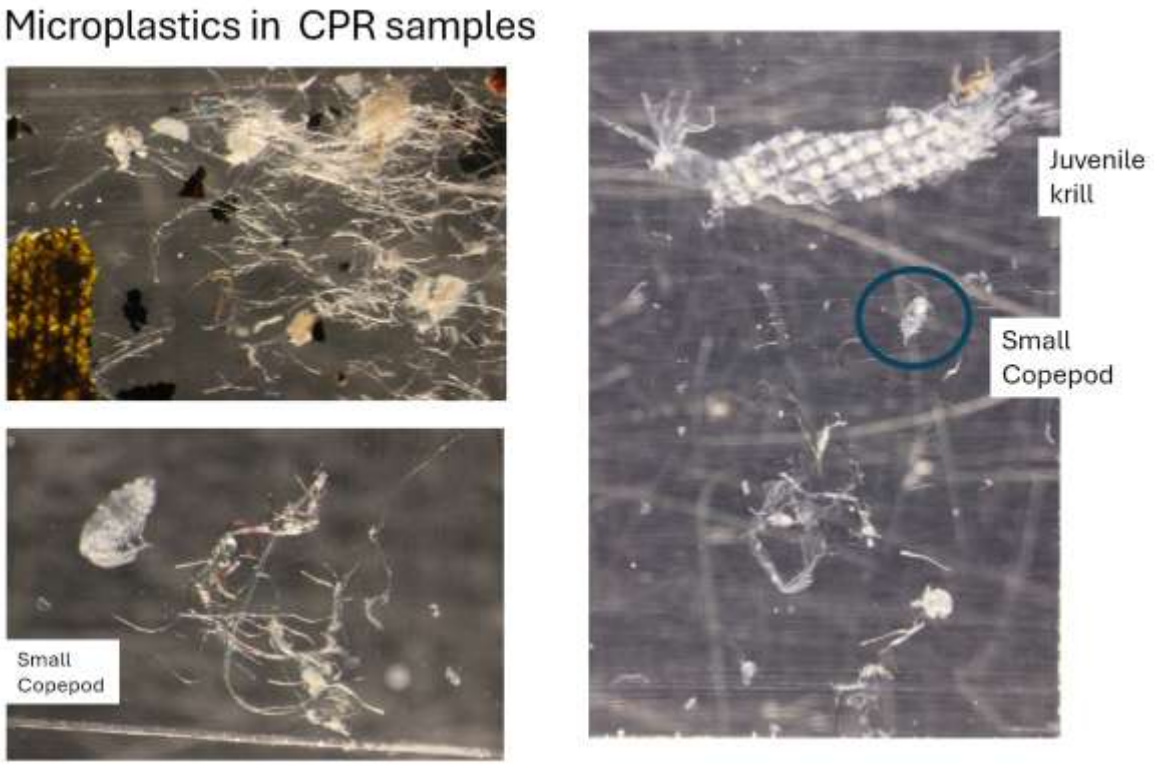


Figure 5: Examples of microplastics in CPR samples.

2.2 Subsampling CPR samples

Dataset used

Analysis of the effects of subsampling CPR samples was based on the dataset described by Pinkerton et al. (2024) (N = 55 245 samples; 911 tows; 276 308 n.mile) as shown in Figure 6. This dataset includes measurements in East Antarctica predominantly collected by Australia with substantial data from Japan, and data in the Ross Sea sector between New Zealand and the Ross Sea which predominantly is from New Zealand, and particularly from the three phases of the Fisheries New Zealand project with NIWA and Sanford Ltd, using the FV *San Aotea II*.

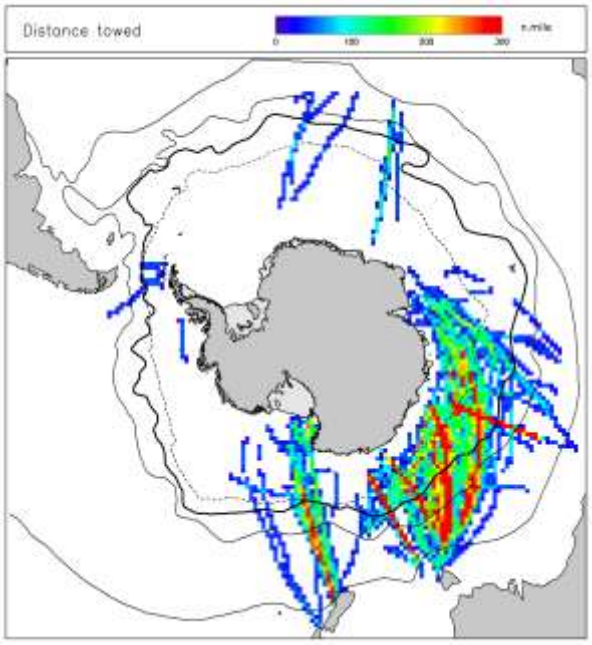


Figure 6: Mean spatial distributions of Southern Ocean CPR data as cumulative total distance towed (in nautical miles). For visualisation of the actual zooplankton measurements by the SO-CPR, data have been grouped into blocks of linear size 9×9 km or about 4.9 n.mile. Fronts are shown as black lines from north: Subtropical Front, Subantarctic Front, Polar Front (bold). The mean northern extent of the seasonal sea-ice is shown dashed.

Spatial resolution for analysis

The analysis approach is aligned to understanding the effects of subsampling on two key measures: (1) the abundance of zooplankton and microplastic particles in space (counts/n.mile); (2) trends (changes between years) of the abundances of zooplankton and microplastics. Typically, CPR samples (which represent 5 n.mile of towed distance) are combined into larger spatial areas to understand spatial patterns and trends, and we followed this approach here. We used a 25 n.mile resolution for analysis of abundances and 5° latitude (about 300 n.mile) for analysis of trends.

Grouping of zooplankton and microplastics

For this study, the zooplankton and microplastic data analysed in Pinkerton et al. (2024) were divided into three groups: (1) 27 "key" zooplankton groups which are those (typically) higher-abundance groups of zooplankton that were the focus of previous modelling and analysis (e.g. Pinkerton et al. 2020, 2024); (2) 70 "fine" zooplankton groups which provide more specific insights into the zooplankton community (see Appendix 1); and (3) microplastics, which are further characterised by type ("strand" or plastic microfibre versus "not strand" which includes flakes, fragments, beads etc), size (small, medium, large)

and colour (black, blue, clear which were the predominant colours of microplastics from previous analysis).

Autocorrelation and the Poisson model

Autocorrelation, also known as serial correlation, measures the agreement of a signal with a delayed copy of itself, and is crucial in analysing series of data where the order of observations matters. If the abundances of zooplankton or microplastics in neighbouring CPR samples are more similar to each other than pairs of samples further apart, we will find a positive autocorrelation and the samples will not be independent. Autocorrelation between neighbouring samples means that subsampling would lose less information. If there is no autocorrelation, then more information will be lost by subsampling.

The way in which the autocorrelation varies with the separation between samples will help guide what level of subsampling may be possible without significant loss of information. For the purposes of this project, we calculate the autocorrelation coefficient for each CPR tow separately and then average across all tows in the dataset. This prevents the autocorrelation coefficients being affected by long-term or large-area variation (i.e. variation between tows) and instead focusses on the small-scale (i.e. within tow) variability.

Autocorrelation analysis provides insight into whether a Poisson model might be suitable for modelling zooplankton or microplastic data from the CPR. A Poisson model is a statistical model used to analyse count data, where the dependent variable represents the number of occurrences of an event (in our case, the number of counts of zooplankton or microplastic particles in a CPR sample) within a specific time or space interval. The Poisson model assumes that the probability of an event happening has a constant rate and occurs independently of other events. The autocorrelation analysis will provide insights into whether the necessary assumption of independence for neighbouring samples for a Poisson model is tenable in the case of CPR sampling. If the Poisson model is appropriate, it can be used to theoretically analyse the effect of subsampling on the agreement between the full and subsampled dataset.

Bootstrap and subsampling resampling

Each CPR sample within a larger spatial unit (or "section" of 25 n.mile or 5° latitude) is considered a sample of the population. In this study, we use bootstrapping to investigate the uncertainty in the estimate of mean abundance based on all the available data. We will generally have 5 measurements within each 25 n.mile section and about 60 samples in each 5° interval, and so we draw this number at random with replacement to create a bootstrap sample. We repeat the bootstrap sampling 50 times to describe the uncertainty.

For subsampling, we first define the subsampling parameter s, where s=1 represents no-subsampling and s=2 represents 2:1 subsampling or using every second sample. Given s, we first randomise the starting point, s_0, (i.e. the first sample which may be element s_0=1,2,..,s) and then draw samples at regular intervals of every s samples. So, with s=3 and a starting point, s_0=2, we pick samples: {2,5,8,…}. (Note that we are numbering elements starting from 1 here.)

Effects of subsampling on mean abundances

We define the "bias" due to bootstrapping as equation 1:

$$bias = \frac{\frac{1}{N_i N_b}\sum_{all\ i\ b}^{all}[\overline{x_i}(b) - \overline{x_i}(no\ bootstrap)]}{x_m} \qquad \text{Equation 1}$$

Where the data (x, counts/n.mile or given zooplankton or microplastic type) is measured in N_i intervals (every 25 n.mile section of every tow), and within each interval (containing n samples) we bootstrap by selecting n=50 samples randomly with replacement a total of N_b times. Here, i is the interval index and

b is the bootstrap index. The scaling factor, x_m is the overall mean value of x across the whole dataset (no bootstrapping or subsampling). The coefficient of variation of the bootstrap estimate, CV(b), is given by equation 2, and is used as a measure of uncertainty of our bootstrapped estimate of mean abundance.

$$CV = \frac{\sqrt{var[\overline{x_i}(b) - \overline{x_i}(no\ bootstrap)]}}{x_m} \qquad \text{Equation 2}$$

Similarly, we define the bias and CV (equal to the root mean square difference divided by the mean) due to subsampling as equation 3 and 4, where the data (x, counts/n.mile or given zooplankton or microplastic type) is measured in N_i intervals, and within each interval we subsample with s=subsampling parameter (i.e. s=2 means 2:1 subsampling or sampling every second element) and s_0=starting points for subsampling, noting that there are s different starting points, and repeating this n=50 times to allow for different starting points in different tows. As before, x_m is the overall mean value of x for the group and is used as a scaling variable.

$$bias(s) = \frac{\frac{1}{N_i sn}\sum_{all\ n,s0}^{all\ i}[\overline{x_i}(s, s_0) - \overline{x_i}(no\ subsampling)]}{x_m} \qquad \text{Equation 3}$$

$$CV(s) = \frac{\sqrt{var[\overline{x_i}(s, s_0) - \overline{x_i}(no\ subsampling)]}}{x_m} \qquad \text{Equation 4}$$

Finally, a linear model was fitted to the bootstrapped data, x(b)~x, and to the subsampled data, x(s,s0)~x(s=0), to investigate the nature of the correspondence between the full-resolution data and the bootstrapped or subsampled data. The offset of the linear model was scaled by x_m for descriptive purposes. We also investigated a log-log model, but because there are so many zero abundances for most groups of zooplankton and microplastics this was not considered as useful as the linear model.

Effects of subsampling on trends in time

For the trend analysis, a coarser spatial resolution is required because otherwise there are not enough samples in a given area to obtain reasonable estimates of multi-year change; 5° latitudinal blocks are appropriate. Also, because the CPR data in East Antarctica has not followed a regular annual route (being predominantly opportunistic CPR data collection from Australian and Japanese research vessels), the data are ill-suited to spatial trend-analysis. Instead, following Pinkerton et al. (2024), we consider trend analysis only in the Ross Sea sector based on data from FV *San Aotea II*. This comprises 6365 samples, covering a total of 106 tows and 31 773 n.mile. We used the Sen slope (Sen 1968), which is insensitive to outliers (Hipel & McLeod 1994), to describe the average trend. All samples from a given tow within the latitudinal area were averaged to avoid a predominance of zeros (absences) for relatively uncommon samples (detailed methodology in Pinkerton et al. 2024).

As for the investigation of the effects of bootstrapping and subsampling on mean abundances explained above, we define the bias and CV of the bootstrapped and subsampled estimates as equations 1–4 where in this case x_i is the Sen slope of abundances in a 5° latitudinal band, N_i is the number of latitudinal bands and here x_m= mean of the absolute trends as a scaling variable. We also fitted linear models between the true trends and the bootstrapped and subsampled trends to investigate their correspondence.

Effects of subsampling on biogeographic analysis

Consistent with recommended international approaches for understanding change in zooplankton (e.g. Ratnarajah et al. 2023) and as in previous work (Pinkerton et al. 2010, 2020, 2024), we related zooplankton abundances from the CPR transects to oceanographic properties. The SO-CPR sampling is heavily biased towards East Antarctica and the Ross Sea region (Figure 6) and the multivariate statistical technique of Boosted Regression Trees (BRT, Elith et al. 2008) can be used to extend the data to give a circumpolar Southern Ocean perspective (Figure 7).

To test the effect of 2:1 subsampling, we reduced the CPR dataset by half by picking every other sample from each tow and refitted the BRT models to the reduced data. We then followed exactly the same biogeographic analysis method as Pinkerton et al. (2024), including the choice of environmental data, the model fitting method and parameters, the extrapolation (projection) method, and masking for missing or unrepresented areas. We consider the "combined" model, which is generated by blending two separate BRT models as described in Pinkerton et al. (2024): (1) the BRT model using monthly environmental data; and (2) the BRT model using monthly climatological and monthly anomaly environmental information. The new combined model results (based on the subsampled data) were compared to the combined model data based on the full dataset and presented in Pinkerton et al. (2024). We calculated the bias, CV, and reduced major axis regression (slope and offset) between the subsampled and full-date models as Equation 3 and 4. As before, a positive bias means the results based on the subsampled data were greater than those based on the full dataset and vice versa. We carried out this comparison for each of the six climatological months (October to March) and also on the long-term average ("cumulative") composites. Next, we calculated the trends for the combined models for each zooplankton group and compared with those based on the non-subsampled data. The bias, CV and reduced major axis regression were calculated.

These analyses were very computationally costly, taking about 5 days of computation time, and so the comparisons were only carried out for 2:1 subsampling.

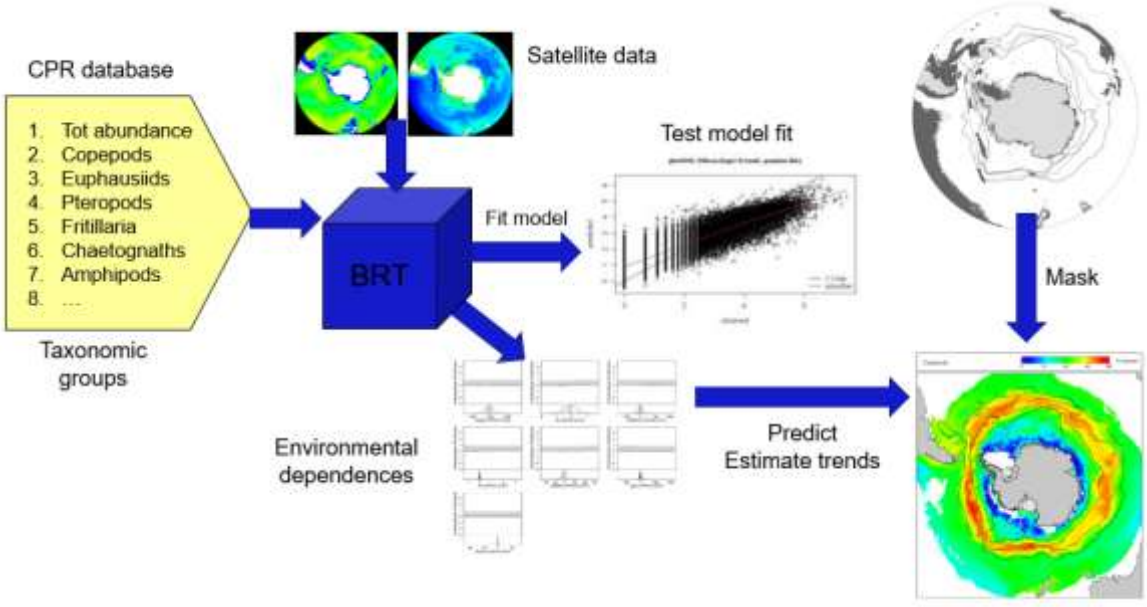


Figure 7: Schematic of biogeographic analysis as used in Pinkerton et al. 2020, 2024.

3. RESULTS

3.1 CPR operations 2023/24, 2024/25, 2025/26

The sampling along the New Zealand-Ross Sea transect was not complete every year because for operational reasons (such as bad weather or the need to go fishing) sometimes it was not possible or not safe to deploy the CPR. In addition, some tows where the CPR was deployed were failures and produced no samples. In Phase 4, Earth Sciences New Zealand, Sanford Ltd and the personnel on *San Aotea II* have worked together to reduce the number of times the equipment malfunctioned, including running training, and demonstrations, and improving maintenance of the CPR, and this has resulted in good results. CPR operations 2023/24, 2024/25 and 2025/26 are shown in Table 2 and Figure 8. CPR sampling was carried out in the 2023/24 season funded by NIWA and the Antarctic Science Platform (ASP), and in the 2024/25 and 2025/26 seasons funded by Fisheries New Zealand (this contract). In the two seasons 2024/25 and 2025/26, Phase 4 of the CPR project carried out 16 tows covering a distance of 4531 n.mile and providing an estimated 906 samples. From the two seasons 2023/24 and 2024/25, Phase 4 of the CPR project analysed data from 15 tows covering a distance of 4476 n.mile and 467 of the available 896 samples (52%).

Table 2: Summary of the tow data for the Continuous Plankton Recorder (CPR) Phase 5, 2023–2026. Distance is recorded in nautical miles (n.mile), with a segment being 5 n.mile. *Analysis not included in this project; numbers given for completeness.

Season	Tow number	Start date	End date	Distance towed n. mile	Number of 5 n. mile samples	Number samples processed
2023–24	1a	19/11/2023	21/11/2023	319	64	33
2023–24	1b	19/11/2023	21/11/2023	86	17	9
2023–24	2	21/11/2023	23/11/2023	447	89	46
2023–24	3	3/02/2024	5/02/2024	433	87	45
2023–24	4	5/02/2024	7/02/2024	437	87	45
2023–24	5	7/02/2024	9/02/2024	409	82	42
2023–24	6	9/02/2024	10/02/2024	229	46	24
2023–24	7a	20/02/2024	21/02/2024	66	13	7
2023–24	7b	20/02/2024	21/02/2024	255	51	26
2023–24	8	29/02/2024	2/03/2024	418	84	43
2024–25	1	18/11/2024	20/11/2024	59	12	7
2024–25	2	20/11/2024	22/11/2024	343	69	35
2024–25	3	22/11/2024	24/11/2024	114	23	12
2024–25	4	3/12/2024	4/12/2024	99	20	11
2024–25	5a	24/01/2025	26/01/2025	118	24	13
2024–25	5b	24/01/2025	26/01/2025	41	8	5
2024–25	6a	26/01/2025	27/01/2025	77	15	9
2024–25	6b	8/02/2025	9/02/2025	137	27	15
2024–25	6c	12/02/2025	13/02/2025	255	51	26
2024–25	7	13/02/2025	15/02/2025	134	27	14
2025–26	1	14/12/2025	15/12/2025	365	73*	0
2025–26	2	15/12/2025	17/12/2025	426	85*	0
2025–26	3	17/12/2025	19/12/2025	439	87*	0
2025–26	4a & b	20/12/2025	21/12/2025	42	8*	0
2025–26	5	5/02/2026	7/02/2026	352	70*	0
2025–26	6	8/02/2026	9/02/2026	130	26*	0
2025–26	7a & b	23/02/2026	26/02/2026	450	90*	0
2025–26	8	26/02/2026	28/02/2026	450	90*	0
2025–26	9	29/02/2024	2/03/2024	500	100*	0

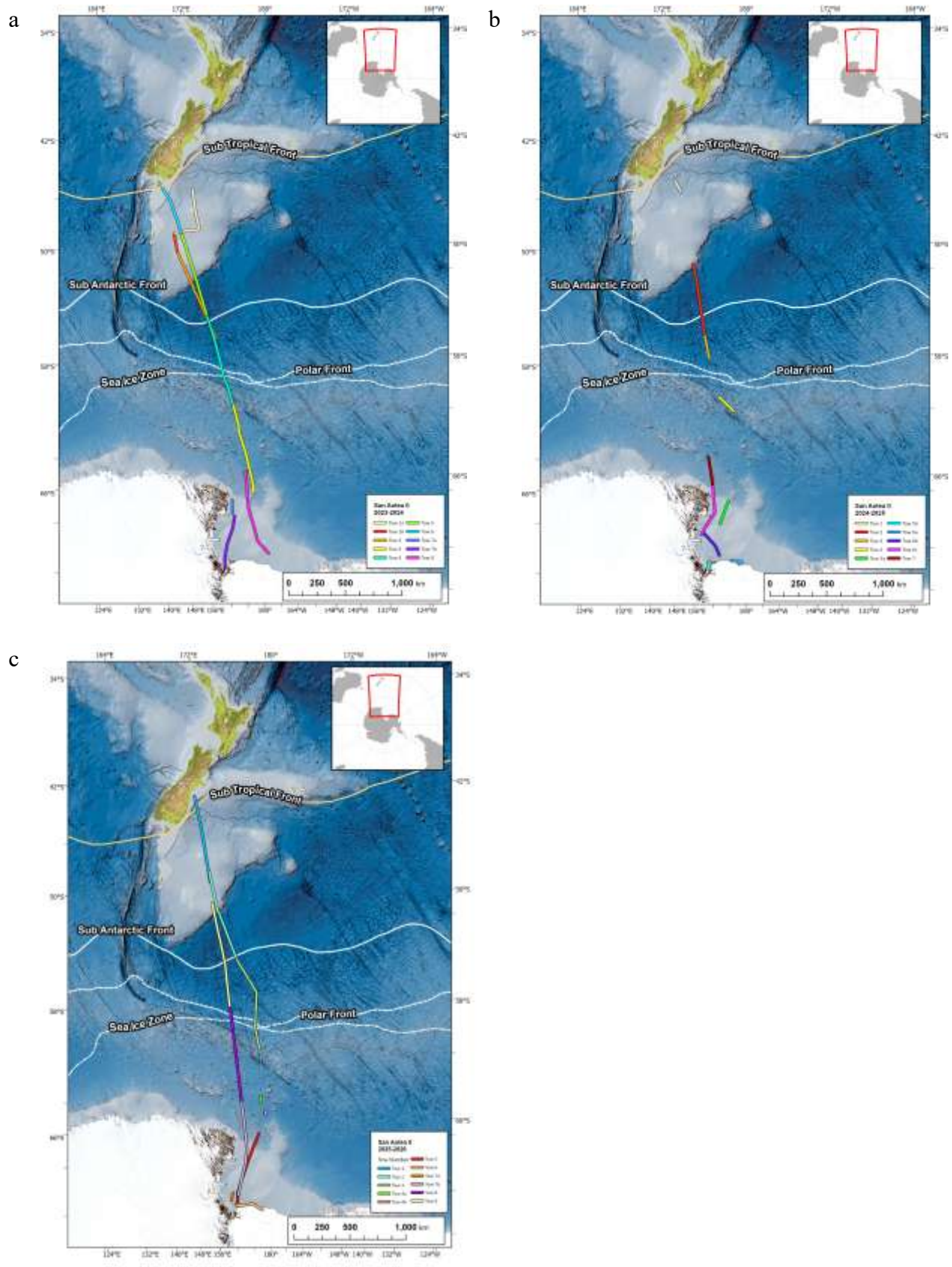


Figure 8: Continuous Plankton Recorder (CPR) tows carried out by FV *San Aotea II* by season. (a) 2023/24; (b) 2024/25; (c) 2025/26. Oceanic fronts are marked.

3.2 Sample analysis 2023/24 and 2024/25

We report on analysis of zooplankton and microplastic from the CPR collected in the 2023/24 and 2024/25 seasons and funded by Fisheries New Zealand (this contract). Sample analysis from the 2025/26 is not currently funded within this project, but samples are stored.

From the 15 tows in the 2023/24 and 2024/25 seasons, we have identified a total of 62 931 zooplankton and 17 516 microplastics. Of the pieces of microplastic that have been recorded, clear plastic threads were the most abundant, followed by yellow, blue and red threads. Across the samples processed, there was an average of 3.6 pieces of microplastic recorded per n. mile, which compares with an average of 3.0 pieces per n. mile (Phases 1 and 2) and 8.8 per n. mile (Phase 3). Microplastics were found in virtually all samples across the tows and latitudes from 2023/24 and 2024/25, consistent with Phase 3 (99.7% occurrence) and higher than 25.4% (Phase 1) and 45.4% (Phase 2).

Data are provided to Fisheries New Zealand and to the SO-CPR project for use in subsequent analyses.

3.3 Subsampling CPR samples

Mean values

For the subsampling sensitivity analysis, the dataset is summarised in Table 3, and for "fine" zooplankton groups in Appendix 1.

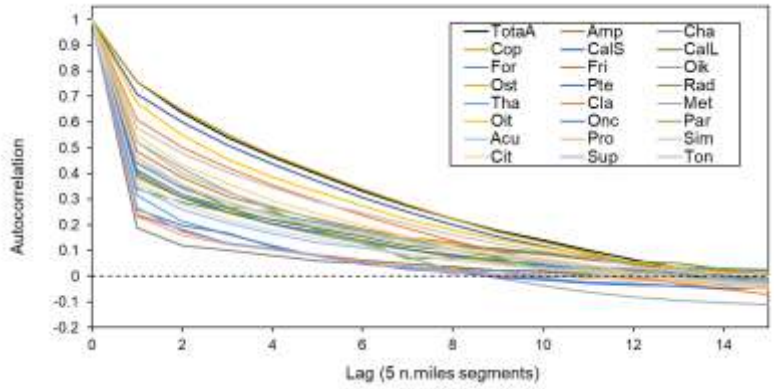
Autocorrelation

Autocorrelation coefficients for "key" groups of zooplankton at lag=1 were between 0.19–0.76 with a mean of 0.45 (Figure 9). For fine zooplankton groups, the mean autocorrelation at lag 1 was 0.28 (-0.01–0.92). For plastic, the lag=1 autocorrelation coefficients were lower, at 0.16 (0.07–0.25), and for environmental properties, they were substantially higher: 0.91 (0.84–0.95). Between the separate groups, there is a clear relationship between the mean abundance and the lag=1 autocorrelation, with more abundance groups having higher autocorrelation (Figure 10). These results imply that a Poisson model is more likely to apply to low abundance (i.e. relatively rare) zooplankton groups and microplastics, whereas the "patch sizes" of more abundant zooplankton groups are generally larger than 5 n.mile giving higher autocorrelation and making the Poisson model less appropriate. More abundant groups with high autocorrelation are also less likely to be substantially affected by 2:1 subsampling than lower abundance zooplankton groups and plastic data. The relatively high autocorrelation of the environmental properties explains why only a modest proportion of the small-scale variability in zooplankton abundance are explicable by biogeographic models: if the granular zooplankton variability is related to environmental conditions, the environmental data used is not at sufficiently fine resolution to capture it.

Table 3: Summary of "key" zooplankton groups and microplastic abundances, giving the number and proportion of CPR samples with non-zero abundances, and the mean and 5–95th percentiles of counts per n.mile (from Pinkerton et al. 2023). Here, "indet"=not further identified.

"Key" group	N (non zero)	Proportion non-zero	Abundance (counts/n.mile)	
		%	Counts	5–95th %
Total abundance	54 806	99.2	29.60	1.0 – 95.6
Copepoda	52 430	94.9	13.58	0.0 – 48.2
Oithona spp.	51 414	93.1	9.49	0.0 – 33.6
Fritillaria spp.	31 123	56.3	2.20	0.0 – 7.9
Pteropods	16 057	29.1	0.65	0.0 – 2.2
Oikopleura spp.	23 332	42.2	0.42	0.0 – 1.8
Amphipoda	12 765	23.1	0.092	0.0 – 0.4
Ostracoda	8 634	15.6	0.087	0.0 – 0.6
Chaetognatha	10 010	18.1	0.065	0.0 – 0.4
Calanoida indet small	49 143	89.0	7.43	0.0 – 28.4
Foraminifera	30 181	54.6	2.21	0.0 – 10.8
Calanus simillimus	24 768	44.8	1.34	0.0 – 6.2
Ctenocalanus citer	19 928	36.1	1.30	0.0 – 7.0
Calanoida indet large	23 793	43.1	0.97	0.0 – 4.4
Clausocalanus spp.	25 332	45.9	0.96	0.0 – 4.4
Rhincalanus gigas	18 775	34.0	0.56	0.0 – 3.0
Neocalanus tonsus	6 104	11.0	0.27	0.0 – 1.0
Thysanoessa macrura	17 762	32.2	0.20	0.0 – 1.0
Radiolaria	6 220	11.3	0.16	0.0 – 0.8
Calanoides acutus	8 803	15.9	0.15	0.0 – 0.8
Metridia spp.	7 506	13.6	0.14	0.0 – 0.6
Thaliacea	2 975	5.4	0.034	0.0 – 0.2
Calanus propinquus	1 783	3.2	0.033	0.0 – 0.0
Paracalanus spp.	240	0.4	0.032	0.0 – 0.0
Oncaea spp.	2 016	3.6	0.032	0.0 – 0.0
Thysanoessa gregaria	1 440	2.6	0.027	0.0 – 0.0
Euphausia superba	1 945	3.5	0.027	0.0 – 0.0
Plastic_total	2560	51.4	3.47	4.4 – 16.0
N_strand	2560	51.4	3.45	4.3 – 16.0
N_notStrand	269	5.4	0.02	0.0 – 0.2
N_black	2090	42.0	0.41	0.6 – 1.8
N_blue	2337	46.9	0.52	0.6 – 2.2
N_clear	1657	33.3	1.77	1.4 – 10.3
N_small	1160	23.3	0.18	0.0 – 1.0
N_medium	2527	50.8	2.30	2.4 – 11.6
N_large	2196	44.1	0.84	1.0 – 4.1

a



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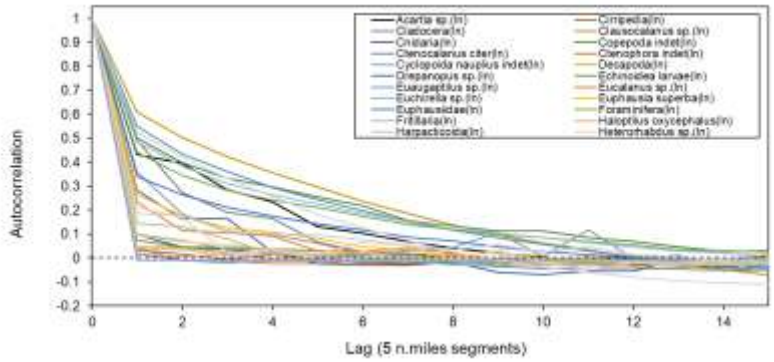

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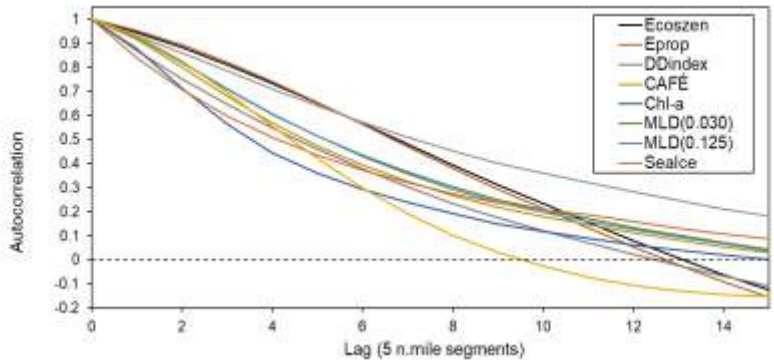

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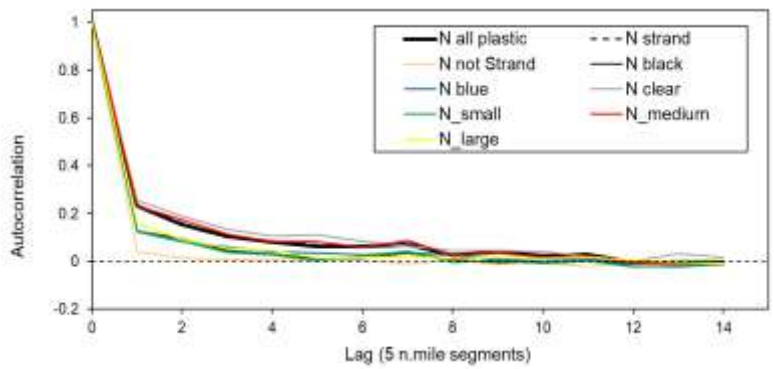


Figure 9: Autocorrelation between: a: "Key" zooplankton groups; b: "Fine" groups; b: c: Environmental covariates; d: Microplastic samples. TotaA=Total_abundance; Amp=Amphipoda; Cha=Chaetognatha; Cop=Copepoda; CalS=Calanoida_indet_small; CalL=Calanoida_indet_large; For=Foraminifera; Fri=*Fritillaria* spp.; Oik=*Oikopleura* spp.; Ost=Ostracoda; Pte=Pteropods; Rad=Radiolaria; Tha=Thaliacea; Cla=*Clausocalanus* spp.; Met=*Metridia* spp.; Oit=*Oithona* spp.; Onc=*Oncaea* spp.; Par=*Paracalanus* spp.; Acu=*Calanoides_acutus*; Pro=*Calanus_propinquus*; Sim=*Calanus_simillimus*; Cit=*Ctenocalanus_citer*; Sup=*Euphausia_superba*; Ton=*Neocalanus_tonsus*; Gig=*Rhincalanus_gigas*; Gre=*Thysanoessa_gregaria*; Mac=*Thysanoessa_macrura*. "N Plastic"=number plastic pieces; "N strand"=number plastic fibres; "N not strand"=number non-fibres; "N black"=number of plastic coloured black (blue, clear); "N small"=number small-sized pieces (medium, large).

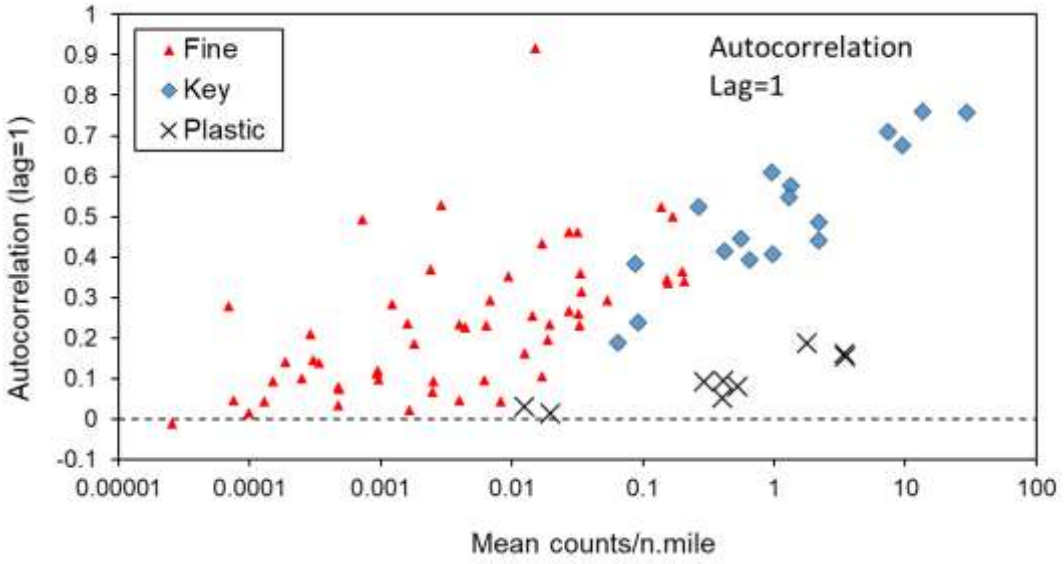


Figure 10: Autocorrelation (lag=1) on the abundance (counts/n.mile) from single CPR samples of: "Key"=higher-abundance species and groups of zooplankton which were the focus of previous modelling work; "Fine"=generally lower abundance individual species and genus groupings of zooplankton; "Plastic"=microplastic particles. The dashed line shows zero autocorrelation at lag=1 (consistent with Poisson model).

Effects of subsampling on abundance

The effects of subsampling on the abundance of zooplankton and microplastics at the 25 n.mile scale were small (Table 4). We found that 2:1 subsampling introduced a mean bias of less than 0.2% of the mean, a CV (root mean square difference divided by the mean abundance) less than 1% and a small departure of the slope from unity (mean slope of 1.06). Differences due to 2:1 subsampling were similar to bootstrap resampling. The effects of 2:1 subsampling on plastic abundances were greater than those on zooplankton abundances (consistent with the lower autocorrelation of plastic distribution compared to zooplankton), but the changes were still relatively minor: mean bias of 0.7%, CV of 0.9% and mean slope of 1.15 (Table 4). Differences (bias, CV, model slope) also showed generally increasing effects of subsampling at 3:1 and 5:1 for both zooplankton and microplastic abundances (Figure 11, Figure 12). All metrics of the effects of subsampling on abundances at the 25 n.mile scale increased at smaller abundances (bias: Figure 13; CV: Figure 14; model offset: Figure 15; model slope: Figure 16).

A Poisson model can be used to explore theoretically how subsampling and changing the spatial analysis scale would inflate the uncertainty (Figure 17). In this plot, the y-axis shows the root mean square difference compared to using the full (non-subsampled) data at full resolution (individual 5 n.mile samples). Increasing the spatial scale of analysis by averaging neighbouring samples into blocks of size given on the x-axis reduces the CVs, whereas subsampling (different lines) increases the CV. An important consideration therefore is the spatial scale that is desired in the analysis of SO-CPR zooplankton and microplastic data. At the 25 n.mile scale of analysis, the increase in CV due to 2:1 subsampling is unlikely to be problematic as the variability will still be about 35% lower than in the individual segments. Further, Figure 17 shows that if the inflation of CV due to subsampling is problematic, we could compensate for 2:1 subsampling by approximately doubling the spatial scale of analysis.

Table 4: Effects of bootstrapping and subsampling on mean abundances in 25 n.mile sections of key groups of zooplankton and microsplastics. "Boot"=bootstrapped full samples; subsampling 2:1, 3:1; 5:1. "CV"=coefficient of variation (standard deviation/mean). The s2 linear model: true=A+B*sub, where "true"= mean abundance (counts/n.mile) based on all data (no subsampling or bootstrapping), and "sub"=subsampled measure for same 25 n.mile section of the same tow, and "mean"= mean true value (all tows) as scaling variable (see Methods).

	Bias (%)				CV				Linear model, 2:1	
	boot	2:1	3:1	5:1	boot	2:1	3:1	5:1	A/mean (%)	B
Total abundance	-0.01	-0.03	0.03	-0.17	0.19	0.86	1.39	2.20	-1.68	1.02
Copepoda	0.01	0.01	0.01	-0.12	0.24	0.83	1.39	2.27	-2.22	1.02
Oithona	0.02	0.01	0.10	0.11	0.22	0.92	1.44	2.24	-2.02	1.02
Fritillaria	-0.05	-0.23	0.12	0.13	0.72	0.74	1.42	2.24	-1.25	1.01
Pteropods	0.04	0.02	0.51	-0.06	1.13	0.77	1.47	2.22	-2.66	1.03
Oikopleura	0.05	0.00	-0.21	-0.01	0.61	0.88	1.36	2.17	-3.39	1.03
Amphipoda	0.14	-0.08	0.31	-0.74	1.07	0.97	1.70	2.11	-17.89	1.18
Ostracoda	0.10	-0.72	0.52	0.21	1.06	0.96	1.65	2.21	-9.30	1.09
Chaetognatha	-0.03	-0.26	-0.30	-0.11	0.84	1.10	1.66	2.23	-14.99	1.15
Calanoida indet small	-0.06	0.02	0.13	0.13	0.26	0.85	1.40	2.27	-1.92	1.02
Foraminifera	-0.05	-0.58	-0.32	-0.01	0.61	1.10	1.46	2.08	-4.58	1.04
Calanus simillimus	-0.18	0.01	-0.08	-0.06	0.71	0.83	1.36	2.28	-3.20	1.03
Ctenocalanus citer	0.01	-0.04	0.24	-0.31	0.66	0.82	1.42	2.17	-2.94	1.03
Calanoida indet large	-0.05	-0.09	-0.21	-0.09	0.58	1.05	1.43	2.30	-3.20	1.03
Clausocalanus	-0.21	-0.04	0.19	0.20	0.85	0.74	1.54	2.38	-4.40	1.04
Rhincalanus gigas	0.03	0.00	0.21	-0.10	0.64	0.98	1.45	2.14	-4.70	1.05
Neocalanus tonsus	-0.18	-0.54	-0.74	-0.10	1.80	0.85	1.51	2.33	-8.25	1.08
Thysanoessa macrura	0.23	-0.30	0.07	0.23	0.84	1.01	1.41	2.10	-13.46	1.13
Radiolaria	0.07	-0.23	0.43	-0.41	0.73	1.01	1.54	2.21	-2.09	1.02
Calanoides acutus	0.05	-0.14	-0.08	0.27	1.27	1.07	1.42	1.99	-7.42	1.07
Metridia	0.82	-0.11	0.07	0.82	2.36	0.79	1.21	2.19	-11.10	1.11
Thaliacea	-0.29	0.06	-0.49	-0.07	1.95	0.89	1.35	2.23	-7.57	1.08
Calanus propinquus	-0.69	-1.30	-3.53	-0.15	4.39	1.02	1.49	2.27	-1.52	1.01
Paracalanus	-0.82	0.08	-2.66	-0.28	8.52	0.64	1.39	2.14	-3.84	1.04
Oncaea	0.22	-0.25	0.89	0.76	2.21	0.96	1.66	2.45	-7.66	1.07
Thysanoessa gregaria	0.24	-0.27	0.75	-0.57	3.27	0.87	1.67	2.24	-7.88	1.08
Euphausia superba	-0.58	-0.09	-2.05	0.32	5.75	0.70	0.94	2.18	-9.27	1.09
Plastic_total	0.10	-1.06	-1.10	0.02	0.37	0.38	0.56	0.79	-5.50	1.04
N_strand	0.08	-0.38	-0.95	-0.34	0.38	0.38	0.57	0.79	-5.93	1.06
N_notStrand	-0.92	1.47	4.85	3.60	2.90	3.32	6.13	5.57	-46.18	1.48
N_black	0.18	-0.78	-0.87	0.58	0.58	0.64	0.92	1.27	-19.96	1.20
N_blue	-0.46	-1.24	-0.69	-1.94	0.49	0.58	0.79	1.08	-12.55	1.11
N_clear	0.29	-0.90	-1.39	-0.53	0.48	0.50	0.74	1.03	-5.33	1.04
N_small	-0.68	-1.70	0.71	-0.02	1.10	1.11	1.65	2.30	-25.13	1.24
N_medium	0.09	-0.63	-0.88	0.16	0.41	0.43	0.60	0.86	-5.30	1.05
N_large	0.51	-1.03	-1.08	-0.25	0.53	0.54	0.87	1.16	-9.84	1.09

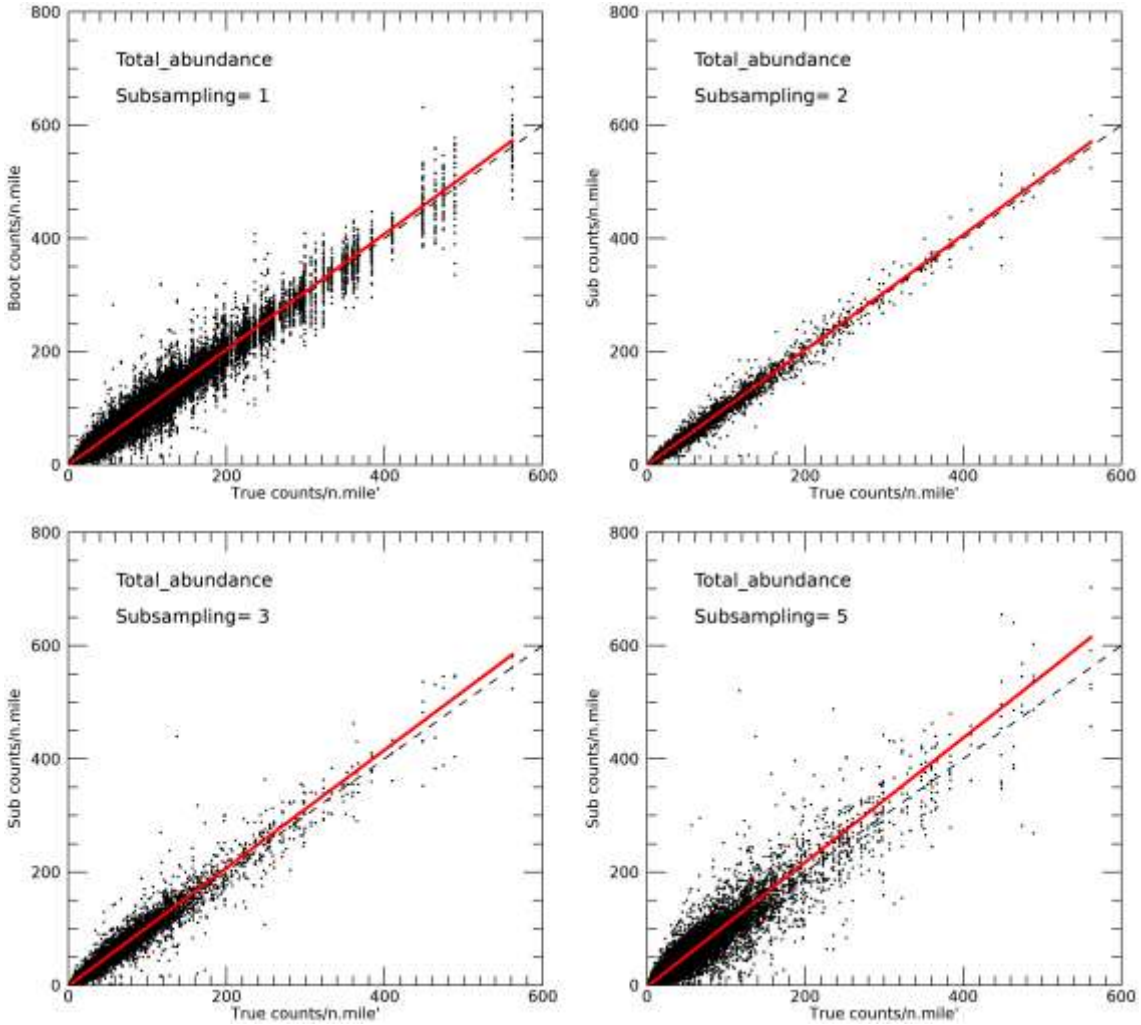


Figure 11: Effects of sub-sampling and bootstrapping on <u>total zooplankton abundance</u> (counts/n.mile), showing x= "True" abundance based on original data, y="Boot" (abundance based on bootstrapped data) or y="Sub" (abundance based on subsampled data) for the same 25 n.mile section of the same tow (see Methods). a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. The dashed line shows 1:1 correspondence and the red-line is the y-on-x least-squares regression. Similar results are shown in Appendix 1 for other "Key groups" (generally higher-abundance groups of zooplankton which were the focus of previous modelling work).

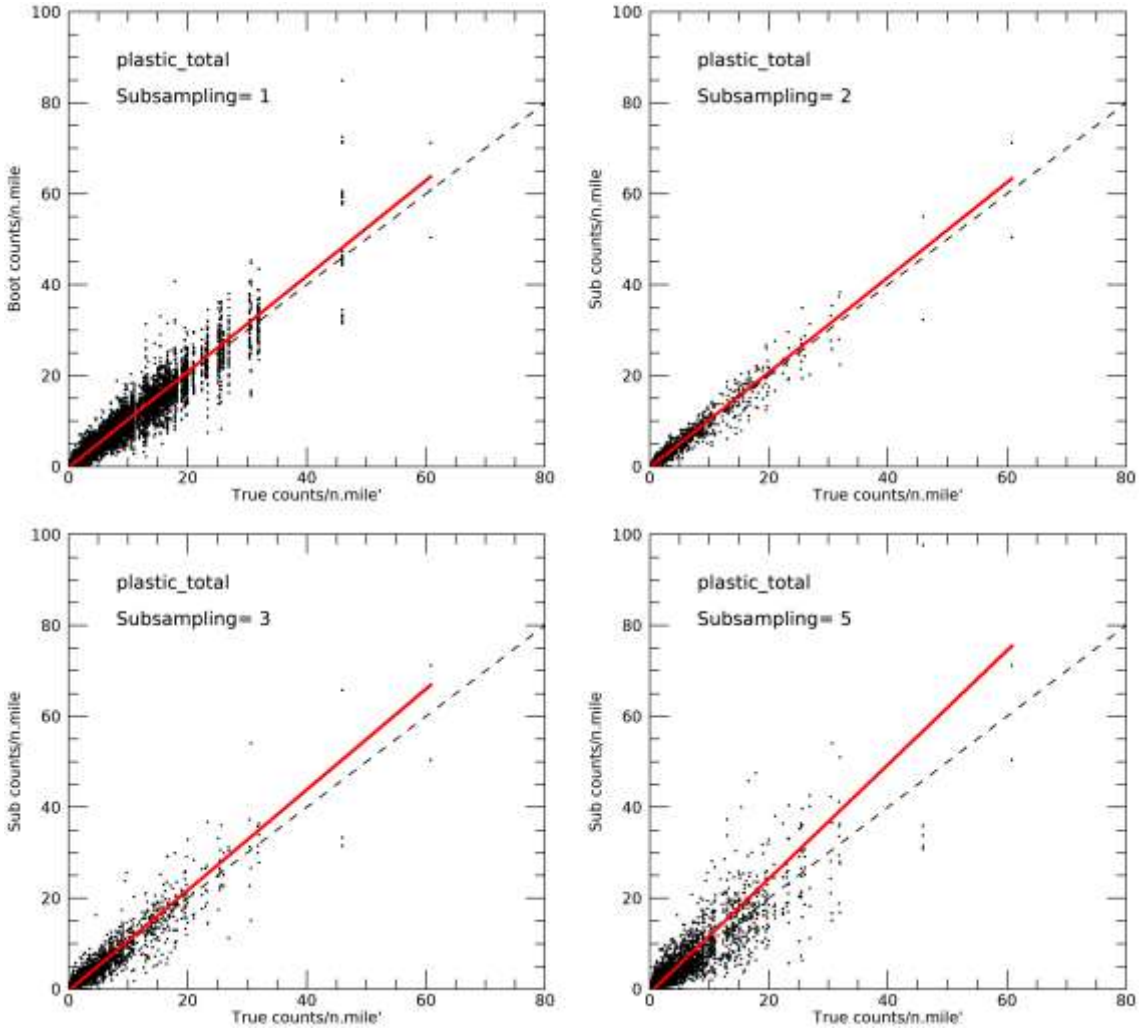
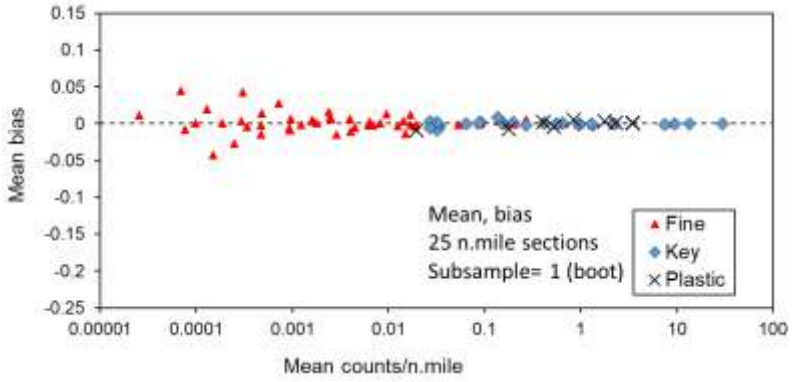


Figure 12: Effects of sub-sampling and boot-strapping on <u>total microplastic abundance</u> (counts/n.mile), showing x= "True" abundance based on original data, y="Boot" (abundance based on bootstrapped data) or y="Sub" (abundance based on subsampled data) for the same 25 n.mile section of the same tow (see Methods). a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. The dashed line shows 1:1 correspondence and the red-line is the y-on-x least-squares regression. Similar results are shown in Appendix 2 for types of microplastics (strand/no-strand, and colours).

a



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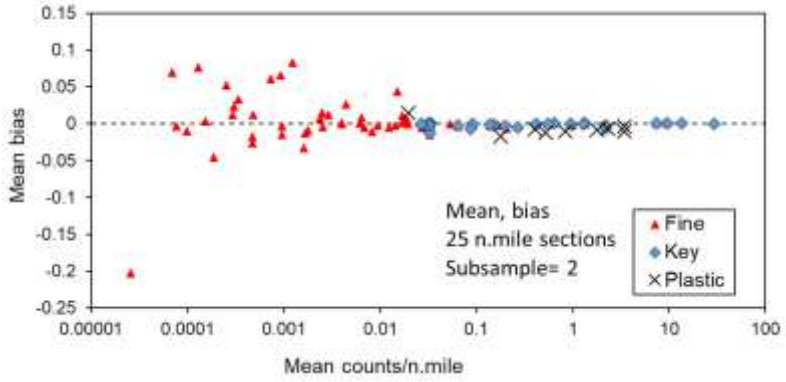

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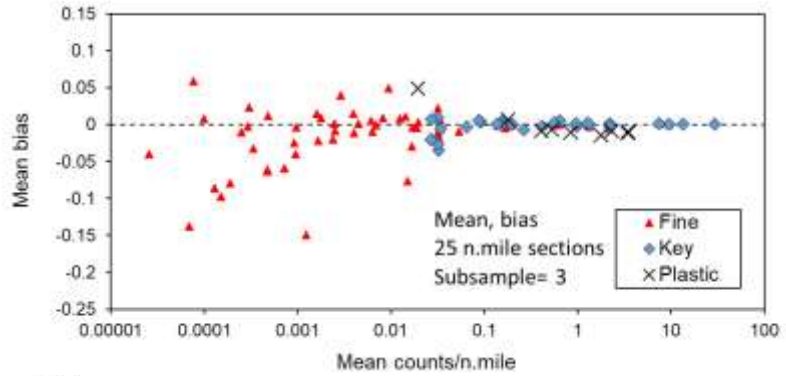

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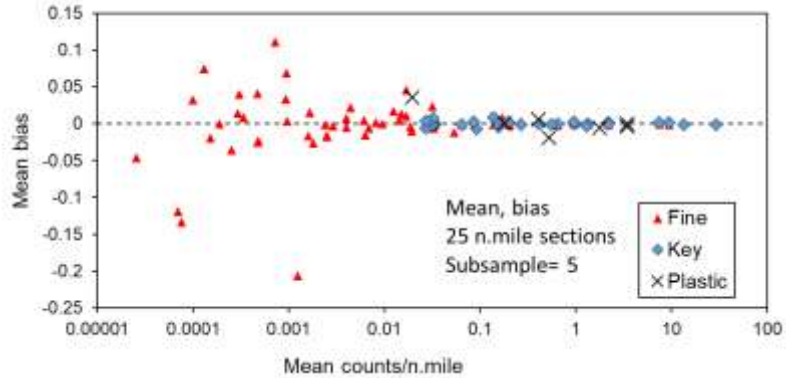
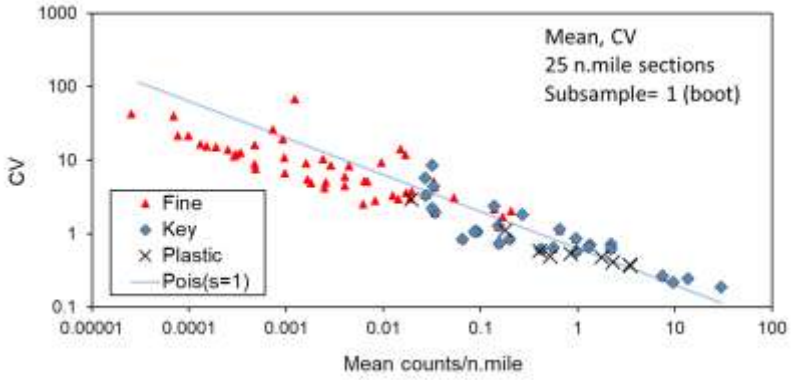


Figure 13: Effects of sub-sampling on the mean abundance (counts/n.mile) showing "Mean bias" = mean(sub–true)/scale where "true"=mean abundance from all data, "sub"=mean abundance from subsampled (or bootstrap) data for same 25 n.mile section of the same tow, and "scale"=mean(true) i.e. the average abundance over all data (see Methods). a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. "Key groups" are generally higher-abundance species and groups of zooplankton the focus of previous modelling work. "Fine" are generally lower abundance species and genus groupings of zooplankton. "Plastic" are microplastic particles. The dashed line shows zero bias.

a



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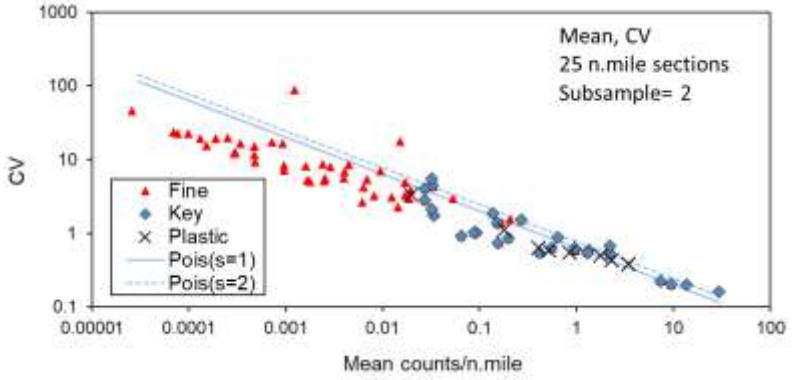

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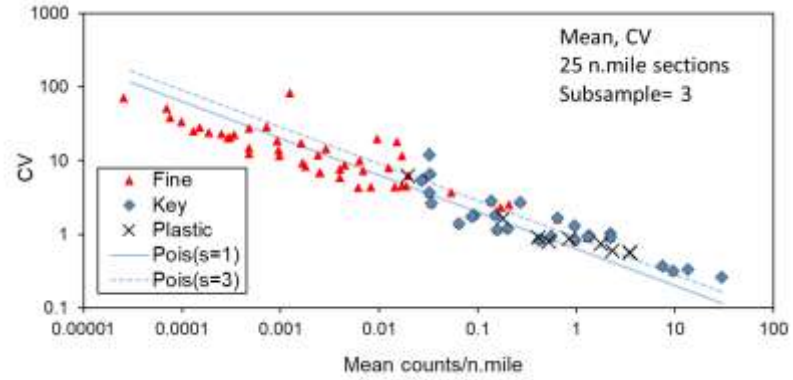

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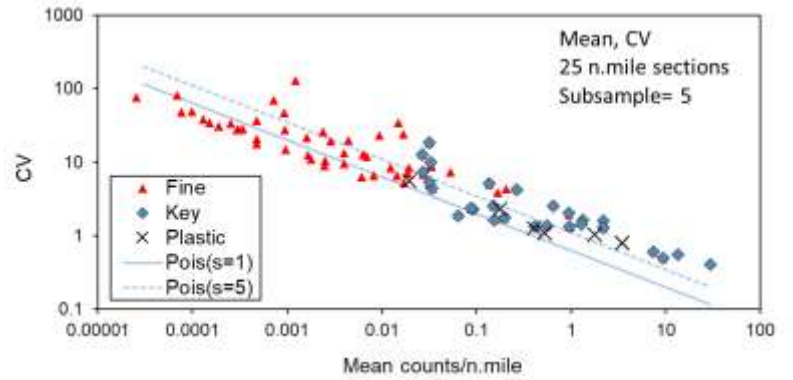
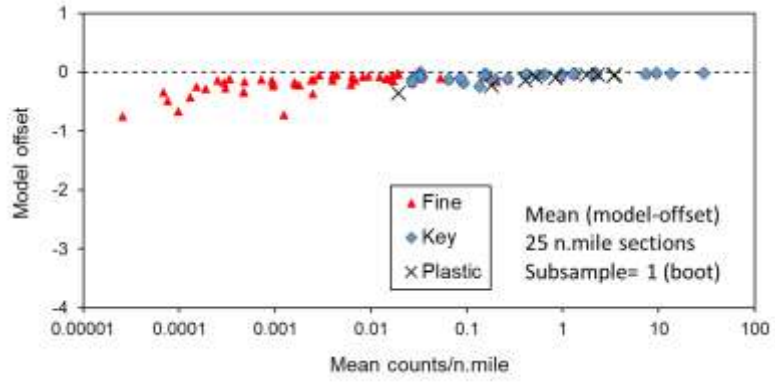


Figure 14: Effects of sub-sampling on the mean CV (coefficient of variation) calculated as the standard deviation(sub–true)/scale where "true"=mean abundance (counts/n.mile) based on all data (no subsampling) and "sub"=subsampled (or bootstrap) measure for same 25 n.mile section of the same tow, and "scale"=mean(true) i.e. the average abundance over all data (see Methods). a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. "Key groups" are generally higher-abundance species and groups of zooplankton the focus of previous modelling work. "Fine" are generally lower abundance species and genus groupings of zooplankton. "Plastic" are microplastic particles. Blue lines are Poisson model estimates of the CV for the full resolution data (subsampling, s=1) and subsampled data (s=2,3,5).

a



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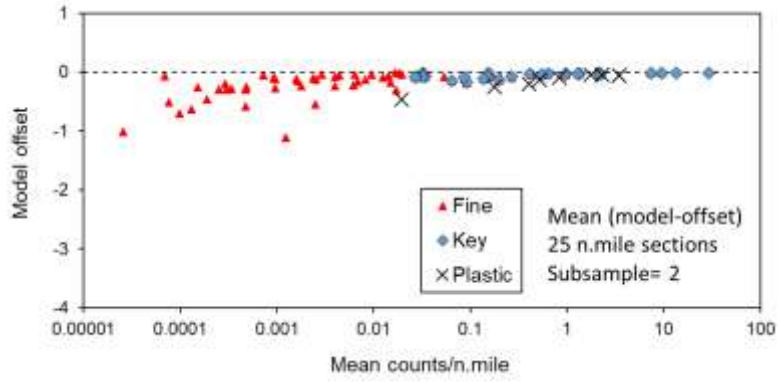

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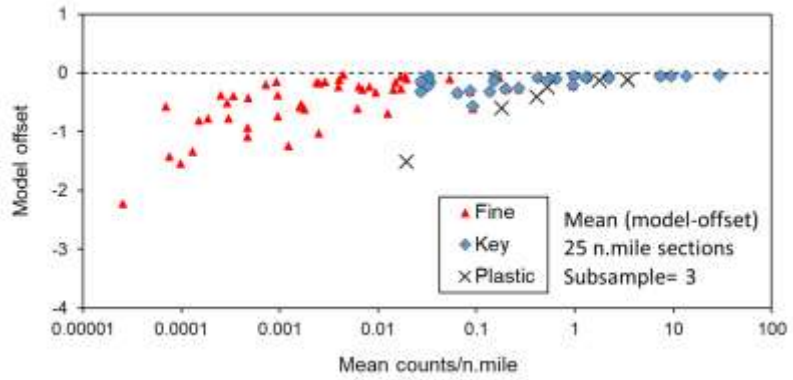

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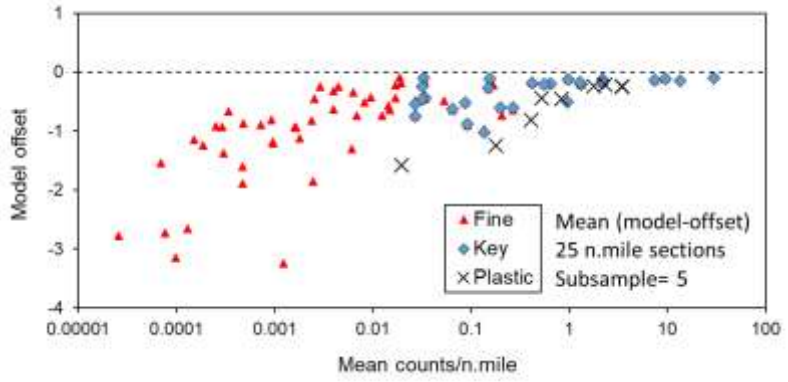
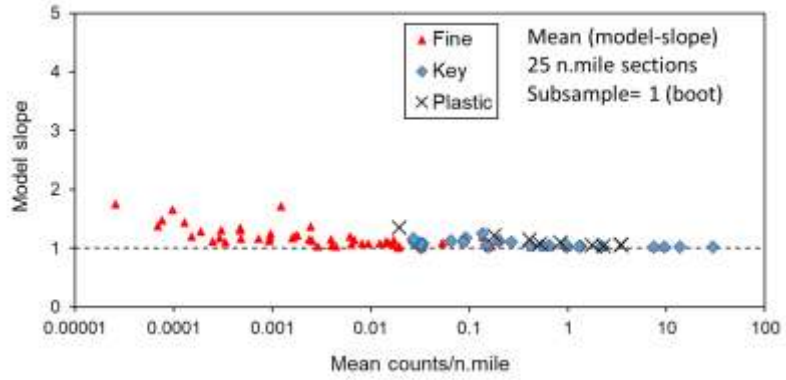


Figure 15: Effects of sub-sampling on the log-log model offset, i.e. "A" in: true=A+B*sub, where "true"= mean abundance (counts/n.mile) based on all data (no subsampling), and "sub"=subsampled (or bootstrap) measure for same 25 n.mile section of the same tow (see Methods). a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. "Key groups" are generally higher-abundance species and groups of zooplankton the focus of previous modelling work. "Fine" are generally lower abundance species and genus groupings of zooplankton. "Plastic" are microplastic particles. The dashed line shows zero log-model offset.

a



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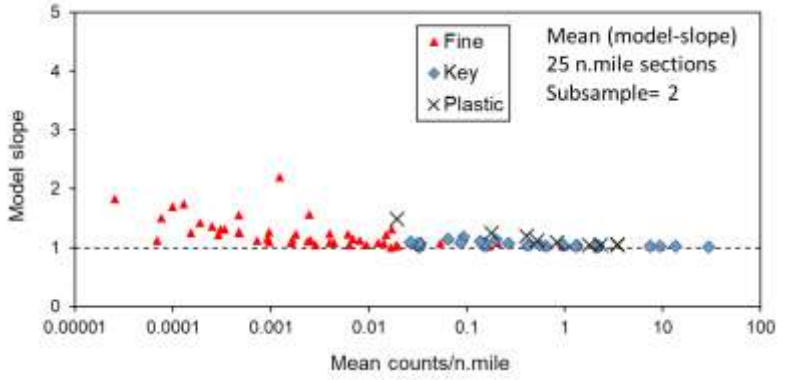
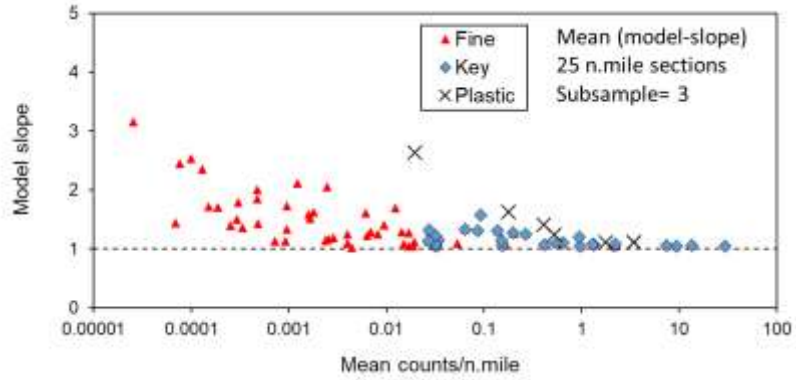


c



d

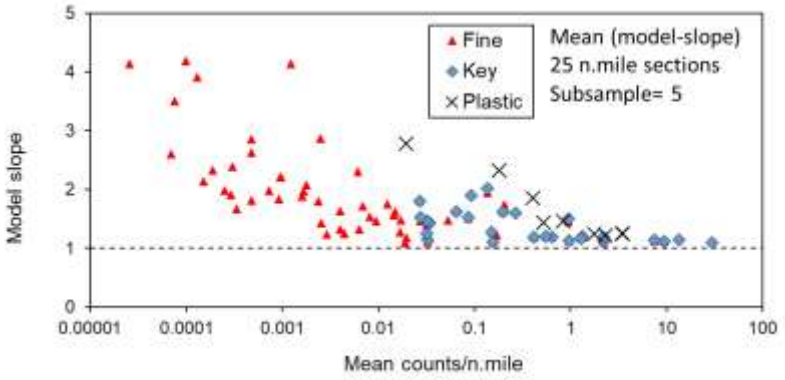


Figure 16: Effects of sub-sampling on the log-log model slope, i.e. "B" in: true=A+B*sub, where "true"= mean abundance (counts/n.mile) based on all data (no subsampling), and "sub"=subsampled (or bootstrap) measure for same 25 n.mile section of the same tow (see Methods). a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. "Key groups" are generally higher-abundance species and groups of zooplankton the focus of previous modelling work. "Fine" are generally lower abundance species and genus groupings of zooplankton. "Plastic" are microplastic particles. The dashed line 1:1 correspondence for the log-model (i.e. linear correspondence between true and sub).

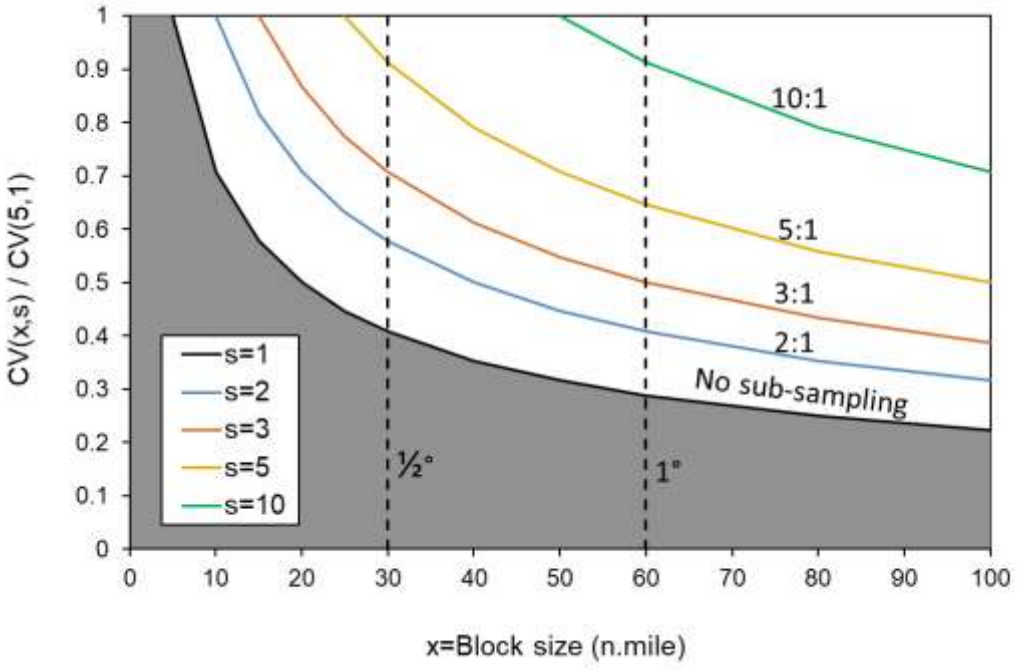


Figure 17: Poisson-model estimates of effects of subsampling and area-averaging on CV (uncertainty) of mean abundances.

Effects of subsampling on trends

The effects of subsampling on the trends in abundance of zooplankton and microplastics at the 5° scale were small (Table 5): we found that 2:1 subsampling introduced an average bias in trends of 3.7% of the mean (bias of 2.2% for total abundance), a CV (root mean square difference in trend divided by the mean abundance) of 0.8% and a small departure of the slope from unity (median slope of 1.03), similar to mean differences in trends due to bootstrap resampling. The mean slope for zooplankton groups was 1.34 due to the influence of one very high value, for *Metridia* spp. which is a relatively minor genus (present in 13.6% samples on average).

The effects of 2:1 subsampling on trends in plastic abundance were similar to the effects of subsampling on zooplankton abundances and also relatively minor: mean bias of 1.5%, mean CV of 0.6%, and a median slope of 1.03 (the mean slope is 1.5 due to the influence of one very high value, for the trend of non-strand plastic particles which are only 8% of the plastic particles).

Differences (bias, CV, model slope) also showed generally increasing effects of subsampling at 3:1 and 5:1 for both zooplankton and microplastic trends (Figure 18, Figure 19). Metrics of the effects of subsampling on trends at the 5° scale increased at smaller mean abundances (bias: Figure 20; CV: Figure 21; model offset: Figure 22; model slope: Figure 23). There seems to be a step up in the effect of subsampling below a mean abundance of about 0.2 counts/n. mile.

Table 5: Effects of bootstrapping and subsampling on trends in abundances in 5° latitude sections of key groups of zooplankton and microplastics. "Boot"=bootstrapped full samples; subsampling 2:1, 3:1; 5:1. "CV"=coefficient of variation (standard deviation/mean). The s2 linear model: true=A+B*sub, where "true"= mean abundance (counts/n.mile) based on all data (no subsampling or bootstrapping), and "sub"=subsampled measure for same 25 n.mile section of the same tow, and "mean"= mean true value (all tows) as scaling variable (see Methods).

	Bias (%)				CV				Linear model, 2:1	
	boot	2:1	3:1	5:1	boot	2:1	3:1	5:1	A/mean (%)	B
Total abundance	-2.6	-2.2	0.9	1.1	0.3	0.5	1.0	1.3	-1.1	1.0
Copepoda	-2.4	-1.3	-4.9	1.5	0.2	0.6	0.9	1.4	-0.3	1.0
Oithona	1.7	-0.8	0.4	0.9	0.2	0.7	1.1	1.4	-0.2	1.0
Fritillaria	4.6	7.1	1.7	-0.4	0.4	0.7	1.2	1.4	14.0	1.1
Pteropods	-16.0	-3.3	-21.9	-46.2	0.6	0.7	1.4	2.3	-0.5	1.1
Oikopleura	8.4	7.2	16.5	20.7	0.5	1.0	1.2	1.6	16.2	1.1
Amphipoda	-6.4	-7.4	1.3	10.8	0.6	0.8	1.3	1.6	-3.9	1.1
Ostracoda	-63.8	-51.4	-75.8	-87.2	1.3	0.9	1.1	1.1	-92.3	1.4
Chaetognatha	50.7	65.9	34.8	65.7	2.4	0.8	1.1	1.4	8.0	1.9
Calanoida indet small	0.1	-1.7	-1.5	-0.7	0.1	0.6	1.0	1.3	-1.5	1.0
Foraminifera	5.3	5.0	8.2	8.4	0.2	1.0	1.0	1.3	6.9	1.0
Calanus simillimus	0.7	-1.3	-8.7	-7.7	0.5	0.6	1.2	1.5	-3.9	1.0
Ctenocalanus citer	-7.7	-10.4	-6.7	-8.0	0.4	0.8	1.0	1.5	-13.2	1.0
Calanoida indet large	-6.3	-7.7	-2.9	-0.6	0.4	0.8	1.3	1.6	-6.2	1.1
Clausocalanus	-4.9	-10.3	-6.9	4.3	0.4	0.8	1.0	1.5	10.5	1.2
Rhincalanus gigas	4.1	0.5	5.8	7.1	0.2	1.0	1.0	1.4	-1.1	1.0
Neocalanus tonsus	-1.2	-3.6	-8.6	-7.0	0.5	0.7	0.6	2.0	-3.9	1.0
Thysanoessa macrura	14.7	11.5	0.2	-27.2	2.9	1.0	1.0	1.1	-117.2	2.3
Radiolaria	-2.8	-8.8	-11.8	-45.4	0.4	1.1	1.8	3.4	-3.1	0.9
Calanoides acutus	7.1	-8.9	16.5	39.6	1.2	1.0	1.3	1.5	-8.6	1.0
Metridia	71.2	-1.3	-25.8	-80.2	6.7	0.5	0.6	1.0	-418.1	5.2
Thaliacea	-7.9	-7.3	-5.3	-16.0	0.8	0.5	1.1	1.5	-7.3	1.0
Calanus propinquus				No data				No data		No data
Paracalanus	18.9	6.7	6.9	15.1	0.8	0.5	0.7	1.4	2.3	1.0
Oncaea	2.0	-5.2	16.2	23.6	0.8	0.3	1.2	1.7	-5.8	1.0
Thysanoessa gregaria				No data				No data		No data
Euphausia superba	-58.3	-64.5	-75.1	-82.5	1.3	1.2	1.3	1.6	-168.6	2.0
Plastic_total	1.2	-2.2	0.9	1.1	0.3	0.1	0.3	0.4	0.2	1.0
N_strand	1.3	-1.3	-4.9	1.5	0.2	0.2	0.2	0.3	-3.4	1.1
N_notStrand	22.2	-0.8	0.4	0.9	0.2	0.2	0.2	0.3	389.8	4.3
N_black	-1.0	7.1	1.7	-0.4	0.4	0.3	0.4	0.5	-16.1	1.2
N_blue	-2.4	-3.3	-21.9	-46.2	0.6	0.4	0.9	1.5	-66.3	1.6
N_clear	3.0	7.2	16.5	20.7	0.5	0.5	0.6	0.8	-9.3	1.2
N_small	10.0	-7.4	1.3	10.8	0.6	0.5	0.8	0.9	31.9	1.2
N_medium	0.7	-51.4	-75.8	-87.2	1.3	1.2	1.5	1.5	-1.0	1.0
N_large	-0.4	65.9	34.8	65.7	2.4	1.9	2.7	3.3	-23.9	1.2

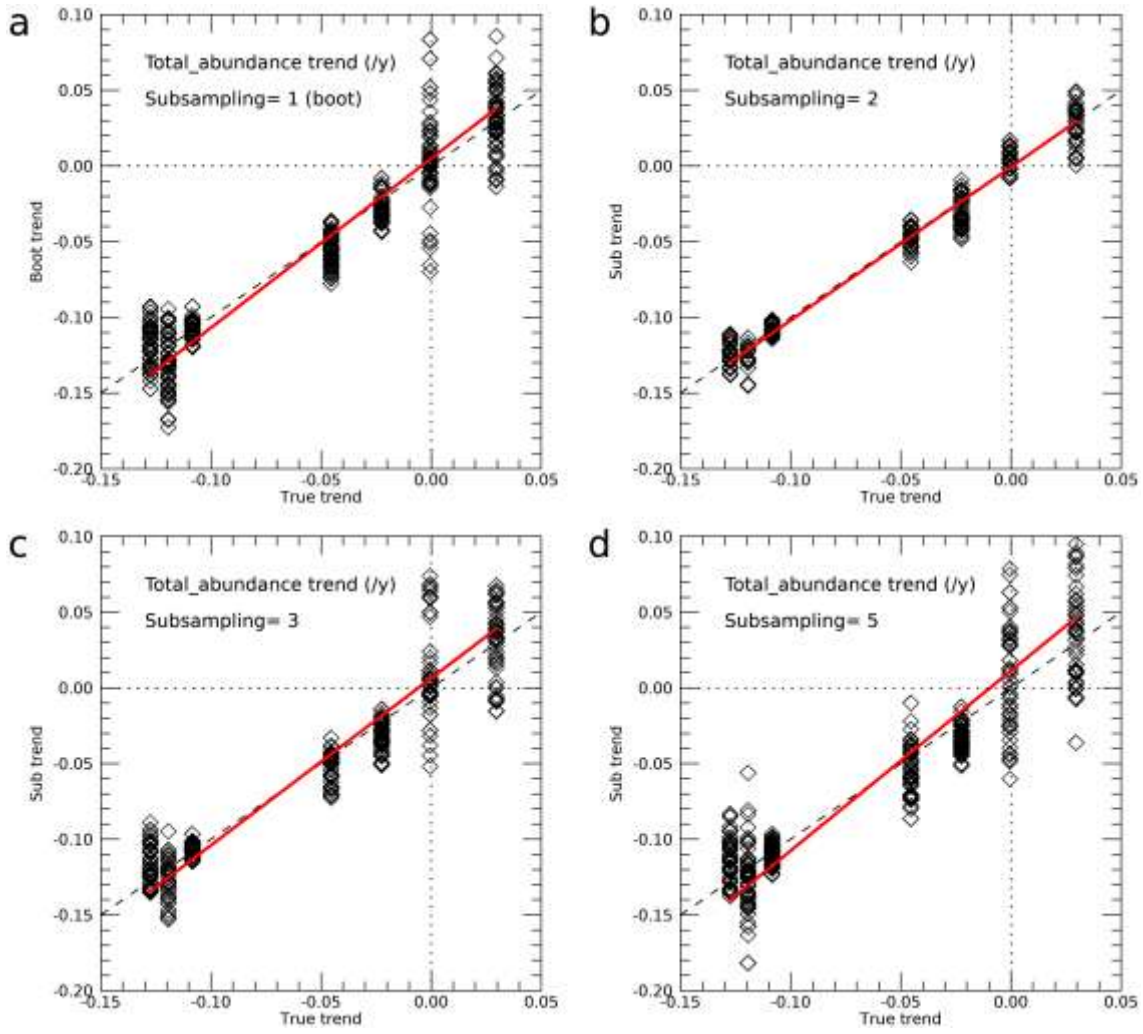


Figure 18: Effects of sub-sampling and bootstrapping on <u>trends in total zooplankton abundance</u> (counts/n.mile/y), showing x="True trend" based on original data, y="Boot trend" (trend based on bootstrapped data) or y="Sub trend" (trend based on subsampled) abundance data for same 5° latitudinal section of data in the Ross Sea region only (see Methods). a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. The dashed line shows 1:1 correspondence and the red-line is the y-on-x least-squares regression. Similar results are shown in Appendix 3 for other "Key groups" (generally higher-abundance groups of zooplankton which were the focus of previous modelling work).

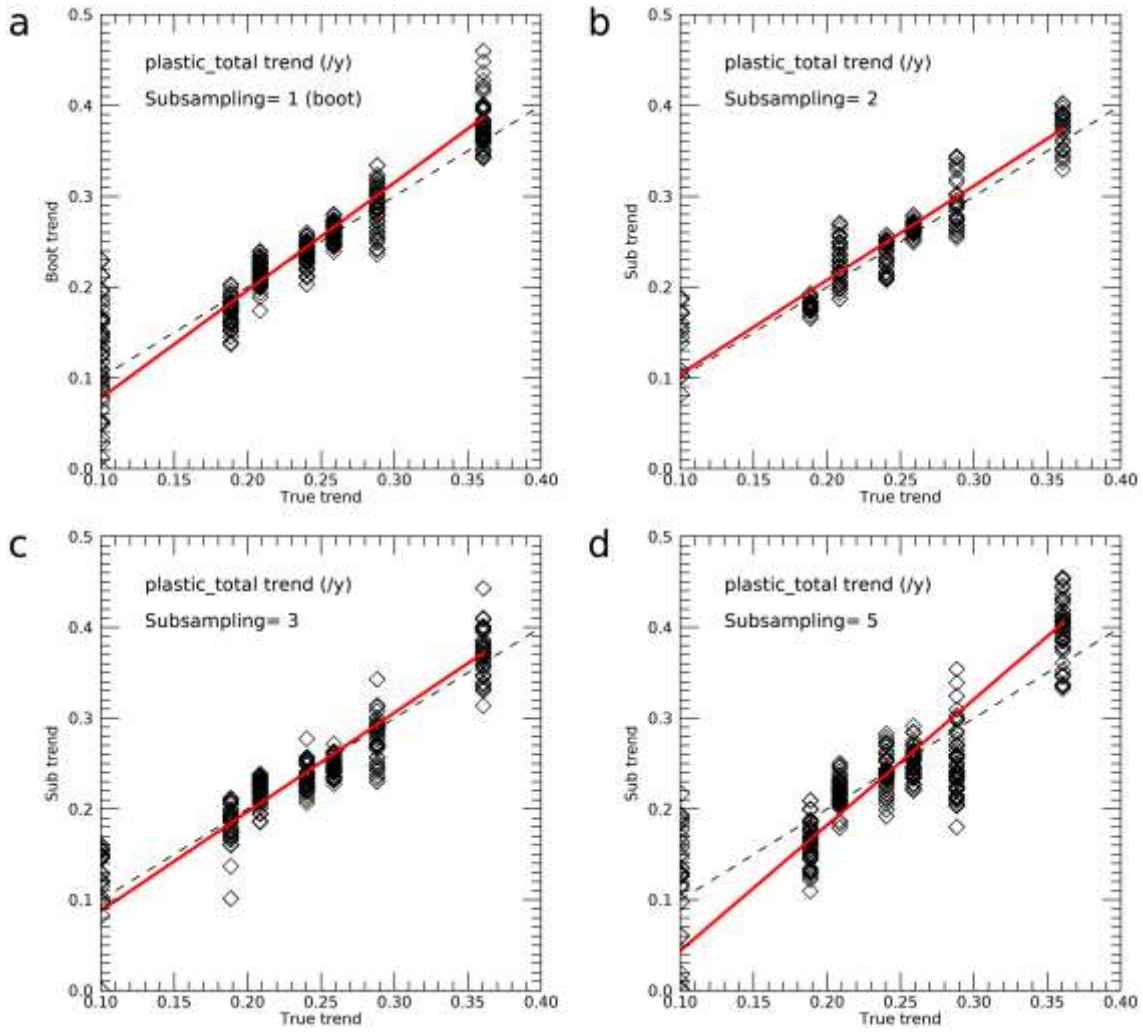
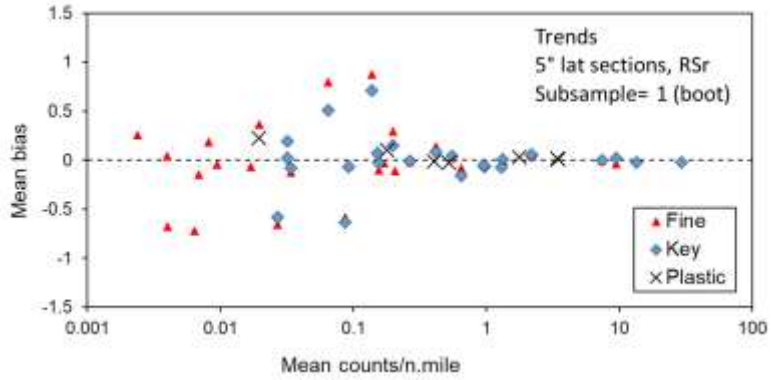


Figure 19: Effects of sub-sampling and bootstrapping on <u>trends in total microplastic abundance</u> (counts/n.mile/y), showing x="True trend" based on original data, y="Boot trend" (trend based on bootstrapped data) or y="Sub trend" (trend based on subsampled) abundance data for same 5° latitudinal section of data in the Ross Sea region only (see Methods). a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. The dashed line shows 1:1 correspondence and the red-line is the y-on-x least-squares regression. Similar results are shown in Appendix 4 for different types of microplastics.

a



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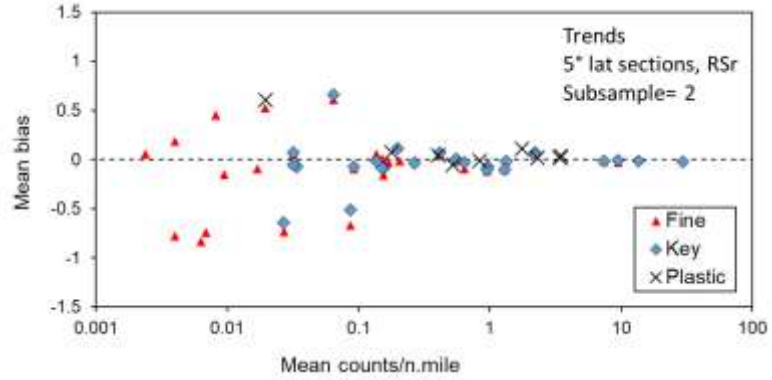

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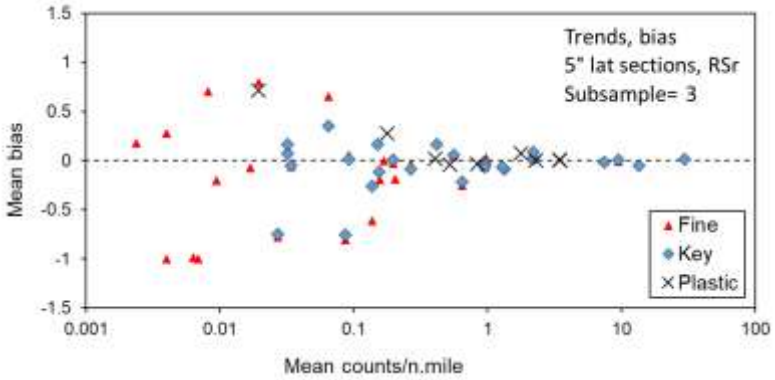

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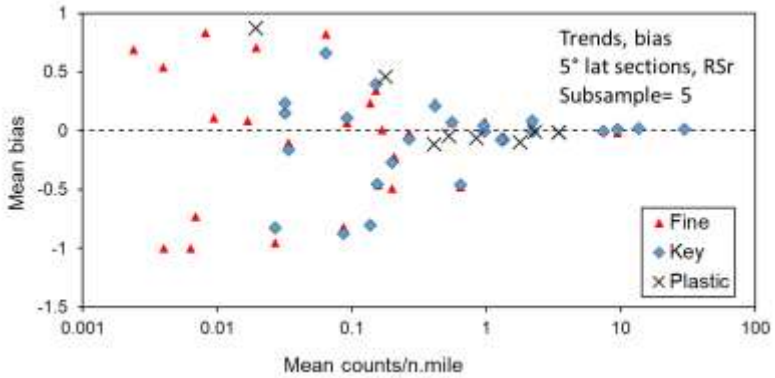
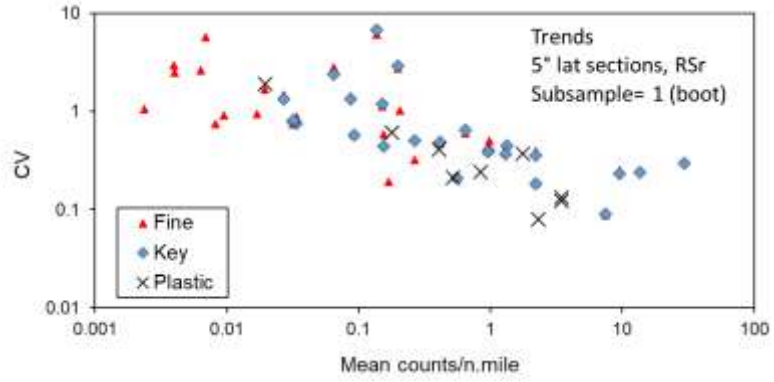


Figure 20: Effects of sub-sampling on the trend in ln(abundance) over time within 5° latitudinal sections in the Ross Sea region (RSr), showing "Mean bias" = mean[trend(true)–trend(sub)]/scale where "trend(true)"=trend in ln(abundance) from all data, "trend(sub)"=trend based on subsampled (or bootstrap) data for same 5° latitudinal section, and "scale"=mean absolute trend over all 5° sections (see Methods). The x-axis shows the average abundance over all data. a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. "Key groups" are generally higher-abundance species and groups of zooplankton the focus of previous modelling work. "Fine" are generally lower abundance species and genus groupings of zooplankton. "Plastic" are microplastic particles. The dashed line shows zero bias.

a



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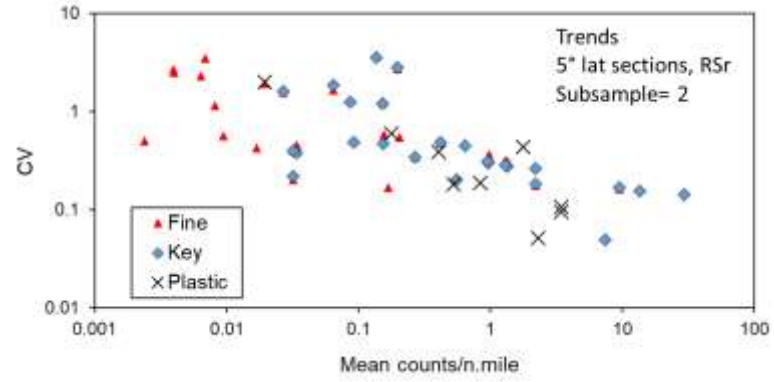

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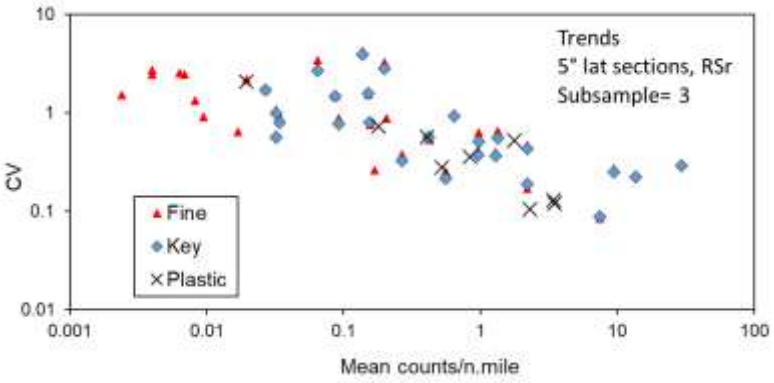

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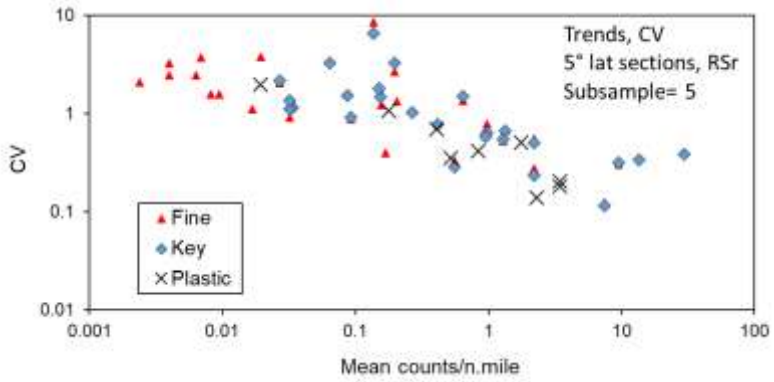
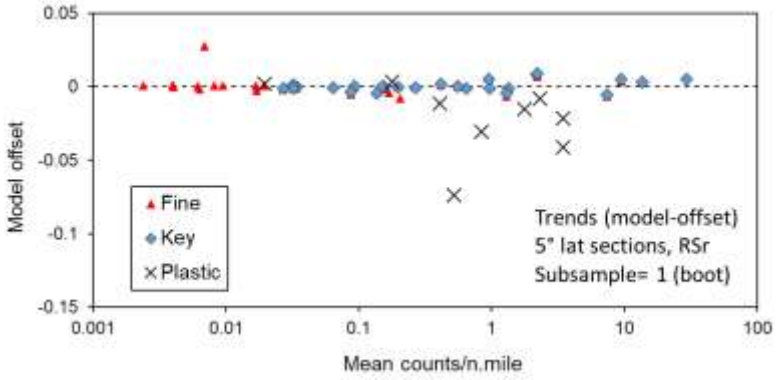


Figure 21: Effects of sub-sampling on the trend CV (coefficient of variation) calculated as the standard deviation[trend(sub)–trend(true)]/scale within 5° latitudinal sections in the Ross Sea region (RSr), where "trend(true)"=trend in ln(abundance) from all data, "trend(sub)"=trend based on subsampled (or bootstrap) data for same 5° latitudinal section, and "scale"=mean absolute trend over all 5° sections (see Methods). The x-axis shows the average abundance over all data. a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. "Key groups" are generally higher-abundance species and groups of zooplankton the focus of previous modelling work. "Fine" are generally lower abundance species and genus groupings of zooplankton. "Plastic" are microplastic particles.

a



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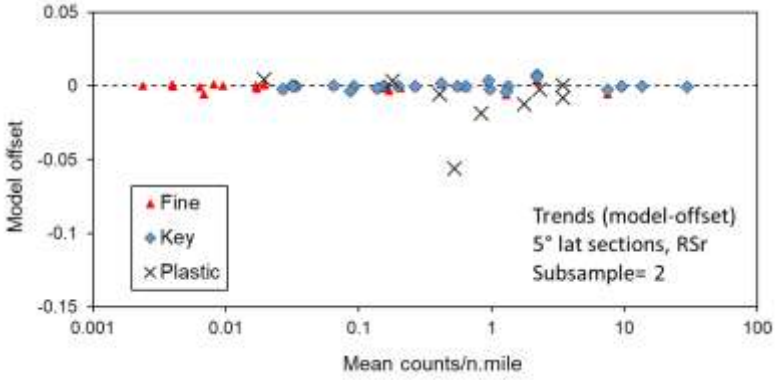

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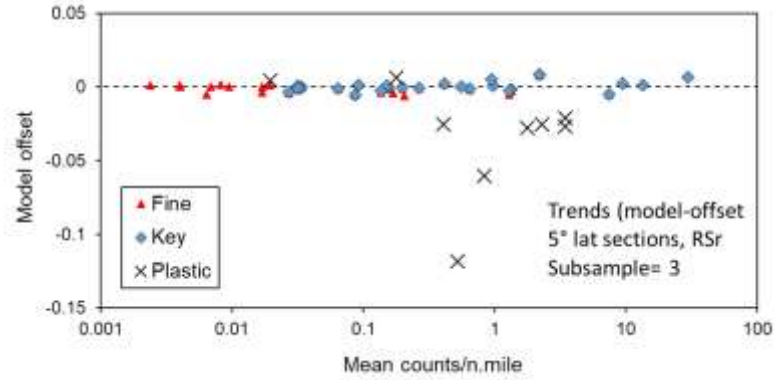

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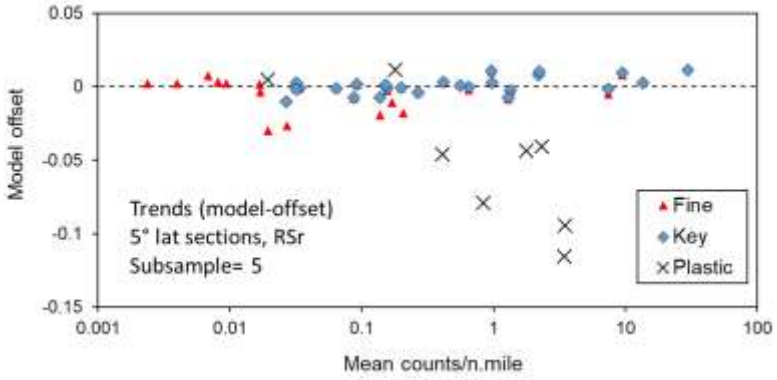
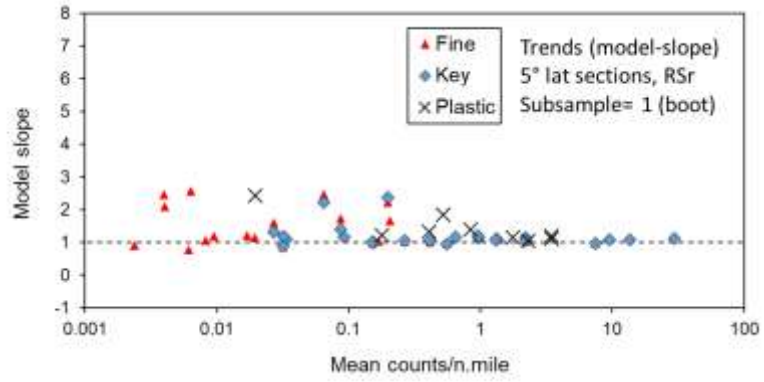


Figure 22: Effects of sub-sampling on the model offset, i.e. "A" in: trend(true)=A+B*trend(sub), where "trend(true)"=trend in ln(abundance) from all data, "trend(sub)"=trend based on subsampled (or bootstrap) data for same 5° latitudinal section (see Methods). a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. "Key groups" are generally higher-abundance species and groups of zooplankton the focus of previous modelling work. "Fine" are generally lower abundance species and genus groupings of zooplankton. "Plastic" are microplastic particles. The dashed line shows zero model offset.

a



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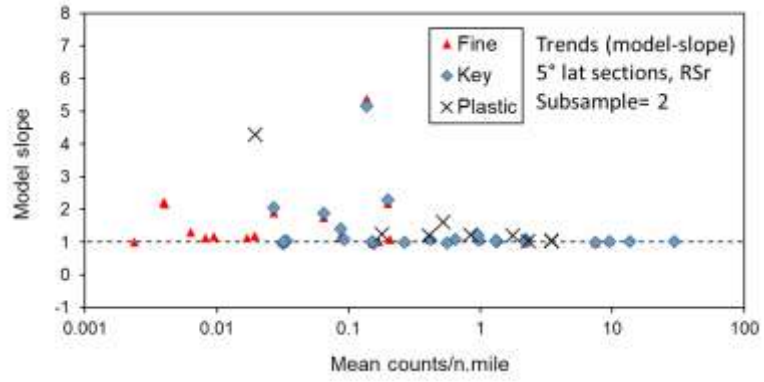

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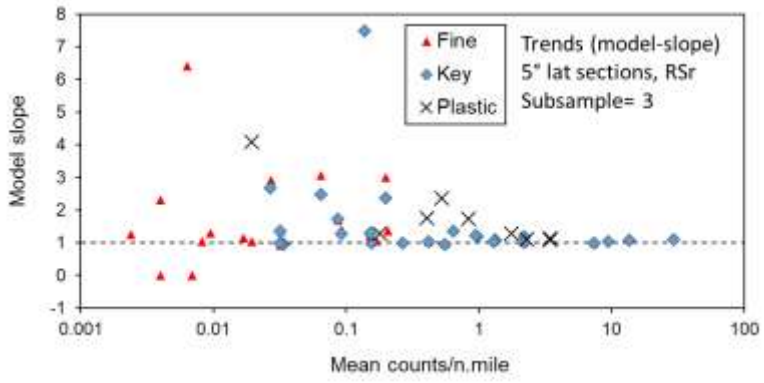

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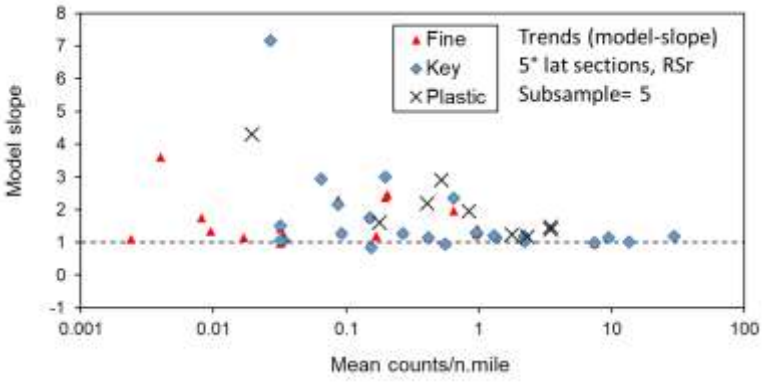

Figure 23: Effects of sub-sampling on the model slope, i.e. "B" in: trend(true)=A+B*trend(sub), where "trend(true)"=trend in ln(abundance) from all data, "trend(sub)"=trend based on subsampled (or bootstrap) data for same 5° latitudinal section (see Methods). a: No subsampling (bootstrap samples); b: Subsampling 2:1; c: Subsampling 3:1; d: Subsampling 5:1. "Key groups" are generally higher-abundance species and groups of zooplankton the focus of previous modelling work. "Fine" are generally lower abundance species and genus groupings of zooplankton. "Plastic" are microplastic particles. The dashed line shows zero model offset.

Effects of subsampling on biogeographic abundances

We found that rebuilding the biogeographic models of "abundance" via the proxy of habitat suitability based on 2:1 subsampled data did introduce bias and uncertainty compared to the models built on full data (Table 6). The pixel-by-pixel correspondence between the full and subsampled models were reasonably good, always being significant at $p<0.001$ and with a median slope close to unity (1.19). The mean R^2 was 0.64, and the mean bias was 22%. Examples for three groups of the spatial correspondence (Figure 24) and pixel-by-pixel comparisons (Figure 25) show that whereas the spatial patterns are very similar in most areas, there are bigger differences in those areas with few in situ measurements (especially the Pacific sector of the Southern Ocean and at the northern limits of the study area). The summary plots at the monthly climatological scale (Figure 26) confirm what is seen at the cumulative level, that generally, the departures from 1:1 correspondence (bias, CV, model slope and offset) are greater at lower mean abundances.

Table 6: Mean abundance: effects of 2:1 subsampling on biogeographic models of habitat suitability. Results shown are the pixel-by-pixel comparisons from the combined BRT models built on the full and subsampled data and analysed at the long-term (cumulative) average time-scale. "RMA model" refers to the reduced major axis linear regression from log(x) versus log(y). All regressions are significant at p<0.001 (F-test, n=1.23E6).

Group				RMA model		
	Mean	Bias	CV	R^2	Offset/mean	Slope
Total_abundance	120.0	0.09	0.23	0.78	-0.11	1.20
Copepoda	67.0	0.02	0.17	0.87	0.12	0.90
Oithona	20.7	0.19	0.54	0.84	-0.28	1.48
Fritillaria	2.43	1.02	2.64	0.46	-1.26	3.27
Pteropods	0.72	0.26	1.60	0.34	0.00	1.26
Oikopleura	1.11	0.17	0.38	0.90	0.02	1.15
Amphipoda	0.41	-0.01	0.16	0.87	-0.16	1.15
Ostracoda	0.52	0.01	0.46	0.62	0.01	0.99
Chaetognatha	0.34	0.25	0.50	0.78	-0.14	1.39
Calanoida_indet_small	30.4	0.27	0.67	0.26	-0.47	1.74
Foraminifera	3.91	-0.13	0.51	0.51	0.14	0.74
Calanus_simillimus	2.10	0.10	0.52	0.91	0.00	1.10
Ctenocalanus_citer	2.04	0.12	0.71	0.40	-0.47	1.59
Calanoida_indet_large	1.52	0.21	0.72	0.70	-0.09	1.30
Clausocalanus	5.35	0.05	0.32	0.79	-0.16	1.21
Rhincalanus_gigas	0.85	0.27	0.75	0.83	0.10	1.16
Neocalanus_tonsus	1.57	0.81	2.28	0.80	-0.33	2.14
Thysanoessa_macrura	0.51	0.04	0.30	0.84	-0.12	1.16
Radiolaria	0.23	-0.14	1.41	0.53	0.21	0.65
Calanoides_acutus	0.30	0.21	1.11	0.63	0.02	1.19
Metridia	1.78	0.03	0.55	0.43	0.29	0.75
Thaliacea	0.39	0.43	1.37	0.47	0.09	1.34
Calanus_propinquus	0.07	-0.05	1.17	0.62	-0.07	1.03
Paracalanus	0.07	0.16	15.42	0.20	0.92	0.18
Oncaea	0.28	0.94	1.75	0.53	0.02	1.92
Thysanoessa_gregaria	0.13	0.33	1.43	0.54	-0.41	1.74
Euphausia_superba	0.16	0.30	4.03	0.81	0.19	1.11

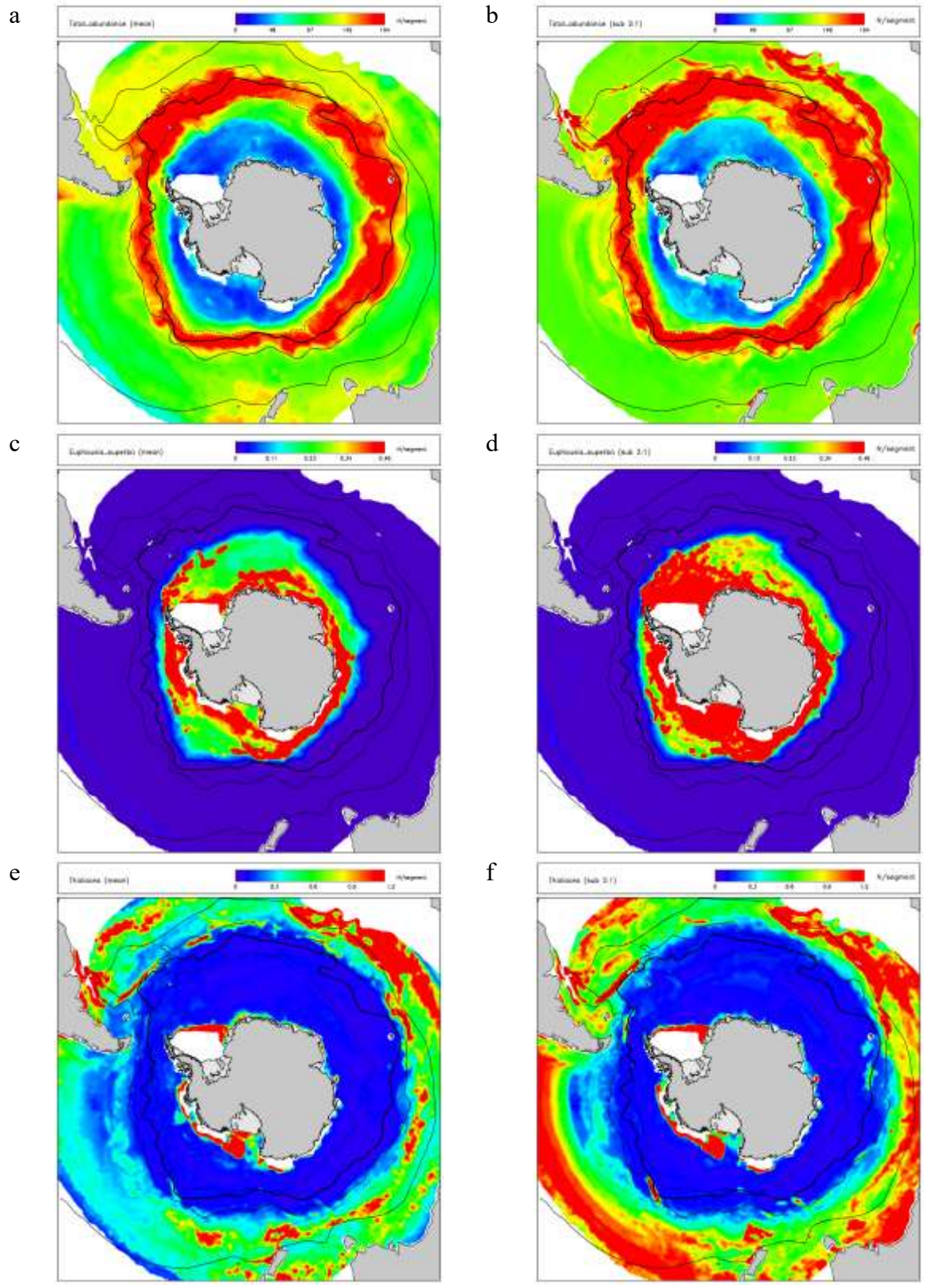

Figure 24: Effects of subsampling on mean abundances of three groups of zooplankton using the combined boosted regression tree (BRT) biogeographic model of habitat suitability, long-term (cumulative) average. Left column gives the modelled suitability based on the full dataset and right column is based on the 2:1 subsampled data. a,b: Total zooplankton abundance; c,d: Antarctic krill, *Euphausia superba*; e,f: Thaliacea (tunicates or gelatinous zooplankton including salps and pyrosomes).

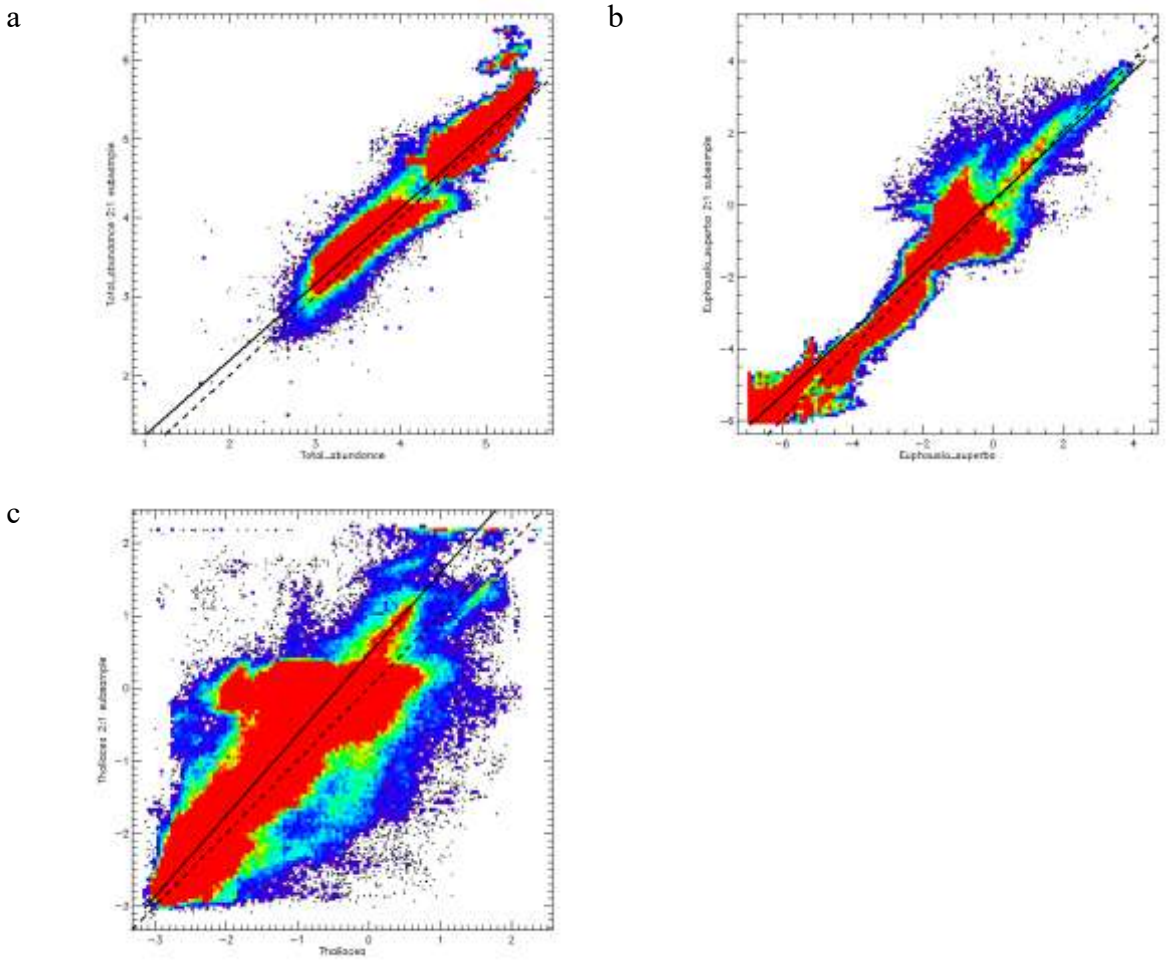

Figure 25: Pixel-by-pixel comparison of effects of subsampling on mean abundances of three groups of zooplankton (as examples) using the combined boosted regression tree (BRT) biogeographic model of habitat suitability, long-term (cumulative) average. The modelled suitability based on the full dataset is plotted on the x-axis and the y-axis shows the value for the same location based on the 2:1 subsampled data. a: Total zooplankton abundance; b: Antarctic krill, *Euphausia superba*; c: Thaliacea (tunicates or gelatinous zooplankton including salps and pyrosomes).

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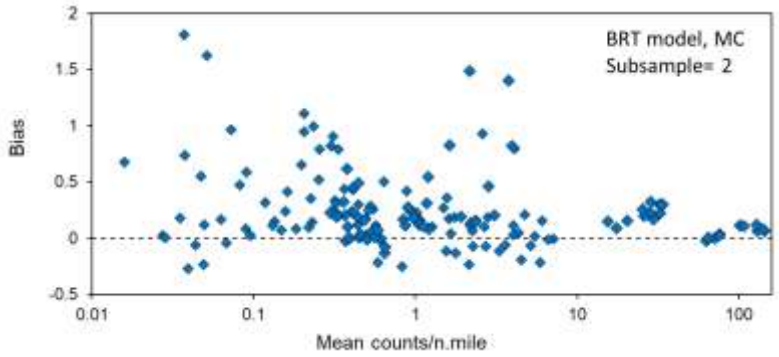

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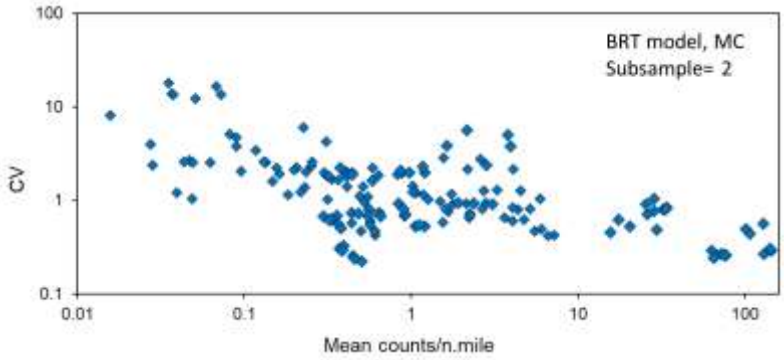

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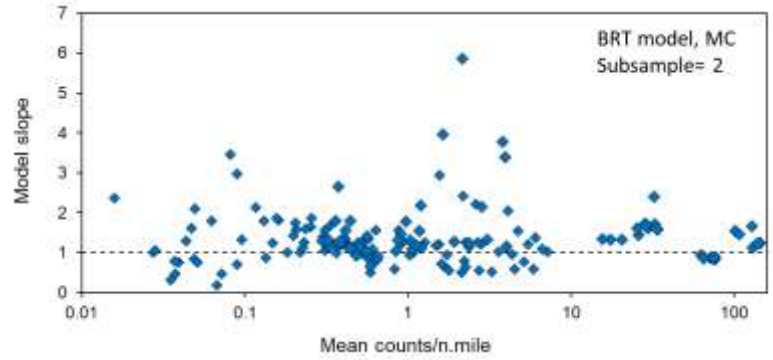

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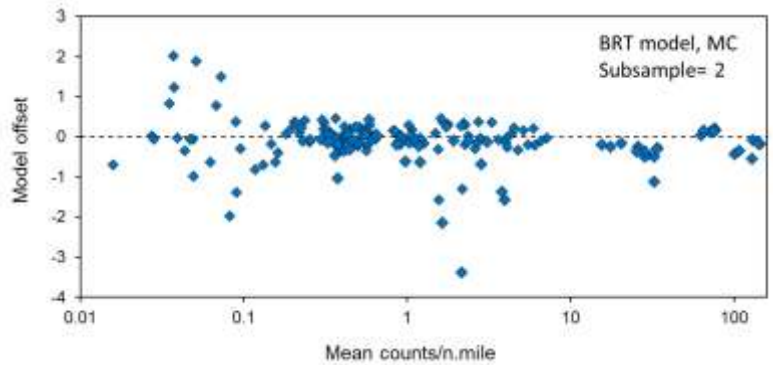

Figure 26: Effects of 2:1 sub-sampling on biogeographic models of monthly climatological abundance (habitat suitability). a: Bias (mean difference as a proportion of overall mean); b: CV; c: Slope of reduced major axis regression, log-log; d: Slope of reduced major axis regression, log-log, as proportion of overall mean. Only "key" groups were modelled. The dashed lines show exact agreement.

Effects of subsampling on biogeographic trends

Linear trends (Sen slopes) built on the monthly biogeographic models of habitat suitability for each of the "key" and "fine" groups of zooplankton were compared before and after subsampling at 2:1 (Table 7). The pixel-by-pixel correspondence between the full and subsampled models were reasonably good, always being significant at $p<0.001$ and the median slope was close to unity (1.06). The mean R^2 was only 0.16 overall, but higher for "high abundance" groups like *Total_abundance* (R^2=0.28) and *Oithona* (R^2=0.48). The mean bias introduced into the modelling as a result of subsampling was less than 1%. Examples for three example groups of the spatial correspondence (Figure 27) and pixel-by-pixel comparisons (Figure 28) show that whereas the spatial patterns are very similar in most areas, there are bigger differences in those areas with few in situ measurements, especially the Pacific sector of the Southern Ocean and at the northern limits of the study area (lower latitudes). Generally, the departures from 1:1 correspondence (bias, CV, model slope and offset) are greater at lower abundances (Figure 29) but the variation is not as pronounced as for previous results.

Table 7: Trends: effects of 2:1 subsampling on trends in habitat suitability from biogeographic models. Results shown are the pixel-by-pixel comparisons from the combined BRT models built on the full and subsampled data. "RMA model" refers to the reduced major axis linear regression from log(x) versus log(y). All regressions are significant at p<0.001 (F-test, n=1.23E6).

				RMA model		
Group	Mean (absolute)	Bias	CV	R^2	Offset/mean	Slope
Total_abundance	0.2419	-0.26	1.75	0.28	-0.20	0.92
Copepoda	0.1486	-0.03	1.95	0.18	-0.01	0.96
Oithona	0.0498	0.27	2.85	0.48	-0.08	1.66
Fritillaria	0.0084	0.48	6.50	0.11	0.09	2.56
Pteropods	0.0019	0.32	12.88	0.03	0.25	1.97
Oikopleura	0.0023	0.39	2.69	0.38	0.24	0.74
Amphipoda	0.0008	0.04	2.74	0.23	-0.11	1.40
Ostracoda	0.0007	-0.45	2.88	0.05	-0.43	0.95
Chaetognatha	0.0008	0.05	2.80	0.16	-0.14	1.30
Calanoida_indet_small	0.0367	-0.41	2.77	0.11	-0.48	1.20
Foraminifera	0.0162	-0.03	1.78	0.23	-0.01	0.88
Calanus_simillimus	0.0029	-0.13	3.87	0.20	-0.13	1.06
Ctenocalanus_citer	0.0047	-0.49	2.47	0.05	-0.49	1.00
Calanoida_indet_large	0.0054	0.07	3.49	0.29	-0.23	1.47
Clausocalanus	0.0267	-0.61	1.92	0.10	-0.31	0.60
Rhincalanus_gigas	0.0039	0.16	2.01	0.53	0.06	1.16
Neocalanus_tonsus	0.0028	-0.58	12.10	0.14	-0.54	1.91
Thysanoessa_macrura	0.0010	-0.09	2.37	0.15	-0.08	0.93
Radiolaria	0.0002	0.23	9.08	0.04	-0.41	2.92
Calanoides_acutus	0.0011	0.31	4.89	0.14	0.15	1.22
Metridia	0.0038	-0.58	4.07	0.05	-0.64	1.32
Thaliacea	0.0010	0.17	5.86	0.03	0.15	1.04
Calanus_propinquus	0.0001	0.18	10.98	0.04	0.33	0.52
Paracalanus	0.0002	0.18	54.86	0.01	0.56	0.23
Oncaea	0.0010	1.00	6.62	0.07	0.91	1.80
Thysanoessa_gregaria	0.0004	-0.88	4.56	0.00	-0.67	0.74
Euphausia_superba	0.0003	0.52	31.84	0.11	0.52	1.01

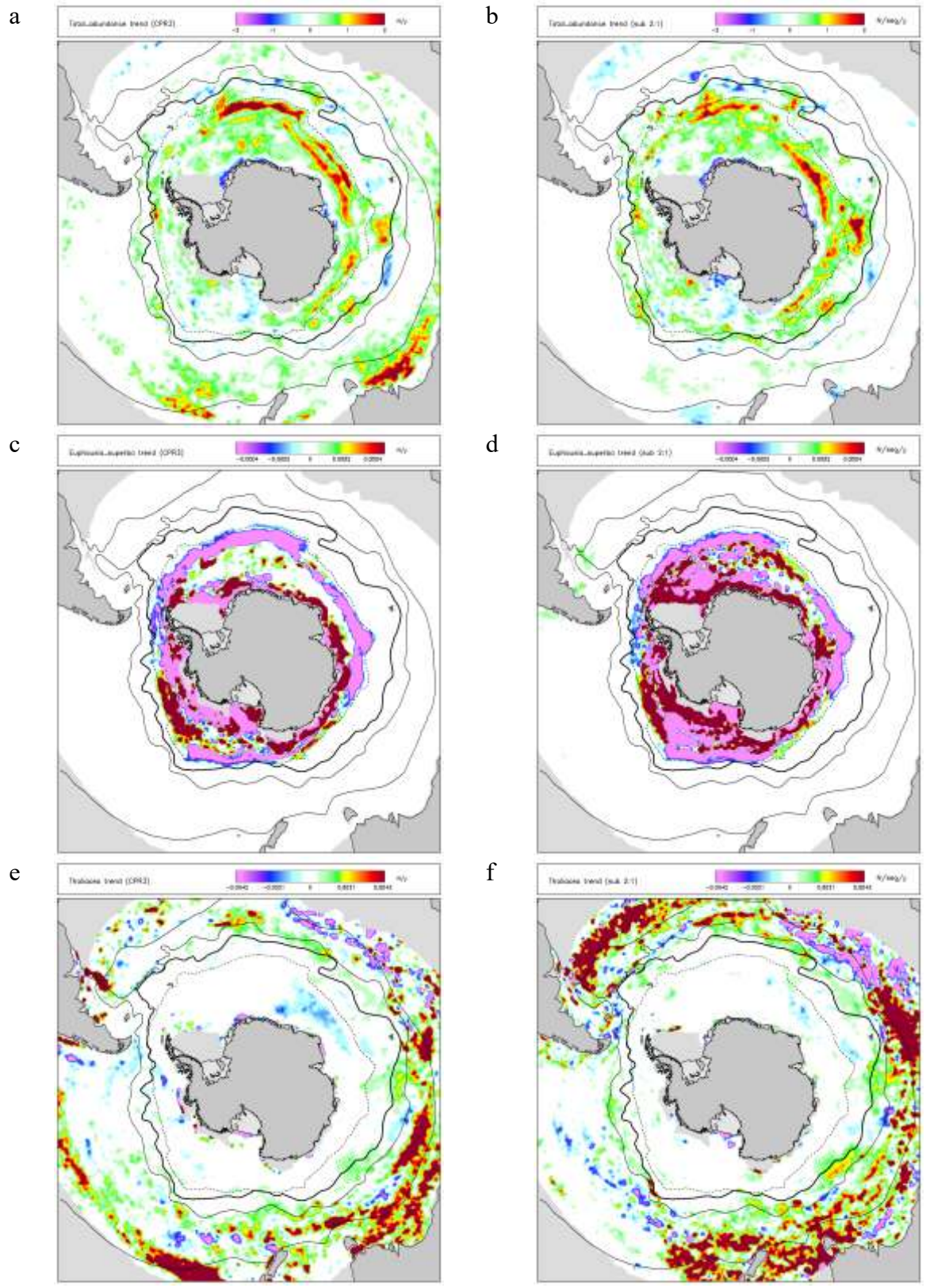

Figure 27: Trends: effects of subsampling on trends of three groups of zooplankton using the combined boosted regression tree (BRT) biogeographic model of habitat suitability. Left column gives the trend in modelled suitability based on the full dataset and right column is based on the 2:1 subsampled data. a,b: Total zooplankton abundance; c,d: Antarctic krill, *Euphausia superba*; e,f: Thaliacea (gelatinous zooplankton including salps and pyrosomes).

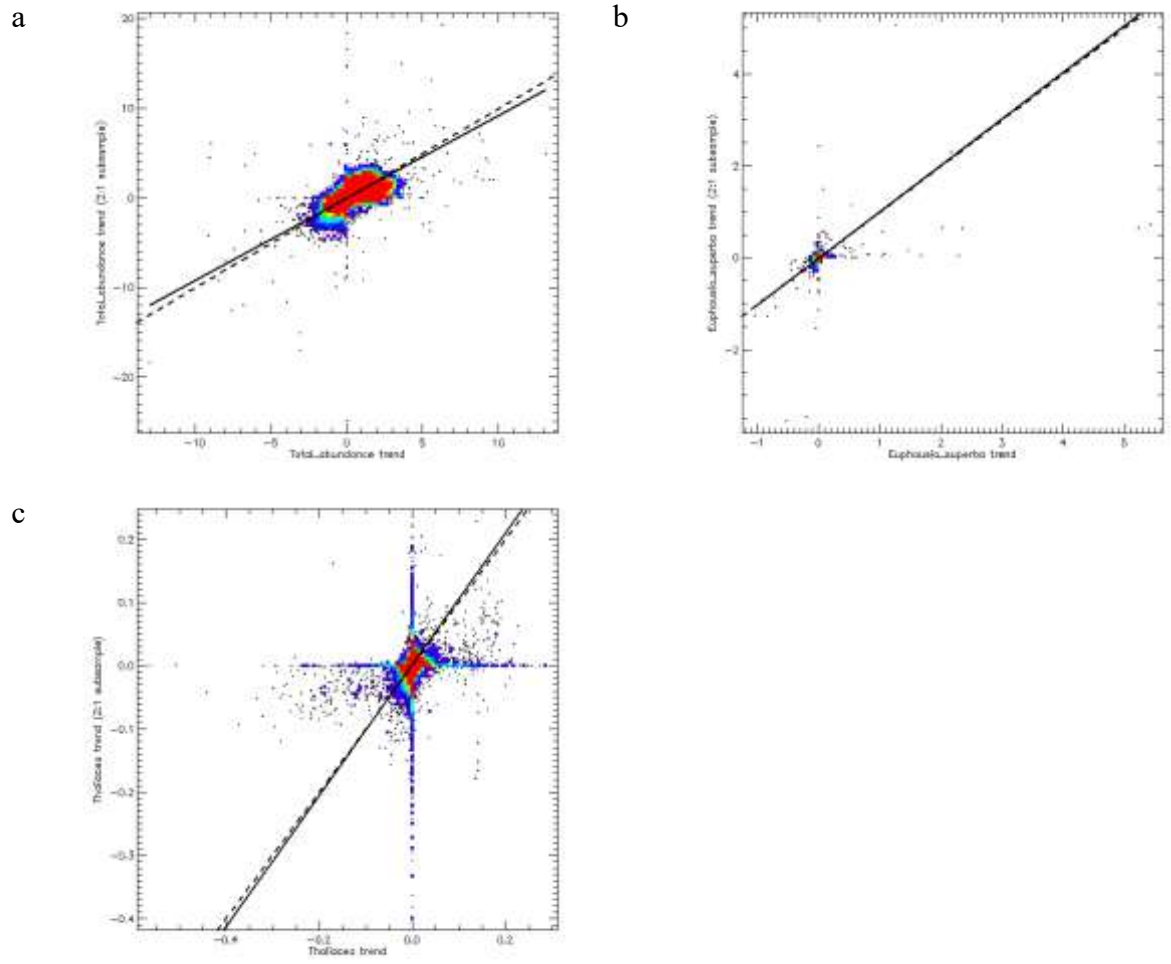

Figure 28: Trends: pixel-by-pixel comparison of effects of subsampling on mean abundances of three groups of zooplankton using the combined boosted regression tree (BRT) biogeographic model of habitat suitability, long-term (cumulative) average. The modelled suitability based on the full dataset is plotted on the x-axis and the y-axis shows the value for the same location based on the 2:1 subsampled data. a: Total zooplankton abundance; b: Antarctic krill, *Euphausia superba*; c: Thaliacea (gelatinous zooplankton including salps and pyrosomes).

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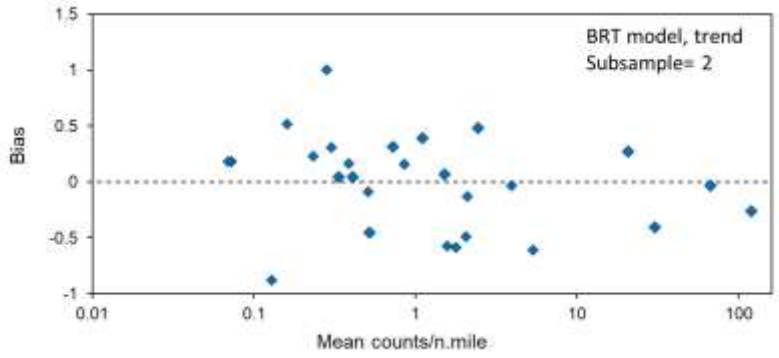

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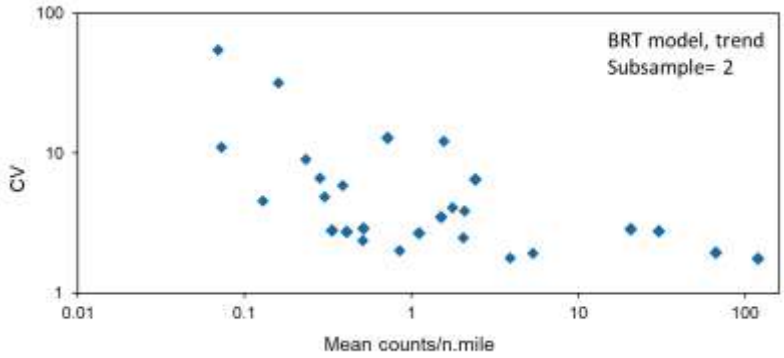

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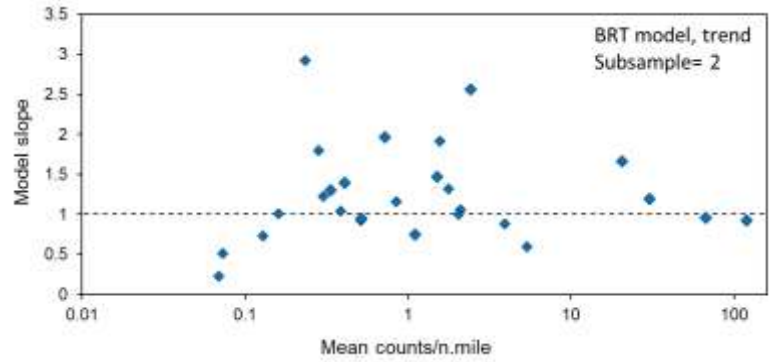

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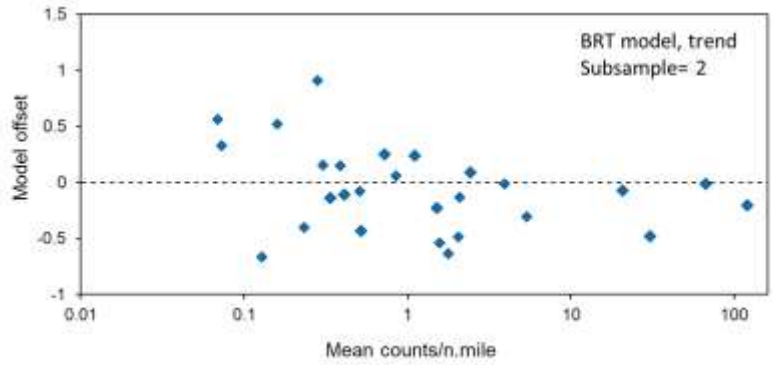

Figure 29: Trends: effects of 2:1 sub-sampling on trends from biogeographic models of monthly habitat suitability. a: Bias (mean difference as a proportion of overall mean absolute trends); b: CV; c: Slope of reduced major axis regression, log-log; d: Slope of reduced major axis regression, log-log, as proportion of overall mean absolute trends. Only "key" groups were modelled. The dashed lines show exact agreement.

4. DISCUSSION

Observation of the patterns and variations in zooplankton abundances over time can show how climate variability and change is affecting marine food webs and will help anticipate climate-driven changes to consumers such as fish, marine mammals and seabirds. Zooplankton are the key trophic link from primary production to higher trophic level organisms so their response to oceanographic drivers is crucial to understanding and predicting food web dynamics. Variations in zooplankton over time can also affect carbon dioxide levels in the atmosphere by changing the amount of carbon transported to the seabed.

This study has advanced the study of zooplankton in the southern part of the New Zealand Exclusive Economic Zone (EEZ) and in the whole Southern Ocean in two ways. Firstly, it has maintained the New Zealand contribution to one of the longest-running and most data-rich biological observation programmes for an additional two years. Second, it has investigated to what extent subsampling laboratory analysis of CPR samples can help to reduce the cost of maintaining the observations and potentially allow sampling in new areas.

Some groups of zooplankton are nearly ubiquitous in the ocean, being present in nearly all 5 n.mile CPR samples, while other groups of zooplankton are much more sparsely distributed and occur in CPR samples only infrequently. The number of individuals of commonly occurring zooplankton groups in neighbouring samples tend to be correlated, suggesting that the "patch sizes" of these common zooplankton have a characteristic spatial dimension of more than 5 n.mile. In this case, 2:1 subsampling does not lose much information, and this is reflected in the low bias, small increase in uncertainty and small departures from 1:1 agreement compared with the non-subsampled data. For the rarer types of zooplankton, their occurrence and numbers in CPR samples tend to follow a Poisson distribution (within a region) where, to a first approximation, they occur randomly and independently in neighbouring samples. Subsampling a Poisson-distributed variable does not introduce bias: if each item is sampled with the same probability, the subsampled data remains a valid, unbiased (though noisier) reflection of the original process. Analysing the Poisson model gives insights into how much additional uncertainty will be introduced by subsampling, and we found it is not very high. Furthermore, uncertainty can be reduced, with or without subsampling, by averaging CPR data over longer spatial scales. For example, carrying out analysis at 25 n.mile resolution reduces the uncertainty for Poisson-distributed samples by about 55% whereas 2:1 subsampling only increases it by about 20%. For trend analysis, it is normal to downscale the spatial resolution even more, perhaps to 5° (300 n.mile) to ensure sufficient data at a geographic location for reliable results. For non-rare zooplankton groups, this spatial averaging more than offsets the additional uncertainty caused by 2:1 laboratory subsampling.

Subsampling will lead to fewer observations of rare zooplankton species or those not generally present in near surface ocean waters (<10 m depth) which could make these data less useful for describing spatial patterns over large scales. However, as discussed in Pinkerton et al. (2020, 2024), it is difficult to use SO-CPR data to study truly rare zooplankton species because it is not known what proportion of unidentified zooplankton species are made up by any particularly rare species. There is also the potential for rare species to be identified with different levels of reliability by different zooplankton analysts, despite the rigorous and ongoing training and cross-checking of SO-CPR analysts. For this reason, previous work (Pinkerton, 2020, 2024) has recommended focussing on groups of the most commonly occurring ("key" groups) and moderately abundant ("fine" groups) of zooplankton and not considering the very rare species of zooplankton. Following this rationale, the increase in uncertainty in observation of rare zooplankton groups due to subsampling is not problematic.

Overall, therefore, the examination of 2:1 subsampling (analysing every second sample) was positive. We found that subsampling laboratory analysis at 2:1 had relatively modest effects on the measurement of absolute zooplankton abundances, trends (long-term rates of change), and on biogeographic models (where the relationships between zooplankton abundances and oceanographic conditions are used to extrapolate measurements in time and space).

The results were also positive for observing plastic particles. The number of plastic particles in CPR samples tended to have low spatial autocorrelation (lag=1), meaning that there was a lot of random variability in the number of plastic particles over small distances (between neighbouring CPR samples within a given CPR tow). However, this small-scale variability in plastic counts was much less than the systematic differences in plastic particle abundances across different time periods and larger spatial scales (between tows). For example, plastic was nearly ubiquitous in recent CPR samples (>90% presence per sample) but relatively uncommon earlier in the CPR time series. This broad-scale variation in plastic abundance means that bias, uncertainty and deviations from 1:1 correspondence as a result of 2:1 subsampling CPR plastic were small, and also means that trends in plastic numbers were robust to subsampling.

In conclusion, our analysis supports 2:1 subsampling analysis for CPR samples and this is recommended for the future. Subsampling at 3:1 and beyond loses more information and is not recommended.

There is one proviso to the analysis and conclusions. We have assumed that the main use of the data is to look at broad-scale patterns of abundance, at spatial scales longer than about 25 n.mile, and down-sampling laboratory analysis will smooth out or entirely miss short-term fluctuations (at the sub 10 n.mile scale). There is evidence from underway "ferry-box" sampling at fine spatial and temporal scales that the choice of resolution can affect both the statistical strength and significance of relationships between zooplankton and the environment (Scott et al. 2023). Their study found that decreasing the sampling resolution lost insights into small-scale drivers of zooplankton changes, and over-emphasized wide-area patterns. For example, features such as "micro-spikes" or response to mesoscale structure might be lost by subsampling. Such fine-scale analysis would be challenging using SO-CPR data as it is currently collected because there are no coincident, fine-scale measurements of environmental properties (such as temperature, salinity, phytoplankton abundance) taken concurrently. This means that, to our knowledge, fine-scale analysis has not been attempted on the SO-CPR dataset to date. If oceanographic measurements are made concurrently and coincidently with SO-CPR zooplankton sampling in the future, this fine-scale analysis becomes possible and then it might be worthwhile to analyse every sample. In this case, it might even be necessary to cut the CPR silks at less than 5 n.mile intervals. This may occur in the future with the new generation of CPR bodies, which include sensors for water temperature, salinity and chl-a fluorescence in the instruments themselves, but is not an issue at the moment.

Following with these subsampling results and our recommendation, we have analysed samples from seasons 2024/24 and 2024/25 at 2:1 subsampling for both zooplankton and plastics. Zooplankton samples analysed were consistent with data from previous phases of this project, with respect to the dominant zooplankton groups present and the type and abundance of microplastics. Analysing every second sample has indeed proved to be substantially quicker overall. Some of the non-analysed samples occasionally contained obvious and visible large species that were not in the analysed sample and on these occasions, this was noted, but will not be in the formal submission of data to the SO-CPR database. This should not cause issues with future analysis of the data.

Our conclusions as to the efficacy of subsampling the Southern Ocean CPR survey has international consequences, as many countries contribute data to this study, especially Australia and Japan, but also historically Russia, Chile and Germany. Changing the protocols for the SO-CPR survey will need agreement from the major partners and we have already reached out to colleagues in Australia and Japan on our preliminary results and received positive feedback. A jointly authored abstract with Australia and Japan on the effects of subsampling has been submitted to the 2026 SCAR Open Science Conference in Oslo in August 2026. International commentary has suggested that keeping the samples which were not analysed is sensible and we concur. In this study, we have preserved all samples so that we can go back and analyse these later if required. The saved and unanalysed samples also potentially provide a resource for training, evaluation and cross-comparison of analysts.

The rapid effects of climate change on oceanographic conditions in the Southern Ocean, and the increasing occurrence and numbers of plastic fibres (Waller et al. 2017; Aves et al. 2022), means that maintaining the time-series of observations is important if we are to understand and potentially respond or adapt to the ecological consequences of these changes. The SO-CPR survey has proved effective and efficient at observing zooplankton communities and microplastic in the surface ocean, but, given the squeeze on research budgets, is under pressure in New Zealand and internationally. We are engaged in research exploring alternative methods of analysis, such as "omic" or genetic approaches for zooplankton (Vezzulli et al. 2022; Pollard et al. 2024, 2025) and image-based/AI-identification methods (e.g. Ohman et al. 2019; Lombard et al. 2019). Until these new methods are proven, cross-calibrated to the SO-CPR timeseries to date, and implemented, the laboratory-based protocol should remain in force. Subsampling will be effective at reducing the cost of maintaining this analysis without sacrificing data quality. Furthermore, our analysis using biogeographic models shows that expanding the observation into unsampled areas of the Southern Ocean, especially the Pacific Sector and towards the northern Subantarctic boundary zone, is crucial to obtain a reliable picture of zooplankton distributions and change.

5. POTENTIAL RESEARCH

Analysing CPR samples collected from the *San Aotea II* on the transit from the Ross Sea region to the Scotia Sea through the Pacific Sector of the Southern Ocean could be considered for one season instead of (or as well as) the normal New Zealand to the Ross Sea route.

6. FULFILMENT OF BROADER OUTCOMES

This project has supported New Zealand's role and strategic aims in the Ross Sea region. Internationally, our research has underscored and consolidated New Zealand's international position as the leader of research on ecosystem change in the Ross Sea region as well as making genuine progress towards the complex issue of managing the ecosystem effects of climate change and fishing in a high-latitude, pristine ecosystem.

This work also contributes to the New Zealand Antarctic Science Platform (ASP) phase 2, Tiaki Moana project. ASP has taken the lead in the development of new genetic methods for Southern Ocean zooplankton analysis. This ASP initiative relied on expertise in zooplankton analysis and CPR operation from the MPI SO-CPR project.

The zooplankton information and insights are directly relevant to New Zealand's commitment to research and monitoring associated with the Ross Sea region Marine Protected Area and have been used in the review of the Special Research Zone (Pardo et al. 2026), during the 10-year review at CCAMLR in 2026/27, including at the Research Coordination Network meeting in Estes Park, Colorado 28 April – 1 May 2026, and as part of the New Zealand 2025–2026 research update to CCAMLR (Pinkerton et al. 2026).

Outputs to date include:

- Pardo, E.; Pinkerton, M.H.; Lamping, S.; Chung, S.; Brooks, C.; Hinke, J.; Ghigliotti, L.; Kim, J.-H.; Tuck, I. (2026). Review of environmental and ecological aspects of the Special Research Zone, part of the Ross Sea region Marine Protected Area. CCAMLR working group paper, WG-EMM-26/75, July 2026.

- Pinkerton, M.H. et al. (2025). Zooplankton and microplastic in the Southern Ocean: Continuous Plankton Recorder sampling to 2023 with special focus on the Ross Sea sector. Poster presentation to ASP science conference, Te Papa Wellington, 1–3 April 2025.

- Pinkerton, M.; Devine, J.; Hawes, I.; Pardo, E.; Robinson, E.; Stevens, C. (2025). New Zealand research and monitoring in support of the Ross Sea region Marine Protected Area: 2023-2025 update. CCAMLR paper WG-EMM-25/45, Hobart, Australia.

- Pinkerton, M.H.; Dawson, G.; Devine, J.; Eisert, R.; Fernandez, D.; Halfter. S.; Hawes, I.; LaRue, M.; Pardo, E.; Robinson, E.; Stevens, C.; Tait, L.; Wiki-bennett, S.; Zhu, J. (2026). New Zealand research and monitoring in support of the Ross Sea region Marine Protected Area: 2025-2026 update. CCAMLR working group paper, WG-EMM-26/55, July 2026.

- Robinson, K.; Halfter, S.; Pinkerton, M.H. (2025). Progress report on Continuous Plankton Recorder sampling 2023/24 and 2024/25. Report for project ZBD2024-01 to FNZ/MPI, June 2025. 20 p.

Future outputs, in draft or preparation include:

- Pinkerton, M.H.; Halfter, S.; Robinson, K.V.; Kitchener, J.A.; Takahashi, K.T.; Hosie, G.W. (2026). Autocorrelation and affordability: how subsampling can help the SCAR Southern Ocean Continuous Plankton Recorder Survey to endure and expand. SCAR (Scientific Committee on Antarctic Research) Open Science Conference: "Diving into Antarctic Science – Making waves for the future", 10-14 August 2026, Oslo, Norway. [Oral]

- Halfter, S.; Pinkerton, M.H.; Stewart, R.; Robinson, K. (2026). Using the Continuous Plankton Recorder to monitor plastics in the Southern Ocean. SCAR (Scientific Committee on Antarctic Research) Open Science Conference 'Diving into Antarctic Science – Making waves for the future', 10-14 August 2026 [Poster] – selected for a travel grant award by the SCAR Plastics Expert Group to allow for further discussions during the conference.

- Pinkerton, M.H.; Halfter, S.; Robinson, K.V. (2026). Subsampling zooplankton and microplastics from the Continuous Plankton Recorder: the effect on abundances, patterns and trends. Polar Biology [or similar international journal]

7. ACKNOWLEDGEMENTS

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8. REFERENCES

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APPENDIX 1: Zooplankton "fine" groups

Table 8: Summary of "fine" zooplankton groups and microplastic abundances, giving the number and proportion of CPR samples with non-zero abundances, and the mean and 5–95th percentiles of counts per n.mile.

"Fine" groups	N (non zero)	Proportion non-zero	Abundance (counts/n.mile)	
		%	Counts	5–95th %
*Acartia*_spp	105	0.2	0.02	0.0 – 0.0
Amphipoda	12 767	23.1	0.09	0.0 – 0.4
Bivalvia_larvae	41	0.1	0.00	0.0 – 0.0
Calanoida_indet_(small)	49 162	89.0	7.44	0.0 – 28.4
Calanoida_indet-(large)	23 818	43.1	0.97	0.0 – 4.4
Calanoides_acutus	8 805	15.9	0.15	0.0 – 0.8
Calanoides_brevicornis	122	0.2	0.00	0.0 – 0.0
Calanus_australis	284	0.5	0.01	0.0 – 0.0
Calanus_australis-Calanus_propinquus-Calanus_simillimus	157	0.3	0.00	0.0 – 0.0
Calanus_propinquus	1 783	3.2	0.03	0.0 – 0.0
Calanus_simillimus	24 775	44.8	1.34	0.0 – 6.2
*Calocalanus*_spp	446	0.8	0.01	0.0 – 0.0
*Candacia*_spp	558	1.0	0.00	0.0 – 0.0
*Centropages*_spp	69	0.1	0.00	0.0 – 0.0
Chaetognatha	10 010	18.1	0.06	0.0 – 0.4
Cirripedia	109	0.2	0.00	0.0 – 0.0
Cladocera	18	0.0	0.00	0.0 – 0.0
*Clausocalanus*_spp	25 338	45.9	0.96	0.0 – 4.4
Cnidaria	1 266	2.3	0.01	0.0 – 0.0
Copepoda_indet	2 338	4.2	0.17	0.0 – 0.0
Ctenocalanus_citer	19944	36.1	1.30	0.0 – 7.0
Ctenophora_indet	33	0.1	0.00	0.0 – 0.0
Cyclopoida_nauplius_indet	260	0.5	0.03	0.0 – 0.0
Decapoda	748	1.4	0.00	0.0 – 0.0
*Drepanopus*_spp	47	0.1	0.00	0.0 – 0.0
Echinoidea_larvae	102	0.2	0.00	0.0 – 0.0
*Euaugaptilus*_spp	24	0.0	0.00	0.0 – 0.0
*Eucalanus*_spp	226	0.4	0.00	0.0 – 0.0
*Euchirella*_spp	191	0.3	0.00	0.0 – 0.0
Euphausia_superba	1 948	3.5	0.03	0.0 – 0.0
Euphausiidae	16 208	29.3	0.21	0.0 – 0.8
Foraminifera	30 185	54.6	2.21	0.0 – 10.8
Fritillaria spp.	31 147	56.4	2.20	0.0 – 7.9
Haloptilus_oxycephalus	378	0.7	0.00	0.0 – 0.0
Harpacticoida	1 344	2.4	0.01	0.0 – 0.0
*Heterorhabdus*_spp	432	0.8	0.00	0.0 – 0.0
Isopoda	7	0.0	0.00	0.0 – 0.0
*Lucicutia*_spp	60	0.1	0.00	0.0 – 0.0
*Mecynocera*_spp	337	0.6	0.00	0.0 – 0.0
*Mesocalanus*_spp	45	0.1	0.00	0.0 – 0.0

"Fine" groups	N (non zero)	Proportion non-zero %	Abundance (counts/n.mile)	
			Counts	5–95th %
*Metridia*_spp	7 507	13.6	0.14	0.0 – 0.6
Microcalanus_pygmaeus	429	0.8	0.01	0.0 – 0.0
Mysidae_indet	10	0.0	0.00	0.0 – 0.0
Nauplius_indet	2 178	3.9	0.05	0.0 – 0.0
Neocalanus_tonsus	6 105	11.1	0.27	0.0 – 1.0
Noctiluca_scintillans	45	0.1	0.02	0.0 – 0.0
Oikopleura spp	23 335	42.2	0.42	0.0 – 1.8
Oithona spp	51 439	93.1	9.50	0.0 – 33.6
*Oncaea*_spp	2 024	3.7	0.03	0.0 – 0.0
Ostracoda	8 636	15.6	0.09	0.0 – 0.6
Other copepods	38	0.1	0.00	0.0 – 0.0
*Paracalanus*_spp	240	0.4	0.03	0.0 – 0.0
*Paraeuchaeta*_spp	1 924	3.5	0.01	0.0 – 0.0
Paralabidocera_antarctica	30	0.1	0.00	0.0 – 0.0
*Pleuromamma*_spp	1 486	2.7	0.01	0.0 – 0.0
Polychaeta	2517	4.6	0.02	0.0 – 0.0
Pteropods	16 064	29.1	0.65	0.0 – 2.2
Radiolaria	6 252	11.3	0.16	0.0 – 0.8
Rhincalanus_gigas	18 787	34.0	0.56	0.0 – 3.0
*Sapphirina*_spp	52	0.1	0.00	0.0 – 0.0
Scolecithricella_minor	114	0.2	0.00	0.0 – 0.0
Stephos_longipes	63	0.1	0.00	0.0 – 0.0
Subeucalanus_longiceps	1 350	2.4	0.02	0.0 – 0.0
Temora_turbinata	81	0.1	0.00	0.0 – 0.0
Thaliacea	2 977	5.4	0.03	0.0 – 0.2
Thysanoessa spp	146	0.3	0.00	0.0 – 0.0
Thysanoessa_gregaria	1 440	2.6	0.03	0.0 – 0.0
Thysanoessa_macrura	17 762	32.2	0.20	0.0 – 1.0
Tintinnina_indet	641	1.2	0.02	0.0 – 0.0

APPENDIX 2: Zooplankton abundances - effects of bootstrapping and subsampling

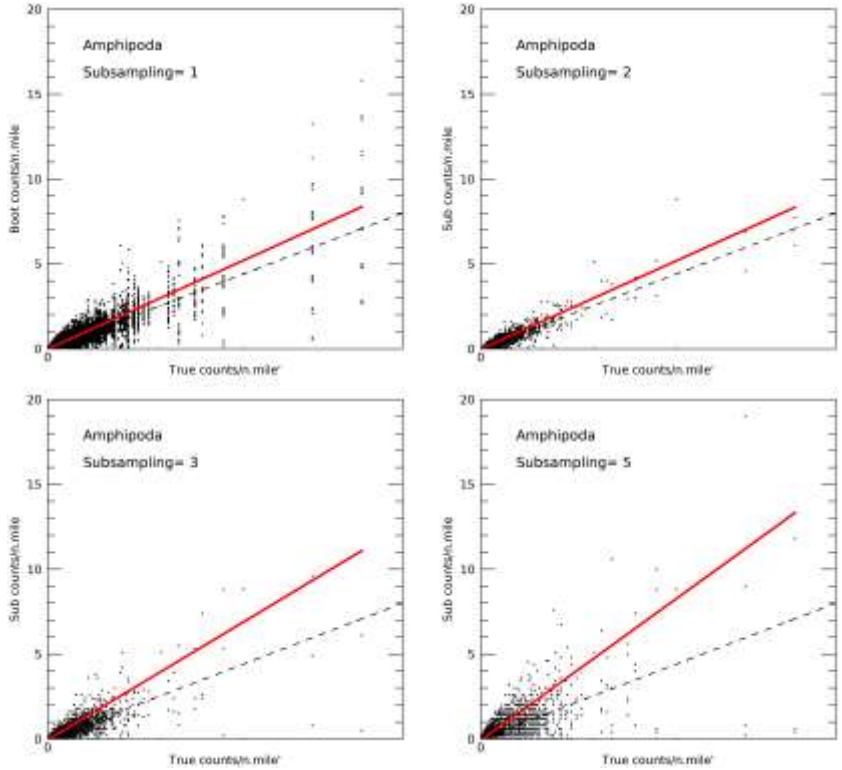

Figure A1: Effects of sub-sampling and bootstrapping on Amphipoda abundance (counts/n.mile). See caption Figure 11 for more details.

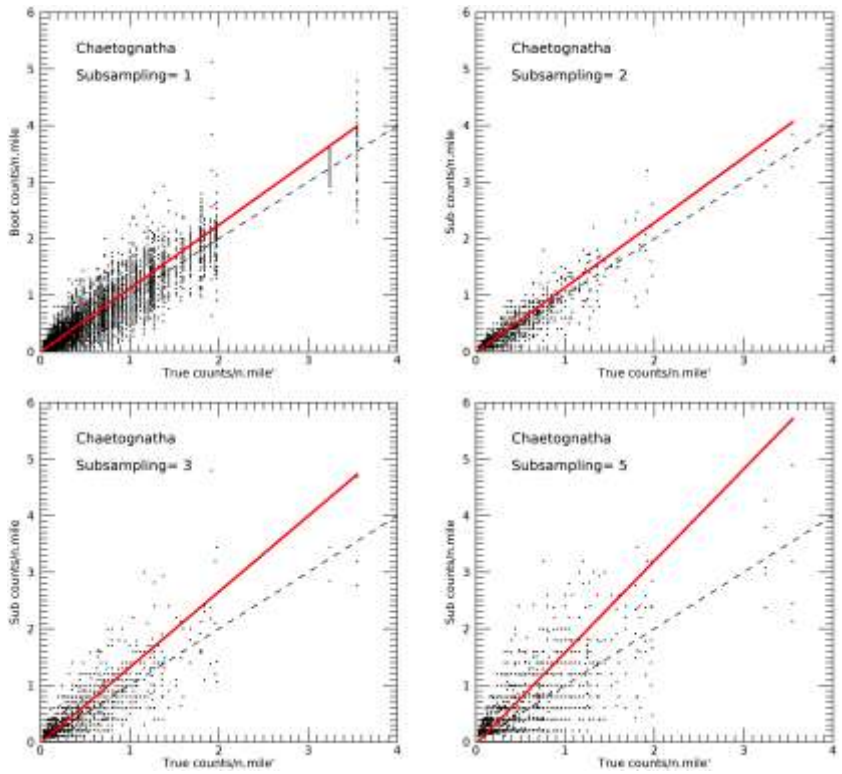

Figure A2: Effects of sub-sampling and bootstrapping on Chaetognatha abundance (counts/n.mile). See caption Figure 11 for more details.

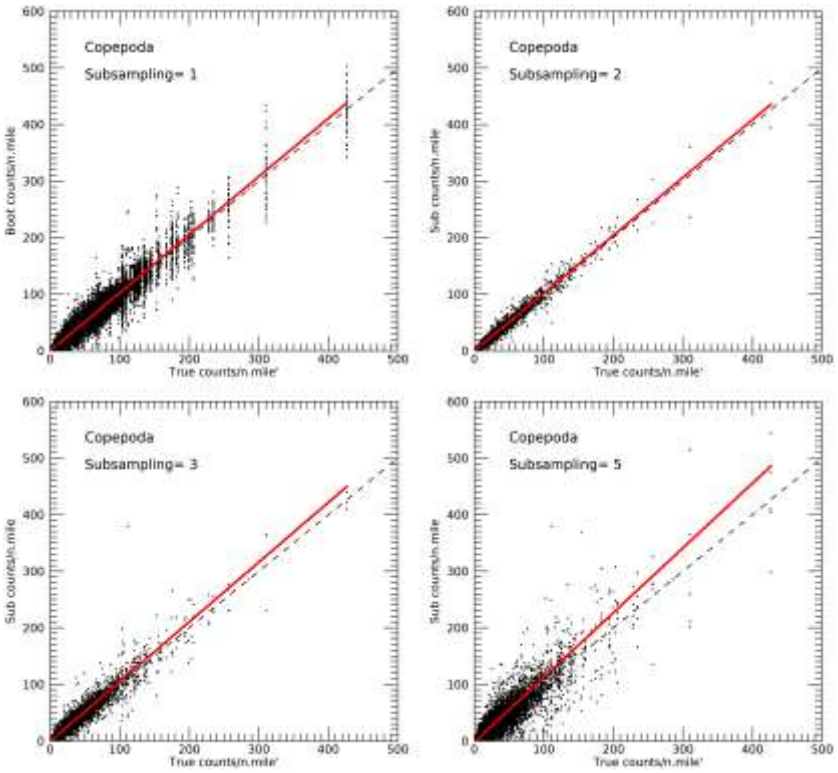

Figure A3: Effects of sub-sampling and bootstrapping on Copepoda abundance (counts/n.mile). See caption Figure 11 for more details.

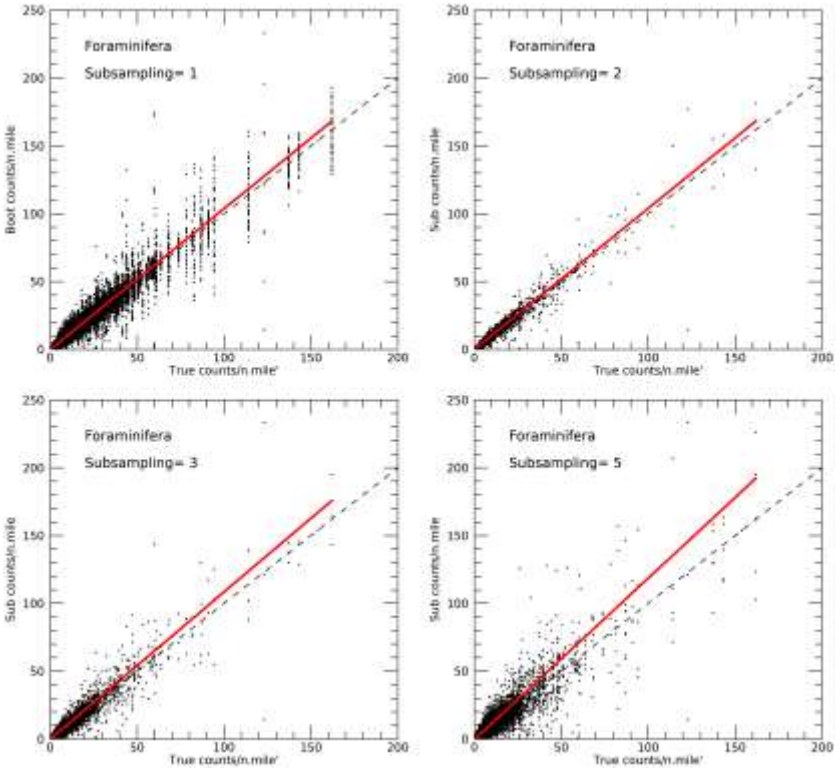

Figure A4: Effects of sub-sampling and bootstrapping on Foraminifera abundance (counts/n.mile). See caption Figure 11 for more details.

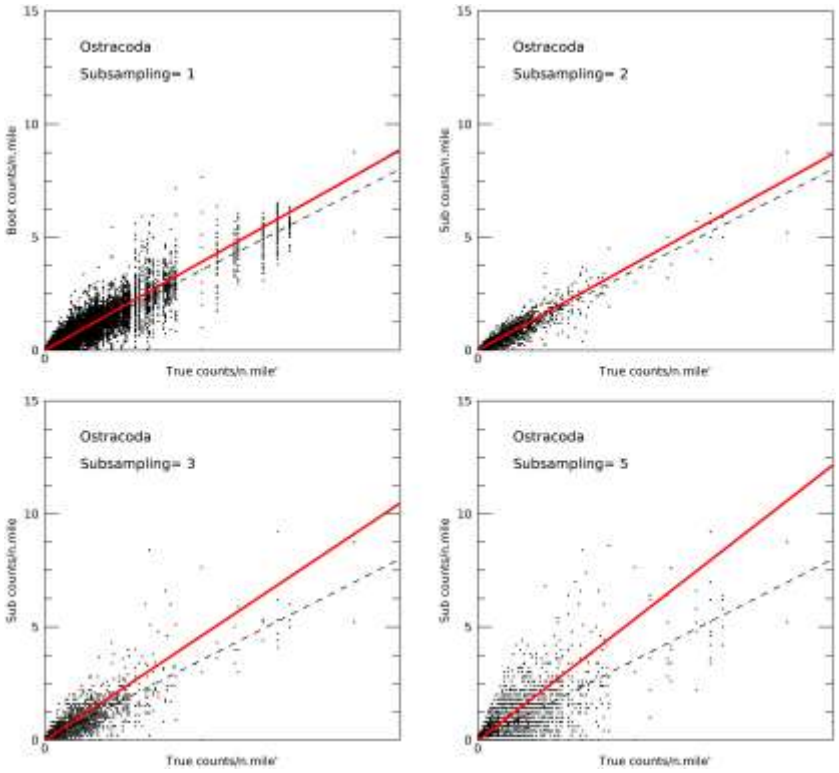

Figure A5: Effects of sub-sampling and bootstrapping on Ostracoda abundance (counts/n.mile). See caption Figure 11 for more details.

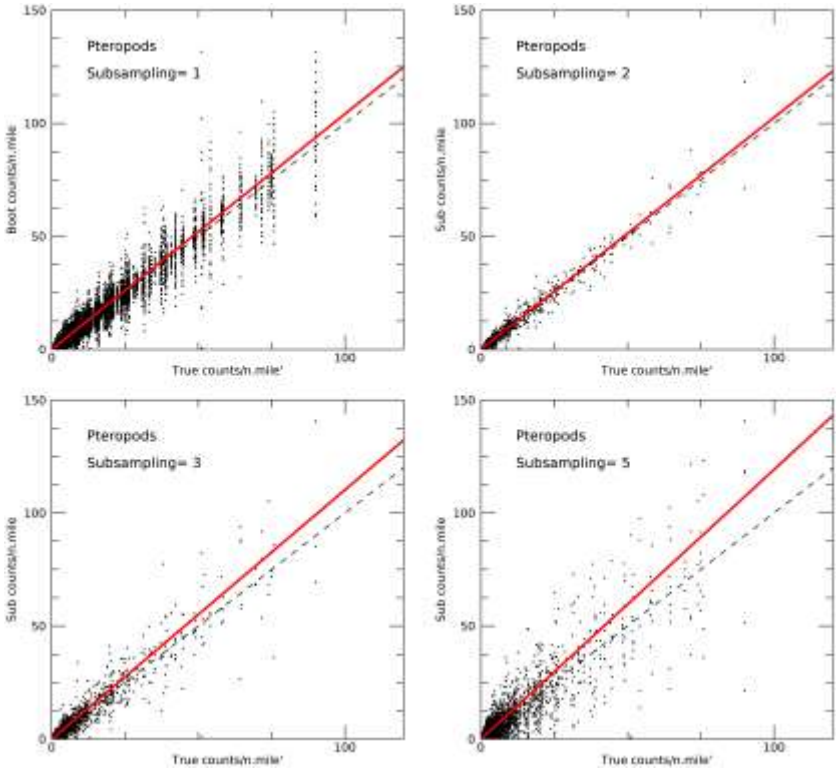

Figure A6: Effects of sub-sampling and bootstrapping on Pteropods abundance (counts/n.mile). See caption Figure 11 for more details.

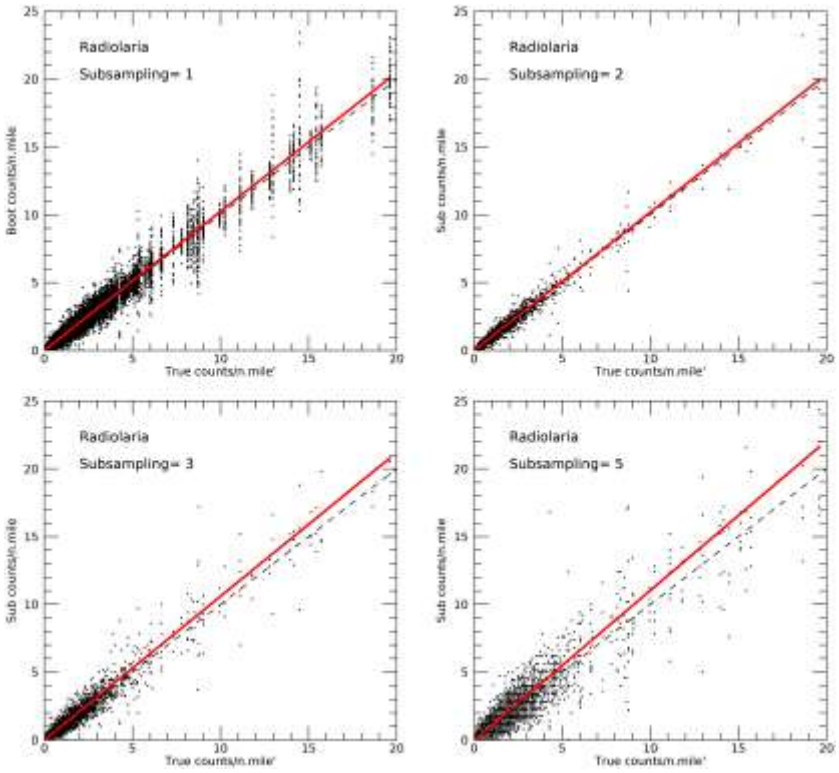

Figure A7: Effects of sub-sampling and bootstrapping on Radiolaria abundance (counts/n.mile). See caption Figure 11 for more details.

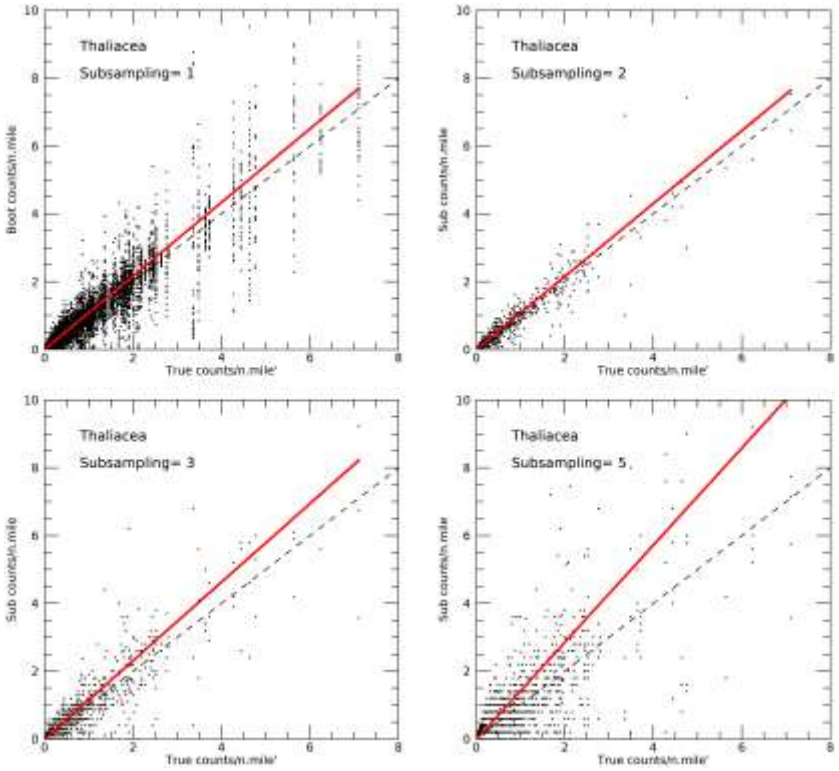

Figure A8: Effects of sub-sampling and bootstrapping on Thaliacea abundance (counts/n.mile). See caption Figure 11 for more details.

APPENDIX 3: Microplastic abundances - effects of bootstrapping and subsampling

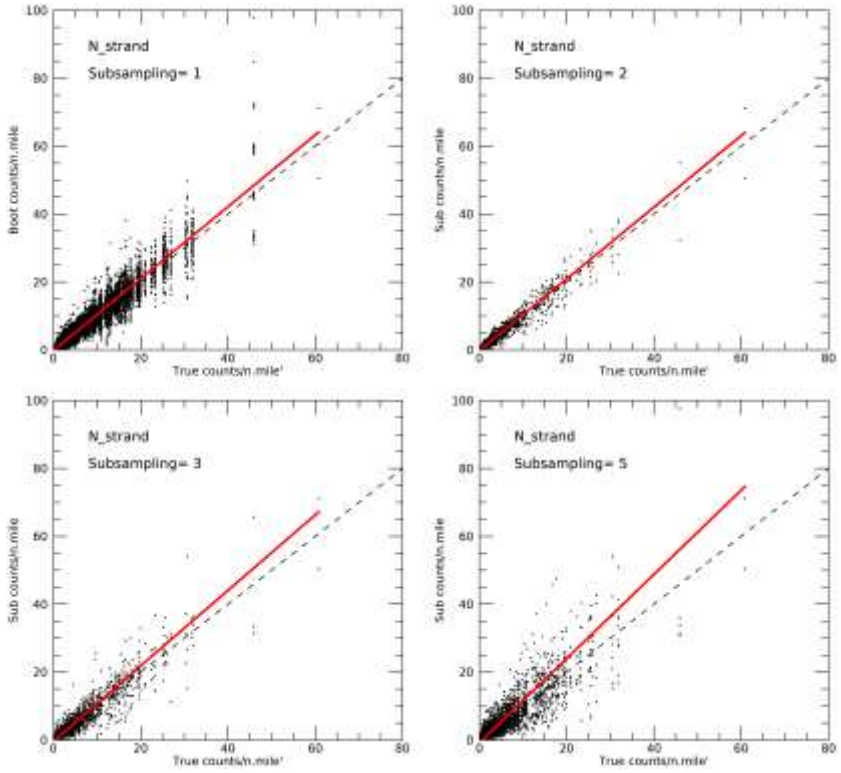

Figure A9: Effects of sub-sampling and bootstrapping on abundance of <u>microplastic strands</u> (counts/n.mile). See caption Figure 12 for more details.

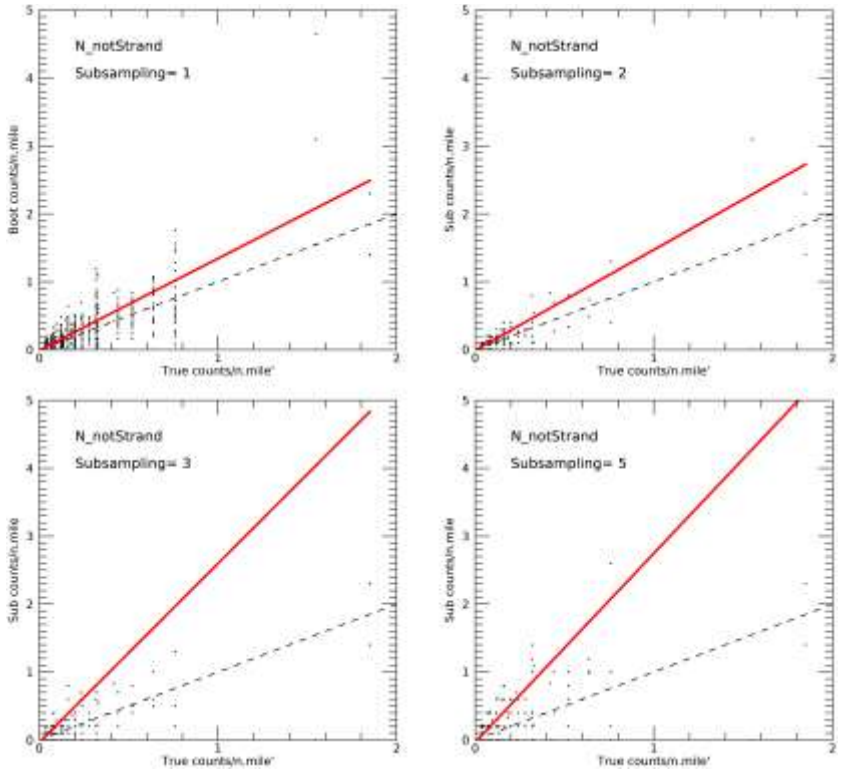

Figure A10: Effects of sub-sampling and bootstrapping on abundance of <u>microplastic non-strands</u> (counts/n.mile). See caption Figure 12 for more details.

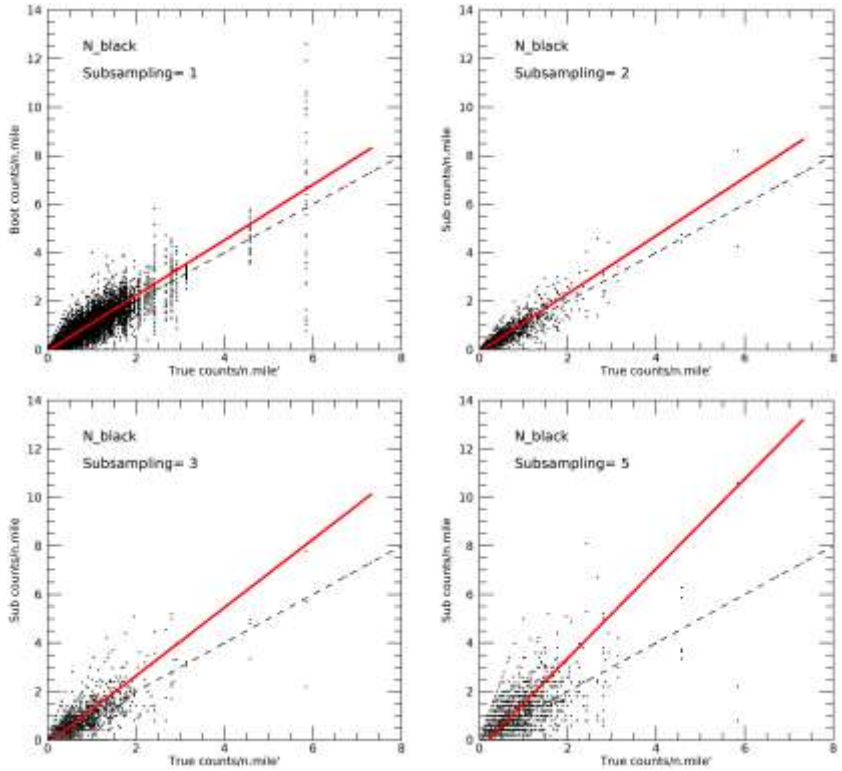

Figure A11: Effects of sub-sampling and bootstrapping on abundance of <u>black microplastic</u> pieces (counts/n.mile). See caption Figure 12 for more details.

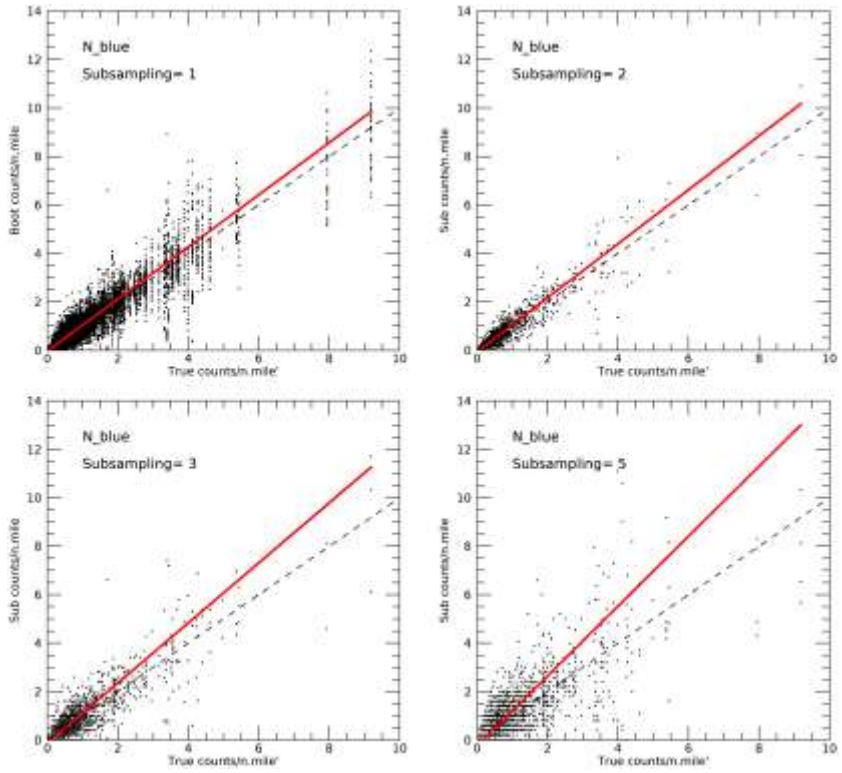

Figure A12: Effects of sub-sampling and bootstrapping on abundance of <u>blue microplastic</u> pieces (counts/n.mile). See caption Figure 12 for more details.

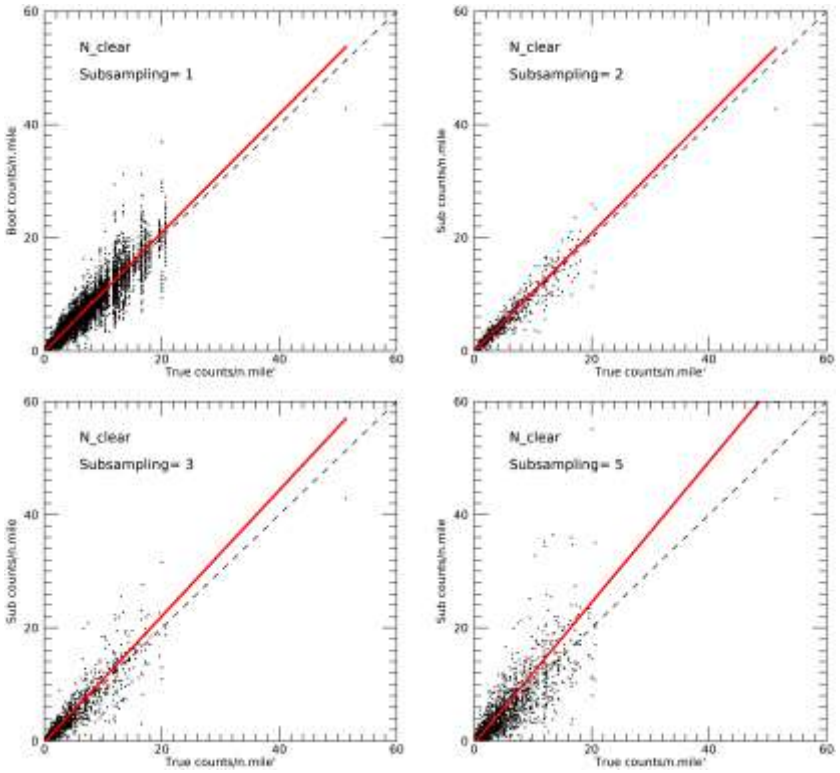

Figure A13: Effects of sub-sampling and bootstrapping on abundance of clear microplastic pieces (counts/n.mile). See caption Figure 12 for more details.

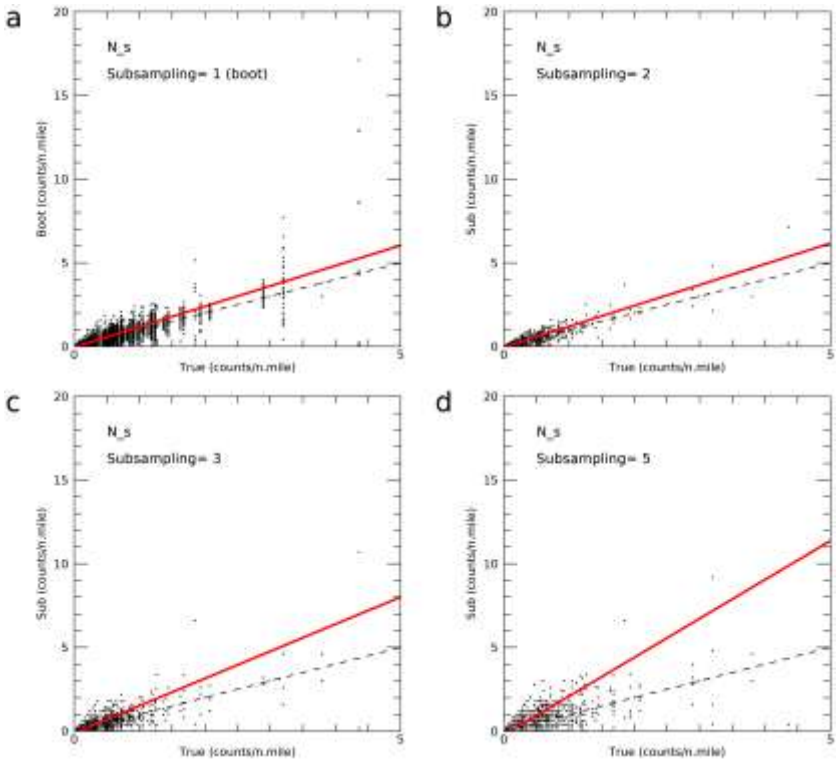

Figure A14: Effects of sub-sampling and bootstrapping on abundance of small microplastic pieces (counts/n.mile). See caption Figure 12 for more details.

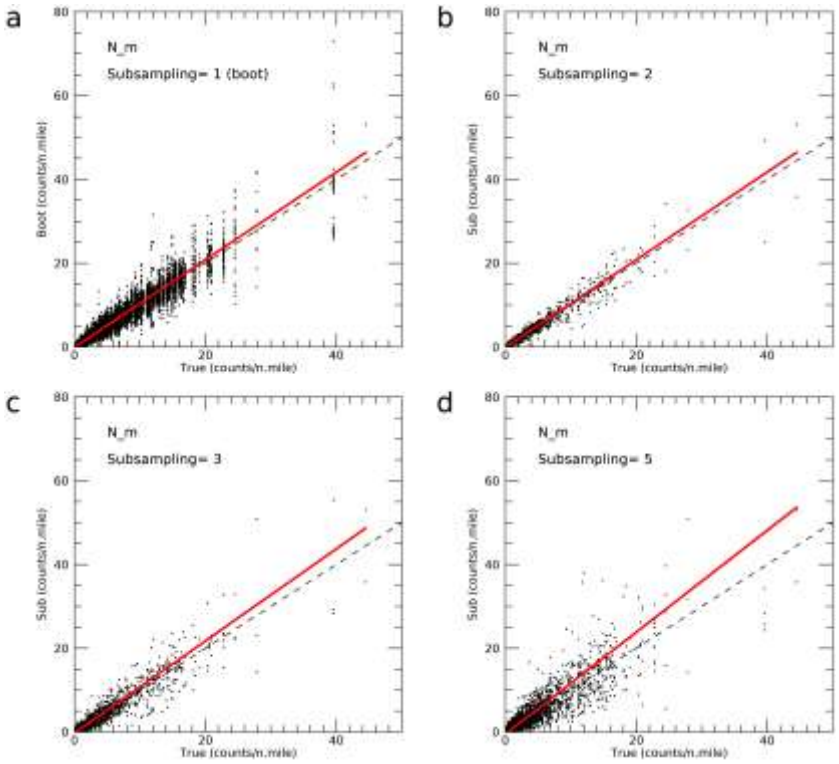

Figure A15: Effects of sub-sampling and bootstrapping on abundance of <u>medium microplastic</u> pieces (counts/n.mile). See caption Figure 12 for more details.

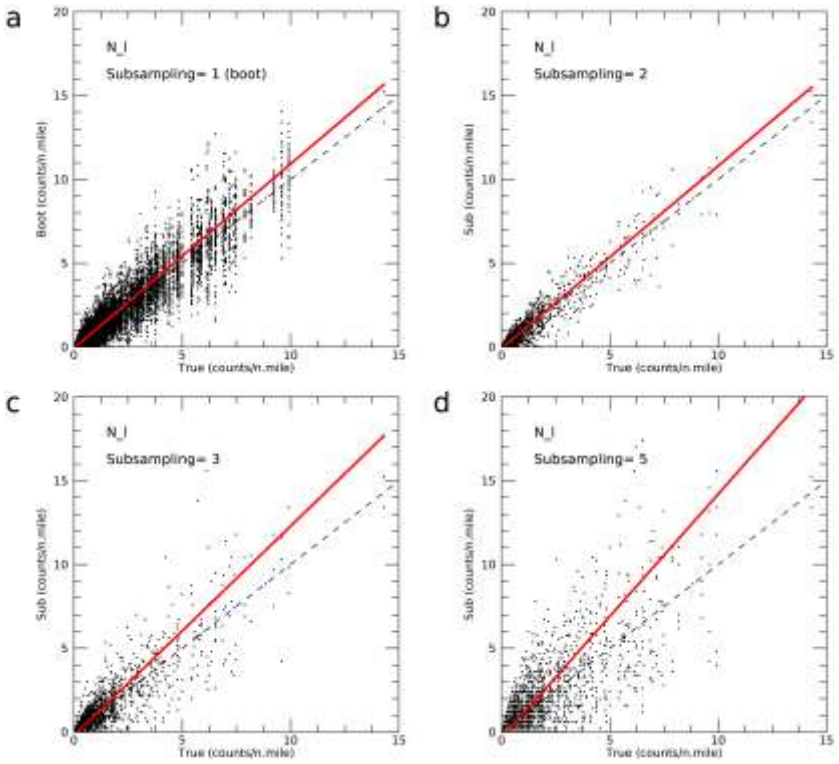

Figure A16: Effects of sub-sampling and bootstrapping on abundance of <u>large microplastic</u> pieces (counts/n.mile). See caption Figure 12 for more details.

APPENDIX 4: Zooplankton trends - effects of bootstrapping and subsampling

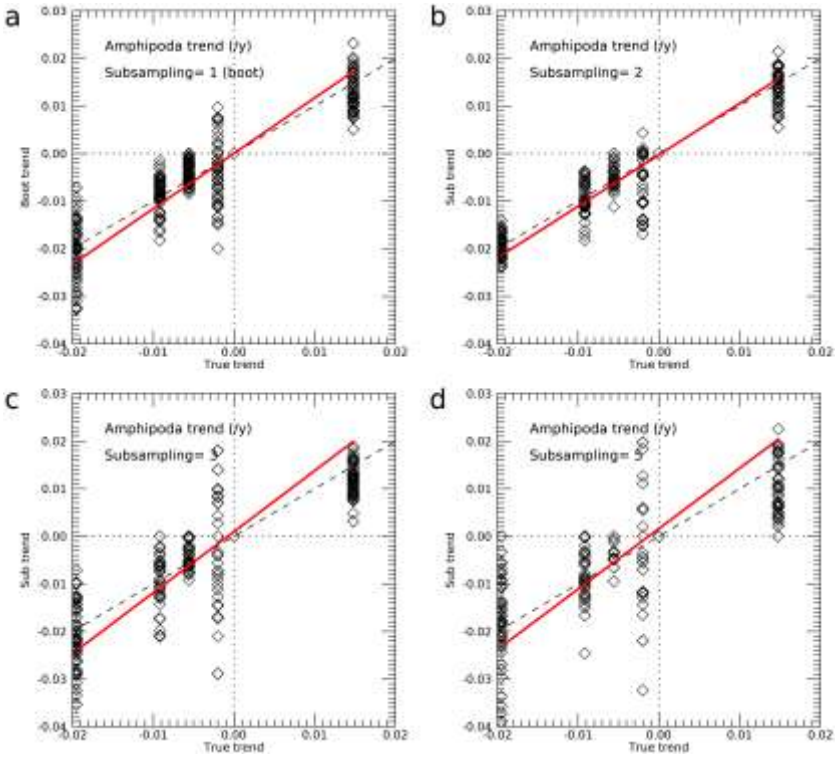

Figure A17: Effects of sub-sampling and bootstrapping on trends in <u>Amphipoda</u> [ln(counts/n.mile)/y]. See caption Figure 18 for more details.

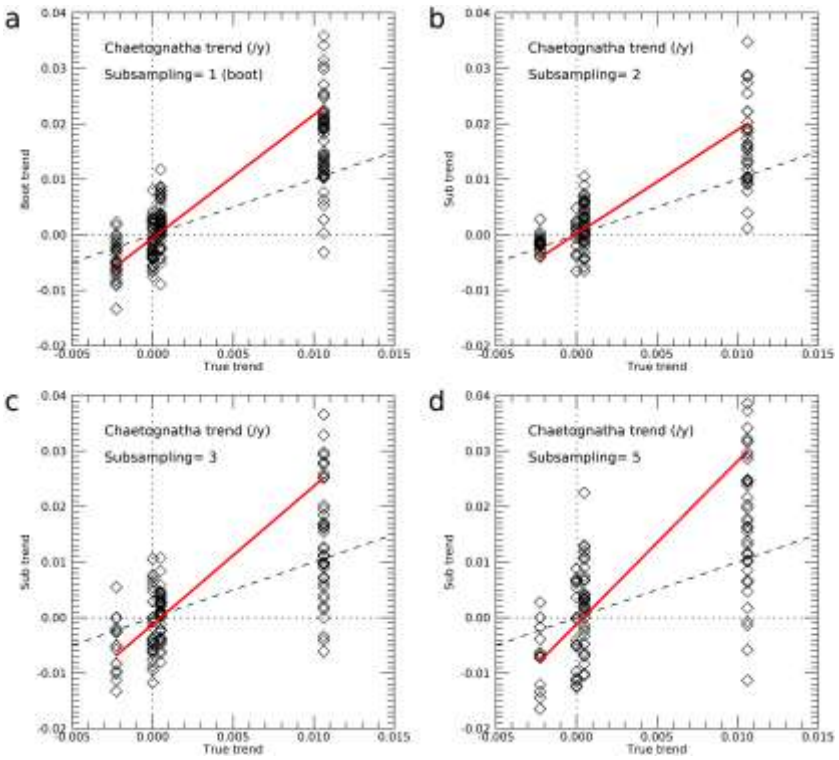

Figure A18: Effects of sub-sampling and bootstrapping on trends in <u>Chaetognatha</u> [ln(counts/n.mile)/y]. See caption Figure 18 for more details.

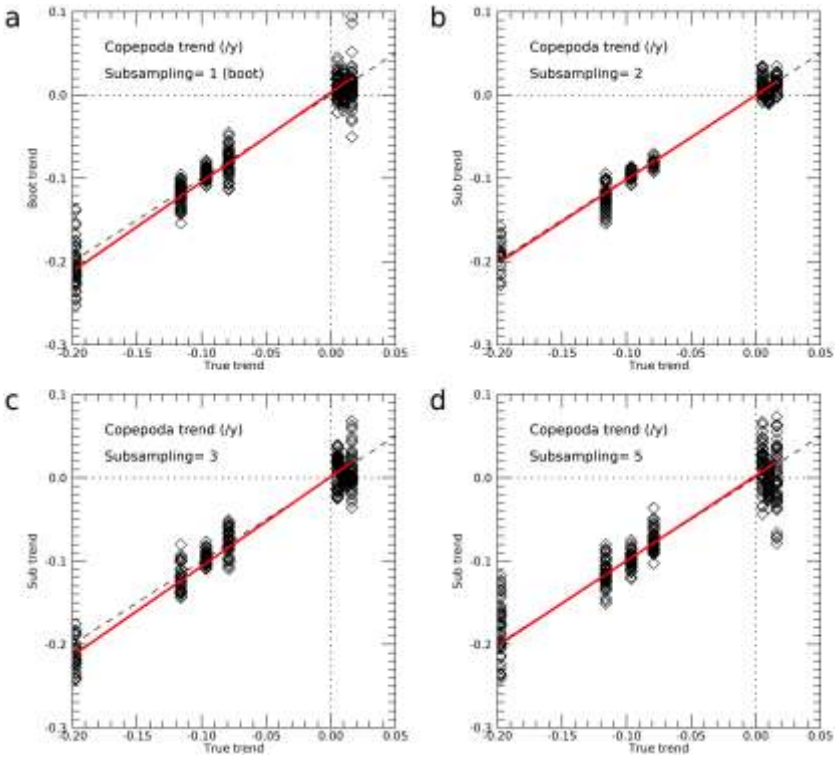

Figure A19: Effects of sub-sampling and bootstrapping on trends in <u>Copepoda</u> [ln(counts/n.mile)/y]. See caption Figure 18 for more details.

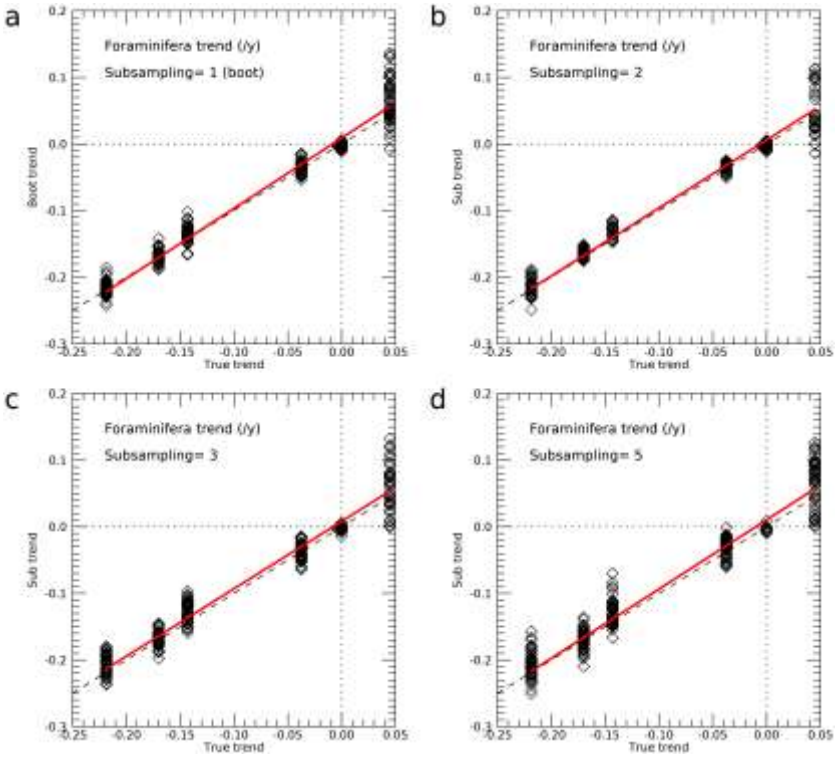

Figure A20: Effects of sub-sampling and bootstrapping on trends in <u>Foraminifera</u> [ln(counts/n.mile)/y]. See caption Figure 18 for more details.

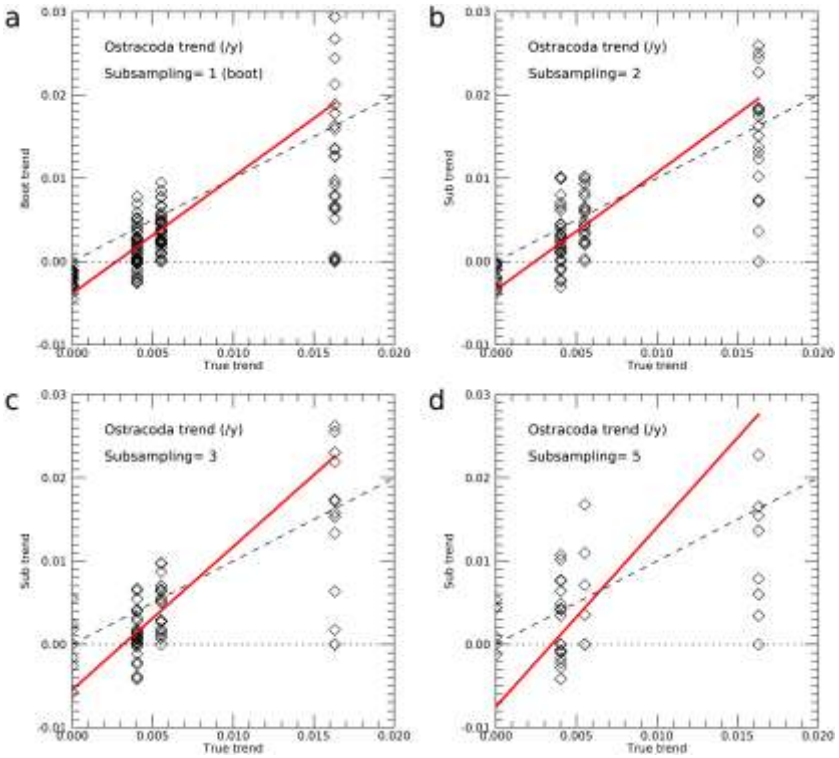

Figure A21: Effects of sub-sampling and bootstrapping on trends in <u>Ostracoda</u> [ln(counts/n.mile)/y]. See caption Figure 18 for more details.

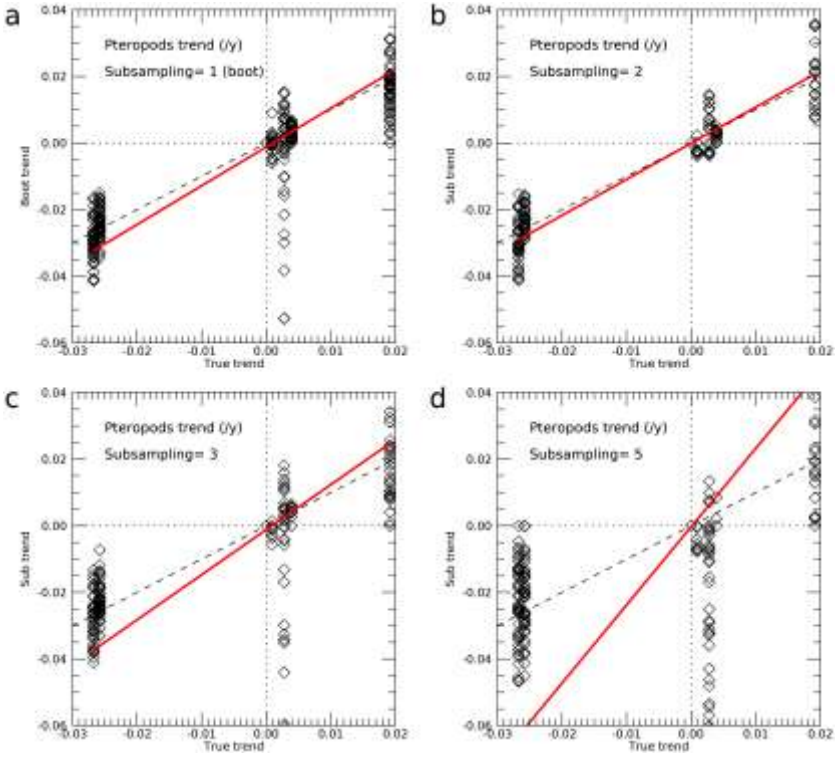

Figure A22: Effects of sub-sampling and bootstrapping on trends in <u>Pteropods</u> [ln(counts/n.mile)/y]. See caption Figure 18 for more details.

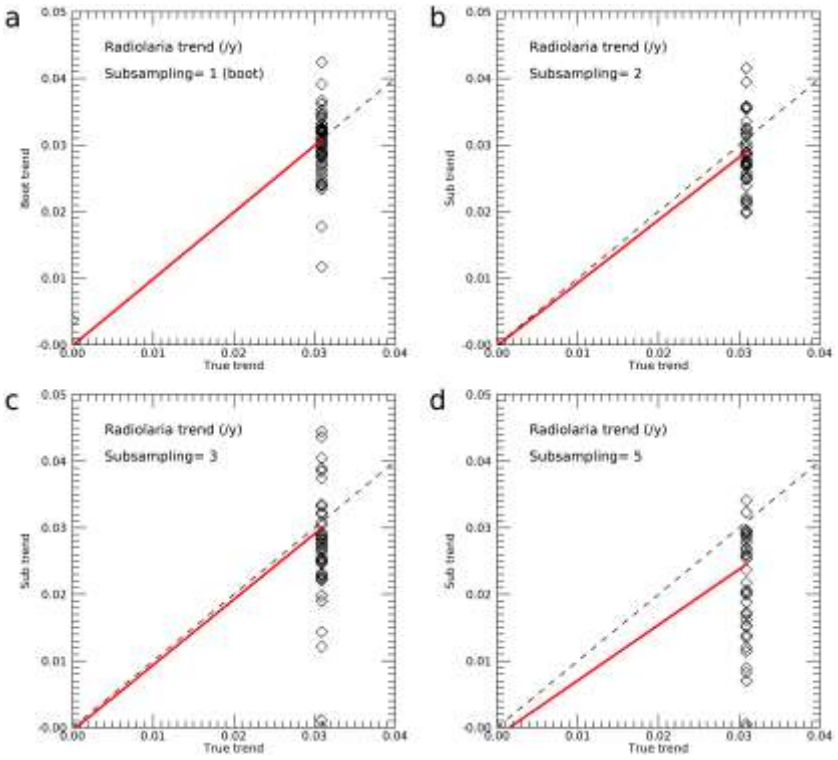

Figure A23: Effects of sub-sampling and bootstrapping on trends in <u>Radiolaria</u> [ln(counts/n.mile)/y]. See caption Figure 18 for more details.

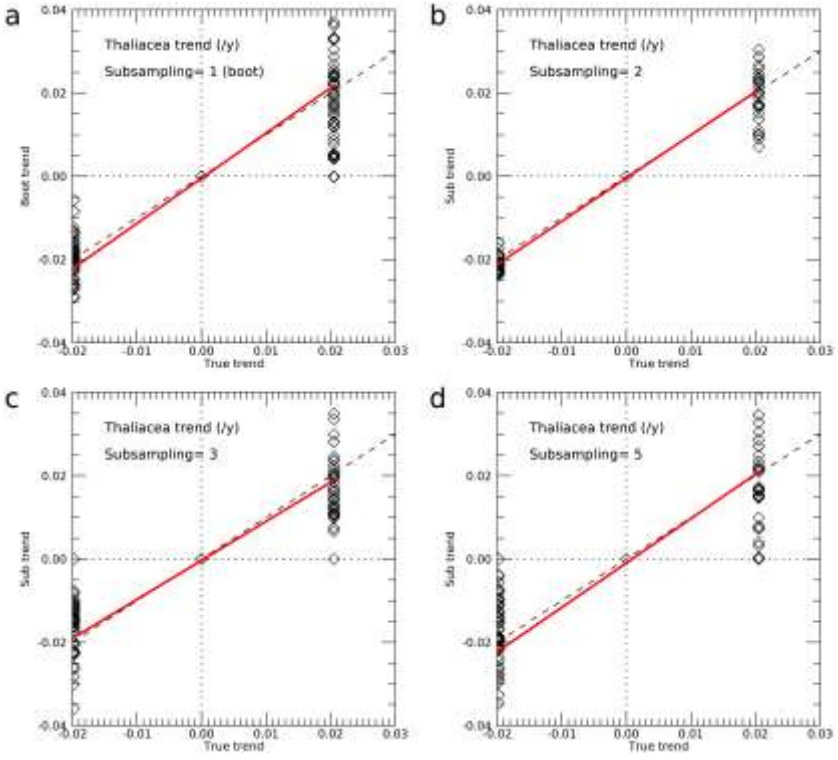

Figure A24: Effects of sub-sampling and bootstrapping on trends in <u>Thaliacea</u> [ln(counts/n.mile)/y]. See caption Figure 18 for more details.

APPENDIX 5: Microplastic trends - effects of bootstrapping and subsampling

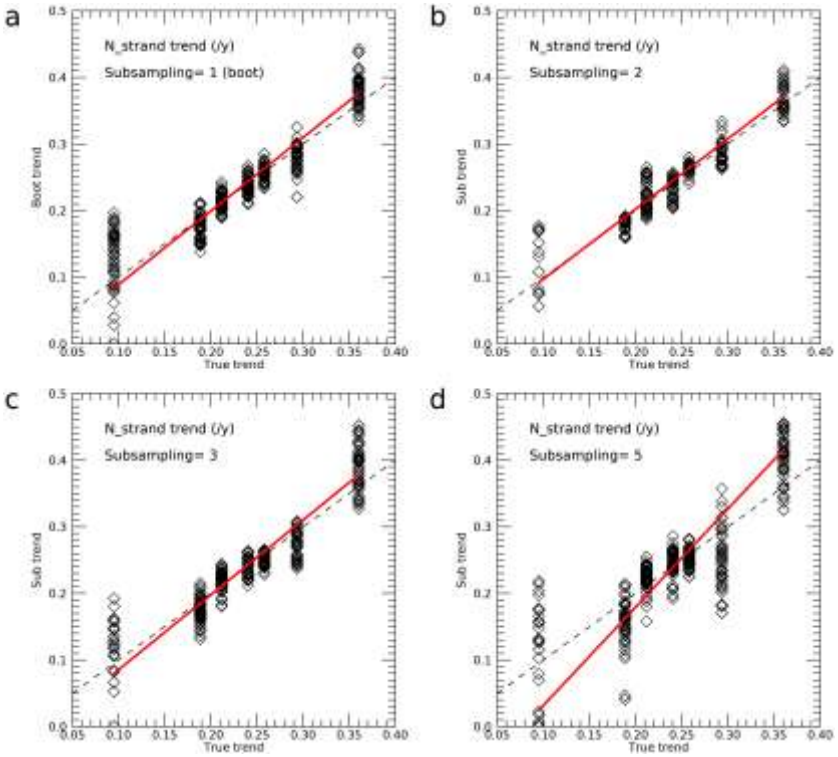

Figure A25: Effects of sub-sampling and bootstrapping on trends in abundance of <u>microplastic strands</u> [ln(counts/n.mile)/y]. See caption Figure 19 for more details.

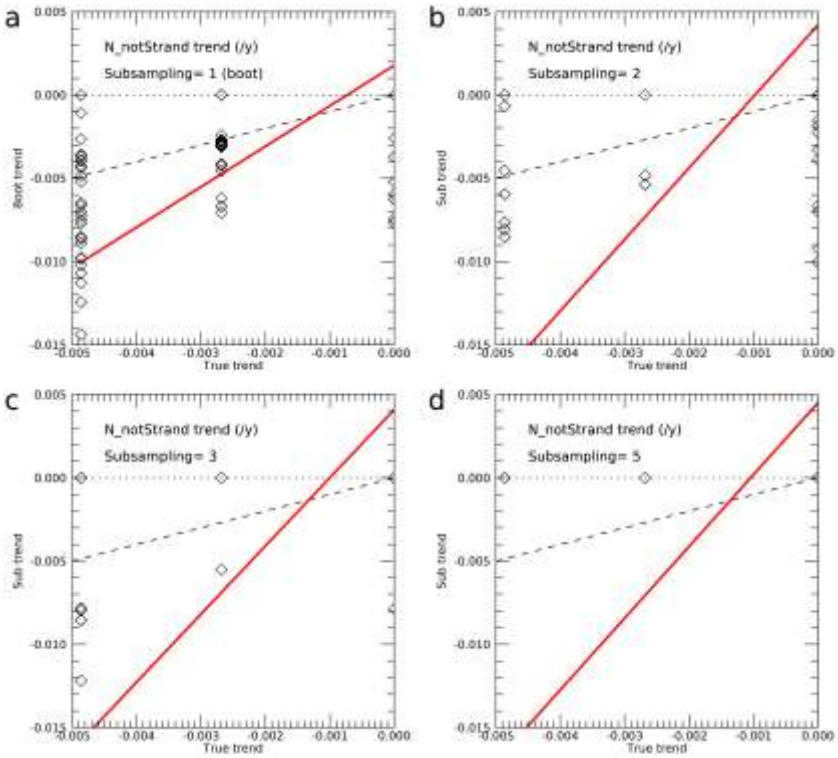

Figure A26: Effects of sub-sampling and bootstrapping on trends in abundance of <u>non-strand microplastics</u> [ln(counts/n.mile)/y]. See caption Figure 19 for more details.

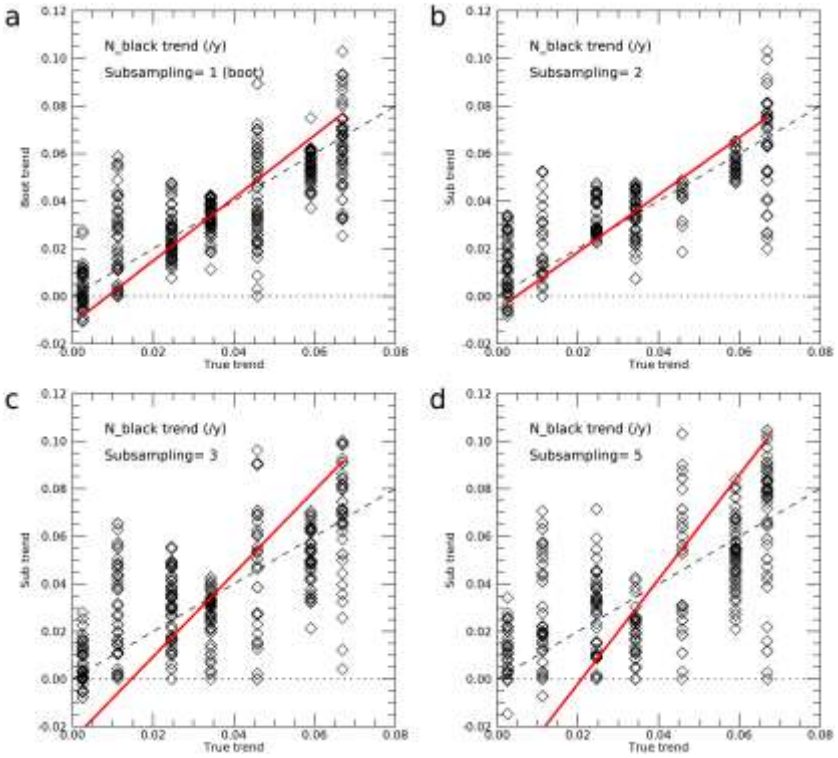

Figure A27: Effects of sub-sampling and bootstrapping on trends in abundance of black microplastic pieces [ln(counts/n.mile)/y]. See caption Figure 19 for more details.

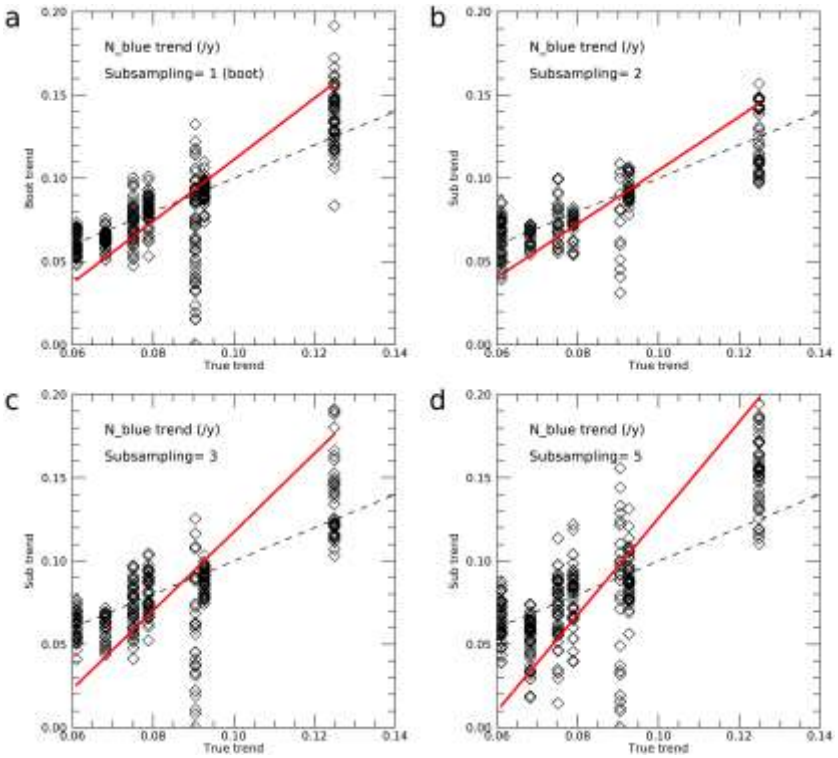

Figure A28: Effects of sub-sampling and bootstrapping on trends in abundance of blue microplastic pieces [ln(counts/n.mile)/y]. See caption Figure 19 for more details.

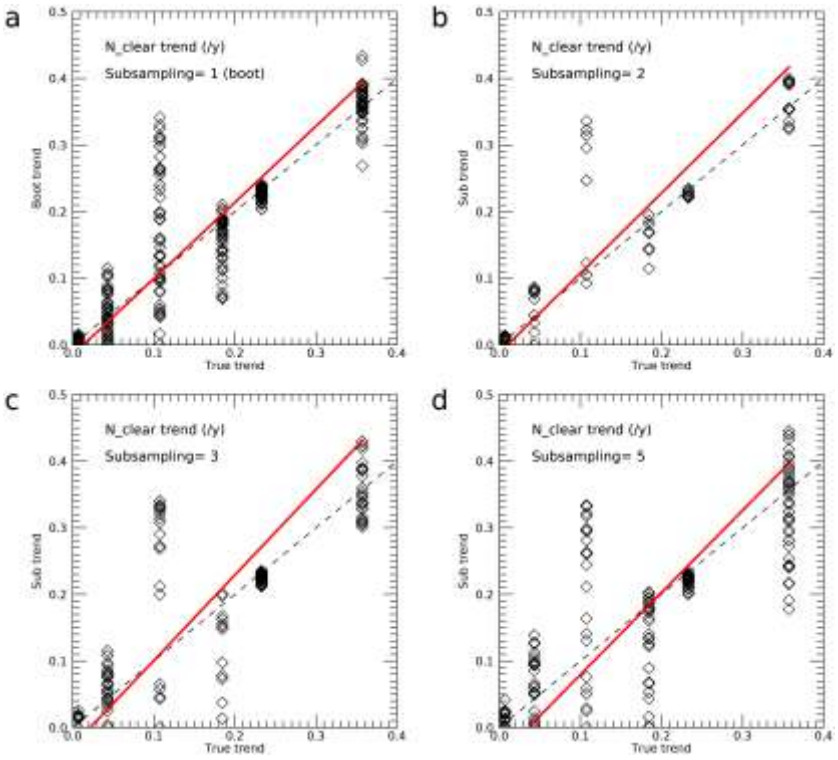

Figure A29: Effects of sub-sampling and bootstrapping on trends in abundance of clear microplastic pieces [ln(counts/n.mile)/y]. See caption Figure 19 for more details.

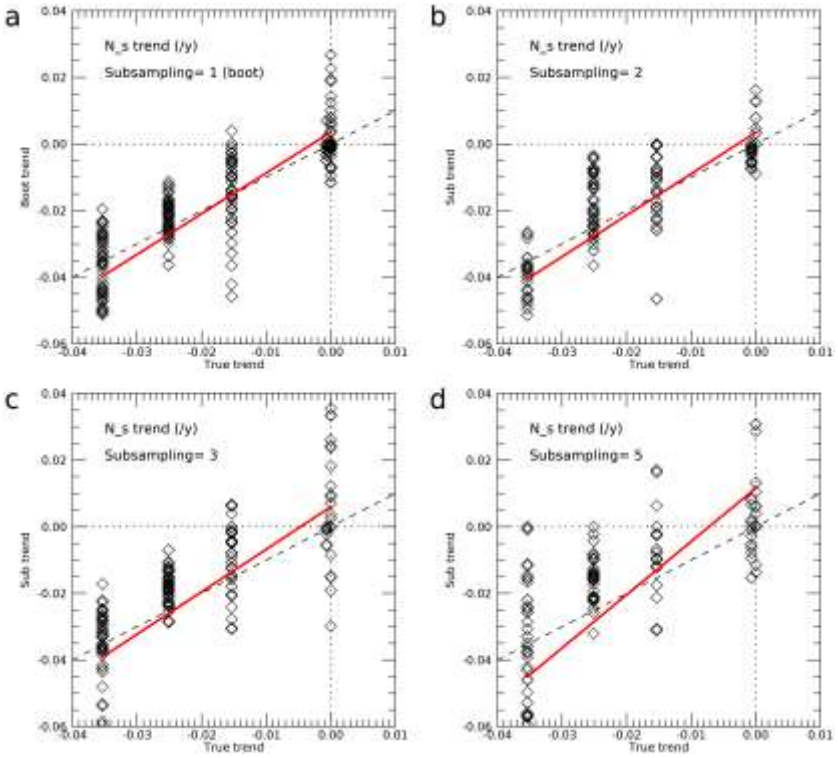

Figure A30: Effects of sub-sampling and bootstrapping on trends in abundance of small microplastic pieces [ln(counts/n.mile)/y]. See caption Figure 19 for more details.

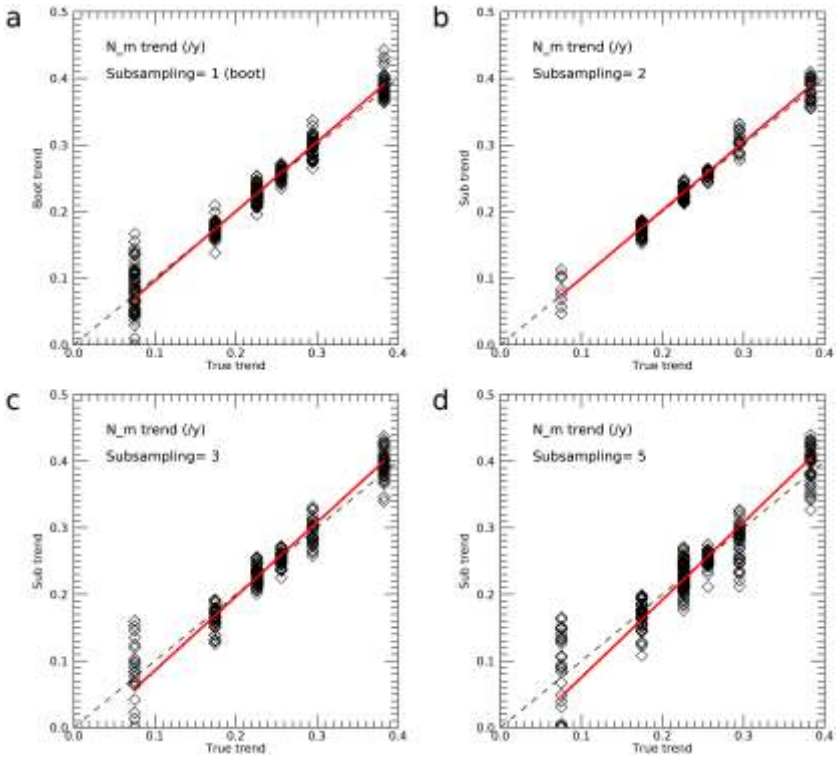

Figure A31: Effects of sub-sampling and bootstrapping on trends in abundance of <u>medium microplastic</u> pieces [ln(counts/n.mile)/y]. See caption Figure 19 for more details.

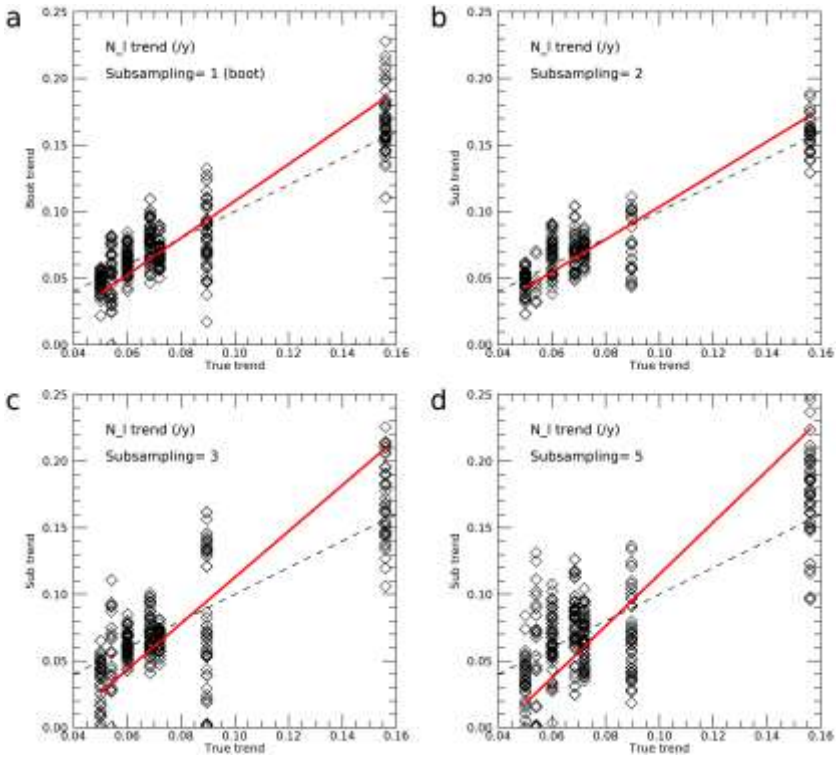

Figure A32: Effects of sub-sampling and bootstrapping on trends in abundance of <u>large</u> microplastic pieces [ln(counts/n.mile)/y]. See caption Figure 19 for more details.