# Microplastic Fibres in Pacific sand lance (*Ammodytes personatus*) burying habitats in the Strait of Georgia, British Columbia, Canada

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# Approval

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# *Abstract*

The ingestion of Microplastic fibres (MF) by forage fish is of growing concern, as these MF have the potential to inhibit ingestion of nutrients, as well as accumulate and magnify at higher trophic levels. In the Strait of Georgia (SoG) on the Pacific Coast of Canada, such accumulation could be significant to the Pacific sand lance (PSL) (*Ammodytes personatus*), a key food source for marine predators such as Pacific salmon, seabirds, and marine mammals. The Pacific sand lance lacks a swim bladder and must bury in shallow low silt, medium coarse sand patches, and these sedimentary habitats may have high MF concentrations.

This research assesses MF concentrations in PSL shallow subtidal burying habitats. Seafloor sediment samples were collected in Spring-Fall 2017, using a Van Veen grab sampler. Samples were collected at distances ranging from ~850 m to 20 kilometers from shore and effluent discharge pipes, and water depths ranging from 5 m to 100 m below the surface. MF concentrations were determined from 112 sediment samples in the laboratory using density extraction methods, while controlling for contamination. We found significantly higher concentrations of MF in suitable PSL burying habitat than in not suitable PSL habitat. Highly suitable PSL habitat had an average of 4.6 MF 10 $g^{-1}$ and a median of 2.3 MF 10 $g^{-1}$. A Kruskal Wallis test revealed that these values were significantly greater than in samples located in non-suitable PSL habitat, which had an average of 1.2 MF 10 $g^{-1}$ and a median of 0.3 MF 10 $g^{-1}$ ($p = 3.5x10^{-5}$, 0-15 fibres 10 $g^{-1}$). Additionally, we observed higher concentrations in shallow water depths (<40 m) than in deeper water depths (>40 m). Congruently, we found distance from estuaries and sewage outflows, as well as proportion of very fine sand in sediment, to be related to MF concentration in seafloor sediment in the SoG. The high concentrations of MF in suitable PSL habitat found in this study could potentially have implications for PSL, such as MF ingestion and the consequent inability to digest organic foods due to the blockage of the digestive tract by MF.

KEYWORDS: Pacific sand lance (PSL), microplastic fibres (MF), Strait of Georgia, very fine sand sediment, estuaries, sewage outflows, suitable PSL habitat

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*Introduction*

Microplastics are defined as any plastic with dimensions less than 5 mm (Andrady 2011, Cole et al 2011, Ballent et al 2016, Anderson et al 2016). Microplastics are generally categorized as a fibre, fragment, or filament (Davidson and Dudas 2016). A fibre is a strand of plastic commonly coming from clothing or rope (Cole et al 2011); a fragment is a piece of plastic that has broken off a larger plastic object (Cole et al 2011); a filament is a thin, soft piece of plastic commonly associated with plastic bags (Cole *et al* 2011). This study has focused on identifying microplastic fibres (MF), as the vast majority of the microplastics identified in many other studies were fibres (Mathalon & Hill 2014, Desforges *et al* 2014, Desforges et al 2015, Stolte et al 2015, Woodall et al 2015, Zobkov & Esiukova 2017).

Coastal ecosystems are thought to be particularly vulnerable to MF because they enter the marine environment from terrestrial pollution via sewage outflow sites and estuaries (Andrady 2011, Anderson *et al* 2016, Ballent *et al* 2016). The highest concentrations of MF are found along polluted coastlines and in mid-ocean gyres, but ongoing research suggests that they are ubiquitous (Allen et al 2019), especially in coastal marine ecosystems (Andrady 2011, Desforges et al 2014, Cluzard *et al* 2015, Stolte *et al* 2015, Ballent *et al* 2016). For example, Desforges et al (2014) found elevated concentrations of MF in coastal waters of British Columbia when compared to the open ocean. In estuarine waters such as the Strait of Georgia (SoG), high concentrations can be attributed to land-based sources such as effluent from wastewater treatment plants (Gies et al 2018), estuaries, and with coastal oceanographic circulation patterns in the SoG that concentrate debris (Crawford et al 1985; Freeland et al 1984).

The potential prevalence of MF in seabed sediments and overlying seawater of the SoG has significant implications for forage fish that rely heavily on these two habitats. At particular risk is the Pacific sand lance (*Ammodytes personatus; PSL*), a forage fish which lacks a swim bladder and buries in coarse, low silt, sandy sediments along the BC coast for habitat (Haynes and Robinson 2011, Robinson *et al* 2013). Thereforee, PSL could be vulnerable to MF in seafloor sediments. If PSL are inadvertently ingesting MF, these indigestible polymers could block the digestive tract, preventing them from digesting real food (Wright et al 2013). This could result in increased mortality or reduced condition of PSL individuals. Although MF concentrations have been found to be widespread in waters of the SoG (Deforges et al 2014), their presence in seabed sediments is not yet studied in this region.

PSL are foraged upon by a wide variety of vertebrate predators such as Pacific salmon (Oncorhynchus species), seabirds such as the Marbled Murrelet (*Brachyramphus marmoratus*), and marine mammals such as humpback whales (*Megaptera novaeangliae*) (Brodeur 1991, Friedlaender et al 2009, Cury et al 2011, Bertram et al 2016). The ingestion of PSL containing MF might result in biomagnification of MF in the vertebrate predators. This amplification of MF particles through the food web means the number of indigestible plastics will increase in higher trophic level species and consequentially could block the digestive track of higher trophic level species and prevent them from digesting food as well (Wright et al 2013, Desforges et al 2015, Davidson and Dudas 2016). MF were already found to be affecting other species in the SoG (Desforges et al 2015, Davidson and Dudas 2016), and if similar effects are impacting PSL in the area then species that rely on them for food, such as the Marbled Murrelet and Pacific Salmon, could also be affected through biomagnification and digestive interference.

While recent research in the SoG has examined the potential influence of MF pollution on water quality (Desforges et al 2014), manila clams (Davidson and Dudas 2016), and zooplankton (Desforges et al 2015), this study researches the extent of MF concentrations in seabed sediment habitats in the SoG. The main objective of the study was to quantify MF concentrations in seafloor sediments that may serve as PSL habitat, thus providing an indicator of the extent of MF in PSL habitat. A secondary objective was to evaluate environmental factors that may influence MF concentrations in seabed sediments, such as depth, sediment composition, and proximity to potential MF sources. We hypothesized that: (1) MF would exhibit similar settlement patterns as very fine sand and silt since MF and these sediment grains are of comparable size and weight, (2) sediment samples from areas closer to potential sources of MF such as sewer outflows, would have higher concentrations of MF than samples collected further away, and (3) MF concentration will increase with ocean depth due to particle settling properties and depth (Hill et al. 2008).

*Study Area*

Seabed sediment samples were collected from shallow (< 100m) subtidal waters within the SoG (Figure 1) and analysed for both PSL habitat suitability and MF concentration. Each grab sampled was classified into one of three PSL burying habitat suitability classes: (1) highly suitable PSL habitat/ PSL present in sample (P/HS), (2) moderately suitable PSL habitat (MS), and (3) not suitable PSL habitat (NS). These classes were based on grain size properties, particularly weight percent silt content, as described in Robinson et al. (2013). Highly suitable sediment grabs had very low percent silt content (< 2%); medium suitable sediment had moderate silt content (2-4%); and not suitable sediment had higher silt content (>4%). Eleven of the 112 samples collected contained PSL individuals. These samples were considered to have near identical grain size compositions to P/HS habitat and as such were classified as that category. A multidimensional scaling model performed in R illustrates the near-identical grain size compositions of HS samples and samples with PSL present (Appendix J).

Bathymetric charts were used to identify possible areas of sandy seafloor sediment with low silt content (P/HS PSL habitat). Sampling locations were selected based on three characteristics, including (1) expected PSL habitat suitability, (2) water depth (all of which were <100m), and (3) distance from known sewage outflow sites (ranging from 0 m to 14 km). Specifically, the 112 grab samples of surface seabed sediment were selected to adequately represent the three suitability classes, as well as the depth range using 20-m depth categories from 0 to 100 m. Fifty-three samples were selected as highly suitable habitat, twenty from medium suitable habitat, and thirty-eight from not suitable habitat.

Sample locations were clustered in three distinct regions of the SoG. One cluster of 61 sample sites was located off the coast of the Saanich Peninsula, between the municipalities of Sidney and Victoria (Haro Strait; Figure 1b). Samples were collected around the Saanich Peninsula sewage system outflow site as it is a potential source of MF. This outflow site is the main one for the entire Saanich Peninsula, which has a population of 383,000 between Sidney and Victoria (crd.bc.ca). This area was considered to be the southern SoG (SSG). A second cluster of 25 sites was located off the coast of the Comox Valley regional district, Vancouver Island (Figure 1a). Again, grab samples were collected around the major sewage outflow site in the area. This discharge site serves the 66,527 residents of the Comox Valley Regional District (crd.bc.ca). Finally, a third cluster of 26 sample sites was located in the northern SoG, (area marked by the town of Lund) (Figure 1a). This area is located between the municipalities of Campbell River on Vancouver Island and Powell River on mainland B.C. Campbell River has a population of 35,000 while there are 13,000 residents in Powell River (Canadian Census, 2018). Both municipalities have a waste water treatment plant and grab samples were collected near both. Collectively, the 50 samples associated with the Comox Valley and Campbell River/Powell River areas were considered the northern SoG (NSG) region.

Maps illustrating sample site locations in relation to depth, nearest estuary, nearest sewage outflow site, nearest finfish aquaculture, nearest shellfish aquaculture, nearest marina, and nearest anchorage site can be found in appendix F.

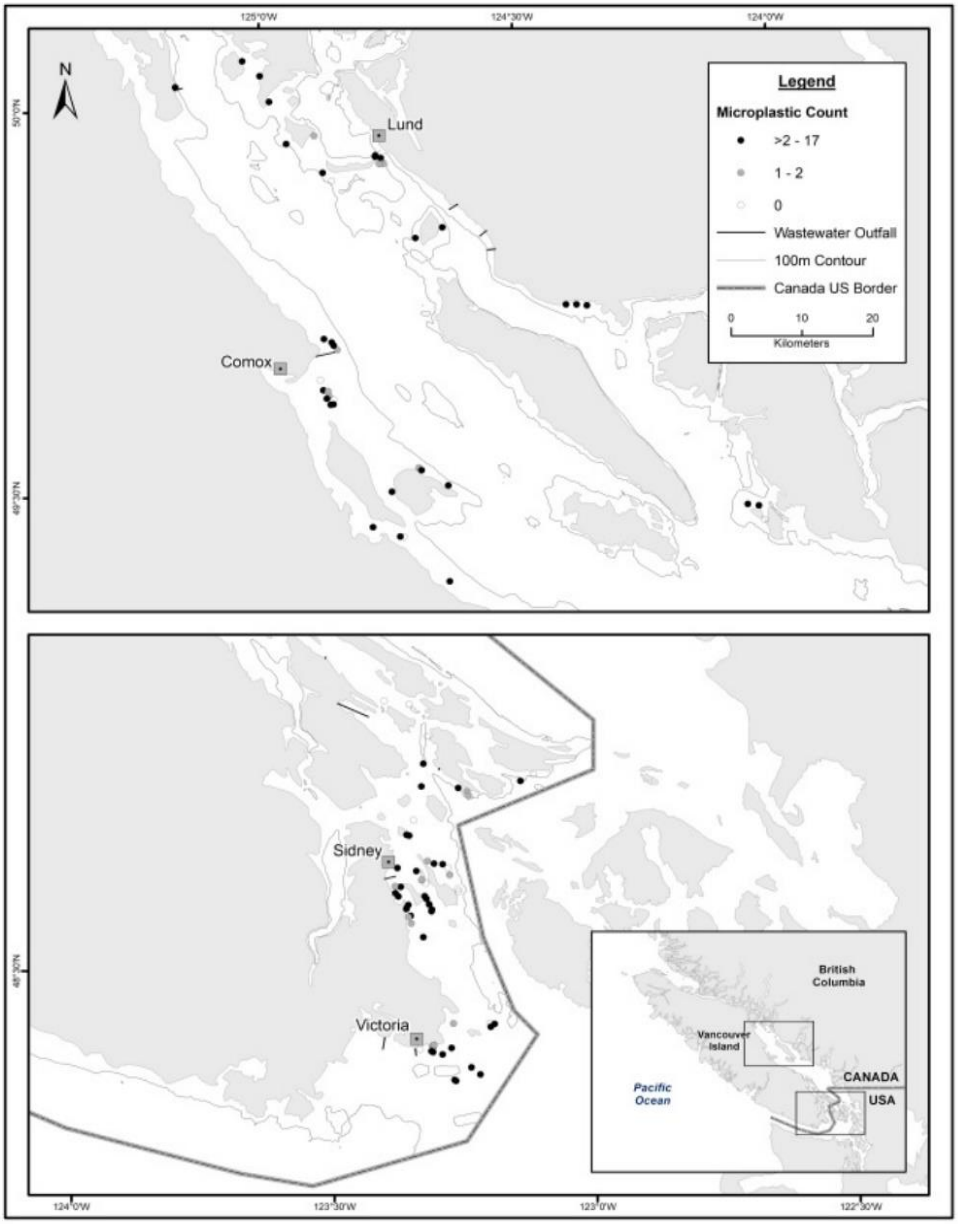

Figure 1 Sample site locations. All samples were taken at depths < 100m and were chosen for their potential to represent suitable variance between sediment types as outlined in Robinson et al. (2013) and depth. The top panel shows what this paper refers to as the NSG, while the bottom panel shows the SSG.

### *Field Methods*

Seabed sediment samples were collected from each region using a 0.3m$^2$ Van Veen benthic grab sampler. Sediment samples were examined for marine species, which were removed. Any PSL found in the samples were separated from the sample and archived. The sediment sample was placed in a Ziploc® bag and frozen until lab analysis. To determine if plastic freezer bags could contaminate a sample with outside plastic, controlled tests were carried out using sediment samples identified to have no MF. Three of these samples were placed in plastic Ziploc® bags and three were placed in glass containers for five days. These samples were then re-examined, and neither set of samples was found to have MF. Thus, as with previous MF studies (Mathalon and Hill 2014, Ballent et al 2016), plastic freezer bags were determined to pose no threat of MF contamination.

*Laboratory Methods*

Samples were removed from the freezer and thawed to room temperature. Half of the sample (250 grams) was separated for analysis and the remainder placed back in the freezer and archived.

Grain size analysis was conducted at Parks Canada laboratory in Vancouver, British Columbia. Samples were oven-dried at 60ºC for 24 h and then poured over a series of graded, brass sieves with sizes of 0.062mm, 0.125mm, 0.250mm, 0.25mm, 0.5mm, 1mm, 2mm, and >2mm. The sieve stack was shaken for 10 minutes. Sieved sediments from each size fraction were weighed to the nearest 0.01 g and the dry-weight proportion of each sediment classification was calculated. Each category of grain size was classified according to the Wentworth grain size classifications (Wentworth 1922): <0.062 mm (Silt-clay), 0.062-0.125mm (very fine sand), 0.125-0.250mm (fine sand), 0.25-0.5mm (medium sand), 0.5-1mm (coarse sand), 1-2mm (fine gravel), >2mm (medium gravel).

Each unsorted sediment sample was placed in a saline solution of sodium polytungstate with a density of 1.4 g $cm^{-3}$ to separate the MF from sediments using a density analysis method as described in Mathalon and Hill (2014), Stolte et al (2015), Zobkov and Esiukova (2017) (Appendix B). 10 grams of sample sediment were placed in a 50 ml centrifuge tube with 20 ml of sodium polytungstate. The solution was then spun at 3000 rpm for 10 minutes. A pipette was used to extract the sodium polytungstate supernatant into 15 ml centrifuge tubes. These tubes were again spun at 3000 rpm for 10 minutes to ensure complete separation of any MF from sediments (Van Cauwenberghe *et al* 2013, Claessens *et al* 2013). The supernatant was again extracted using a pipette and placed on a Whatman 50 (2.7 µm pore size) filter paper that was resting over a beaker. The filter paper was quickly covered by a petri dish cover to prevent both contamination from MF in the ambient air and from extracted MF blowing off the filter paper. When the sodium polytungstate completely filtered through the paper, usually after 24 hours, the filter paper was placed in a petri dish and placed under a microscope for examination. Mostly MF larger than 2.7 µm were extracted as the rest would pass through the pores of the filter paper.

MF were visually identified, examined, quantified, and categorized; a subset of MF was archived and the size (in mm) and colour was recorded. The length of the MF ranged from 1 µm to 5 mm.

*Contamination control*

To control for contamination of samples from airborne MF during the extraction process, materials were rinsed with filtered deionized water after every use, white cotton clothing (instead of synthetic material) was worn during all stages of the analysis, and ceiling fans were running at all times to help prevent airborne MF from settling. Additionally, filter papers were placed at every work station and analysed for the number of airborne MF settling on the work surfaces.

Procedural blank samples of only deionized water were subject to the entire extraction process to determine the level of contamination during analysis.

Of the 6 procedural blank tests run, 3 contained MF (2, 2, and 4 MF, respectively). Separately, 5 filter papers were left exposed on surfaces near work stations in the lab, including the centrifuge, the pipette and filter stations, and the microscope station. Of the 5 test filter papers, 4 contained MF after 24 hours of exposure. These MF were assumed to have settled on the filter paper from the ambient air. A total of 7 MF was found on the 5 filter papers. The two contamination tests were considered separately because one tested for contamination during the analysis procedure while the other tested for contamination from ambient air. The averages from the two tests were combined and the total average of contamination calculated for the whole analysis was 2.7 MF per sample. This contamination average was accounted for in the MF counts for each sample, and every count in the results has been reduced by this amount.

### *Statistical analysis*

All statistical analyses were conducted using R version 3.4.2 (R Core Team, 2017). A Shapiro-Wilk test (Ghasemi and Zahediasl 2012) indicated the data were not normally distributed, even after being log transformed. As such, non-parametric tests were used to analyze the data (Appendix D).

The data set was analyzed for significant differences in MF concentrations between habitat suitability classes and differences in MF concentrations between depth categories. A Kruskal-Wallis test was used to assess the significance of differences in medians for MF concentration in sediments categorized into the 3 burying habitat classes (P/HS, MS, and NS, where samples with PSL present were classified as P/HS?). This test is commonly used to determine whether two variables, one dependent and the other independent, differ in their distributions. A Wilcox rank sum test (for non-parametric data) was used in place of an independent samples two tailed t-test to assess if the medians for MF concentration differed between depth classes (Lam and Longnecker 1983).

A General Additive Model (GAM) with a “scat distribution family” (for non-parametric data) was used to assess the significance of the influence of six possible MF sources and three environmental factors on MF concentrations in seafloor sediment in the SoG. The scat distribution family is used for heavy tailed response variables, y, and uses a scaled t model (Wood 2017). The potential source factors included in the GAM model were known locations of boat marinas, anchorage sites, sewage outflow sites, finfish aquaculture, shellfish aquaculture, and estuaries. The distance (meters) between each potential source and the nearest grab location was used to assess their significance of influence. The three environmental factors included were ocean depth, bathymetric tidal speed, and sediment grain size composition, which included the 7 classes of grain size from Wentworth (1922). These covariates were chosen because of their potential significance as outlined in Robinson et al (2013). Robinson (2019, personal communication) provided the environmental information for these covariates.

The GAM analysis was performed using the R-package “mgcv” (version 1.8-24). The response variable was MF concentration while the covariates in the model were the environmental factors and distance to possible MF sources listed above, with smoothing functions applied to the covariates. The GAM selected the smoothing functions needed to produce the model (Wood 2017). Each covariate is added into the GAM one at a time, and the influence that each covariate has on the changes in MF concentration is smoothed so a continuous relationship between MF concentrations and each covariate can be observed. When all covariates are added, the strength of influence ($r^2$) of the model and the significance (p-value) of each covariate is indicated. Covariates determined to influence MF with a 95% confidence interval were considered significant for this study. "Deviance explained" is analogous to variance in a linear regression model (Murase et al 2009) and was used to assess the suitability of the GAM for modeling the dynamics influencing MF concentration in the SoG. Outlying data (residual data) within covariates can have a disproportionate influence on the GAM results and so a goodness of fit test was performed on the GAM to determine the influence of residuals. If residual data of a covariate were shown to influence the results significantly ($p$ value $< 0.05$) then this covariate was not considered in this paper (Wood 2017). Additionally, a collinearity bias can be introduced in a GAM if two covariates are closely correlated. To account for collinearity bias of covariates, a "select07" method was used, such that one covariate from any pair that had a Pearson correlation coefficient of $> 0.7$ was left out of the GAM analysis (Dormann et al 2013). Appendix H has tables indicating the collinearity between all pairs of covariates. When a pair of covariates exhibits high collinearity, one covariate of that pair should be eliminated from the GAM to prevent collinearity bias. Distance from shellfish aquaculture and tidal speed were two covariates left out of the SoG GAM due to collinearity $> 0.7$. Multiple iterations of GAM were run with various combinations of covariates to verify that the model with greatest deviance explained was chosen (appendix I).

ArcMap (version 10.6) with an ArcGIS-R bridge was used to produce a predictive map visualizing the results of the GAM analysis.

### *Results*

Overall, 91 of the 112 (81%) samples contained MF(s). After subtracting the average contamination of 2.7 MF, the highest number of MF identified in one sample was 15 MF 10 $g^{-1}$, the average per sample was 3.0 MF 10 $g^{-1}$ and the median was 1.3 MF 10 $g^{-1}$. The dominant colours were blue (56%), and black (23%). Other colours recorded were white (9%), red (4%), purple (3%), brown (4%), and green (1%).

### *MF concentration and PSL habitat suitability classes*

A comparison of MF concentrations between PSL burying habitat classes demonstrated that MF concentrations, adjusted for potential lab contamination, were significantly higher in highly suitable PSL burying habitats than in medium and non-suitable PSL habitats (Figure 2). A Kruskal-Wallis test comparing the three categories, highly suitable, medium suitable, and not suitable habitats, indicated statistically significant differences in the medians of MF concentrations ($p = 3.5x10^{-5}$).· Highly suitable PSL habitat had an average of 4.6 MF 10 $g^{-1}$ and a median of 2.3 MF 10 $g^{-1}$. These values were significantly greater than in samples located in non-suitable PSL habitat, which had an average of 1.2 MF 10 $g^{-1}$ and a median of 0.3 MF 10 $g^{-1}$.

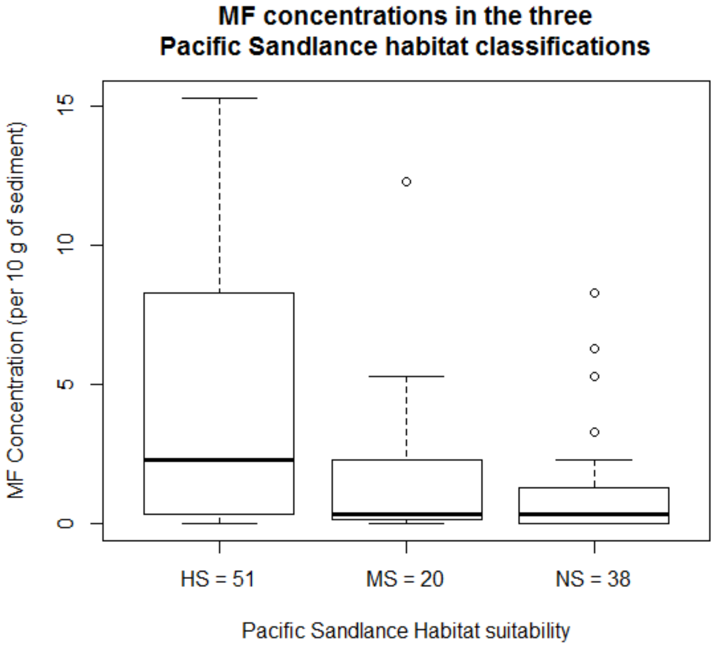

Figure 2. The MF concentrations found within each PSL burying habitat suitability class; P/HS (highly suitable), MS (Medium Suitability), and NS (Not Suitable). The black line within each box represents the median MF concentration (# of MF 10 $g^{-1}$) found across all samples within the respective habitat classifications. The upper and lower extents of each box represent 75th and 25th quantiles, respectively. Lines above and below each box represent the maximum and minimum numbers (excluding outliers), respectively. Dots above the boxes represent data that fall at least 1.5 times outside of the interquartile range.

### *MF concentration and ocean depth*

MF concentrations were also higher in depts shallower than 40-50 m (Figure 3). Samples collected at depths of <40-50 m had a higher median (2.3 MF 10 $g^{-1}$) than in depths >40-50 m (0.3 MF 10 $g^{-1}$). A Wilcox rank sum test indicates that MF median concentrations were significantly different between each depth category (five 20 m intervals including 0-20 m, 20-40 m, 40-60 m, 60-80 m, and 80-100 m) ($p = 2.2\times10^{-16}$).

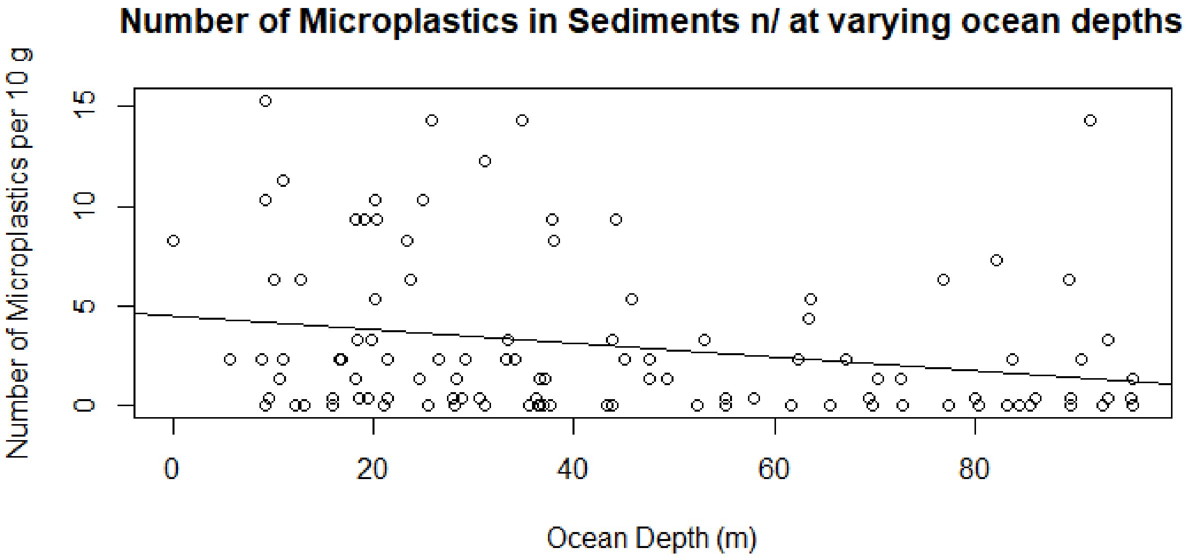

Figure 3. Scatter plot with a regression line illustrating that MF concentrations are higher in shallower depths. The correlation coefficient for this relationship was determined using a spearman rank sum test and is $R^2 = -0.24$.

### *Influences on MF Concentration*

A generalized additive model (GAM) was used to assess several variables that could influence MF concentration in seabed sediments. These variables included five potential sources of MF as well as two environmental factors (as listed in *statistical analysis* section).

The GAM identified three potential MF sources as having a significant influence on MF concentration: proximity to estuaries, sewage outflows, and marinas. When these covariates were included in the GAM, a moderate strength of influence on MF concentration ($r^2 = 0.28$) was determined. Of these covariates, estuaries ($p = 0.04$), sewage outflow sites ($p = 0.02$), and marinas ($p = 1.6\text{x}10^{-3}$) were the only statistically significant sources in this model. However, when a goodness of fit test was performed on the model, the residual data for proximity to marinas was shown to exert significant influence on the result from the model, so it was not considered. This is because residuals (or outliers) in the marina data have disproportionally influenced the relationship between marina proximity and MF concentrations. It is therefore not an accurate depiction of the actual marina/MF relationship occurring in the SoG.

The GAM suggests that the distance from estuaries (Figure 4) and sewage outflows (Figure 5) exerts significant influences on MF concentration. The probability of MF being present in seafloor sediment at specific distances from these two potential sources is represented by the solid black line on the plot, with the greyed area encompassing the standard error for a 95% confidence interval. There is a higher probability that MF concentrations with be higher in seafloor sediments closer to estuaries (Figure 4), but a lower likelihood for it to be higher in sediment close to sewage outflows (Figure 5).

The GAM also identified two environmental factors as a significant influence on MF concentrations: depth and grain size. The model indicated a negative relationship between depth and MF concentrations, suggesting that higher MF concentrations are found in shallower depths (Figure 3).

Additionally, sediment grain sizes between 0.125mm and 0.063mm, or very fine sand (Wentworth 1922), were shown to influence MF concentrations in sediments in the SoG. The GAM suggests that as the proportion of very fine sand in sediment samples increases, the probability of MF contaminating that sediment decreases (Figure 6).

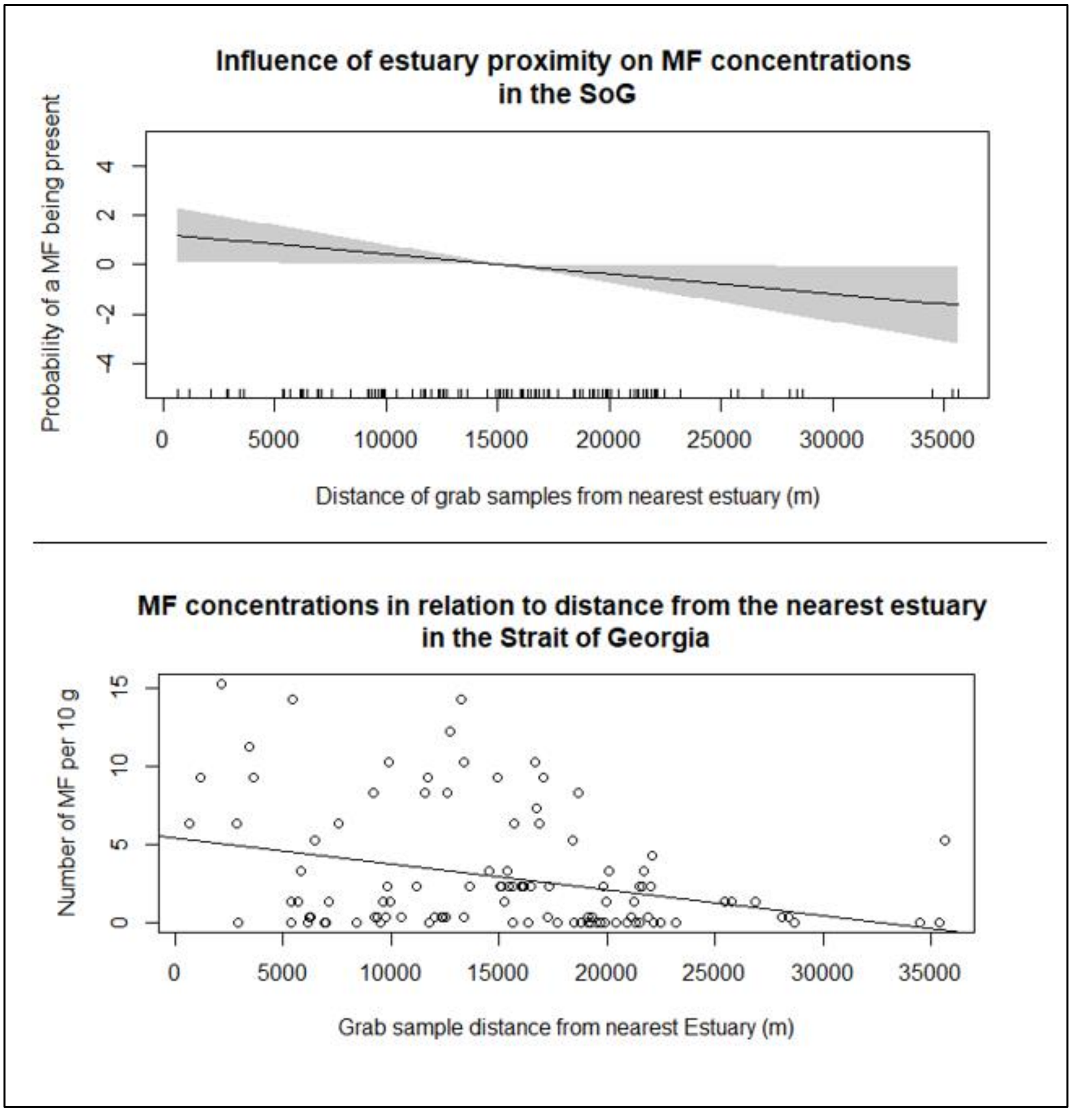

Figure 4. The top panel depicts the GAM model relationship between MF concentrations in seafloor sediments and proximity to estuaries. X-axis indicates distance of sampling sites from estuaries. Y-axis indicates probability of seafloor sediment being contaminated by MF, on a logit scale (0 represents a probability of 0.5). The dashed lines on the x-axis are the locations of the sampling sites. The solid black line is the relationship between MF and estuary proximity, with the grey area representing a standard error encompassing a 95% confidence interval.

The bottom panel depicts a scatter plot illustrating the relationship between MF concentrations (# of MF 10 $g^{-1}$) in each sediment sample and the proximity to estuaries. A regression line was fitted to showcase the slope. The correlation coefficient for this relationship was determined using a spearman rank sum test and is $R^2 = -0.29$.

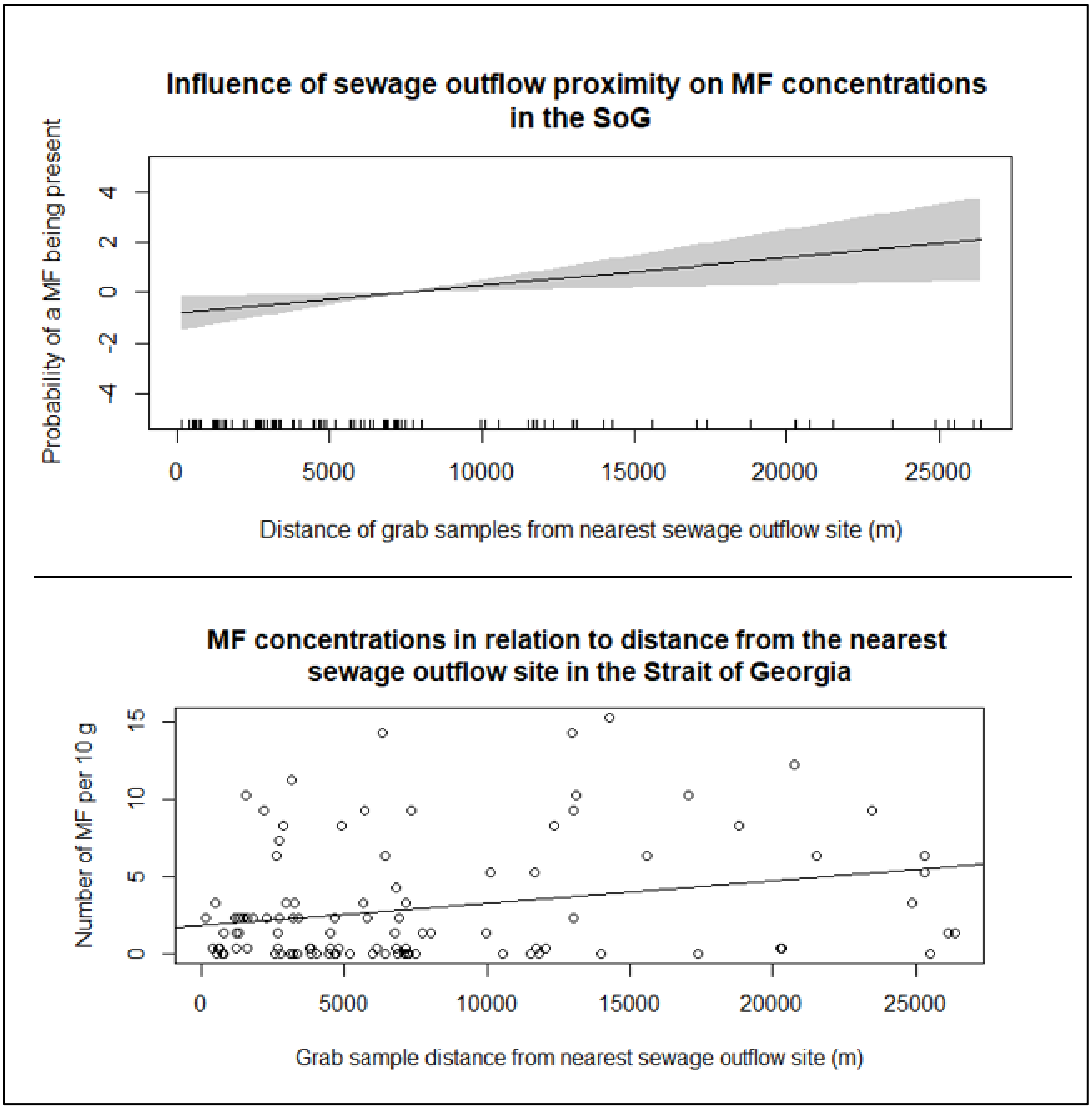

Figure 5. Top panel depicts the influence sewage outflow sites have on MF concentrations in seafloor sediment in relation to proximity of sewage outflow sites. X-axis indicates distance of sampling sites from sewage outflow. Y-axis indicates probability of seafloor sediment being contaminated by MF, on a logit scale (0 represents a probability of 0.5). The dashed lines on the x-axis are the locations of the sampling sites. The solid black line is the relationship between MF and outflow proximity, with the grey area representing a standard error encompassing a 95% confidence interval.

The bottom panel depicts a scatter plot illustrating the relationship between MF concentrations (# of MF 10 $g^{-1}$) in each sediment sample and the proximity to sewage outflow sites. A regression line was fitted to showcase the slope. The correlation coefficient for this relationship was determined using a spearman rank sum test and is $R^2 = 0.15$.

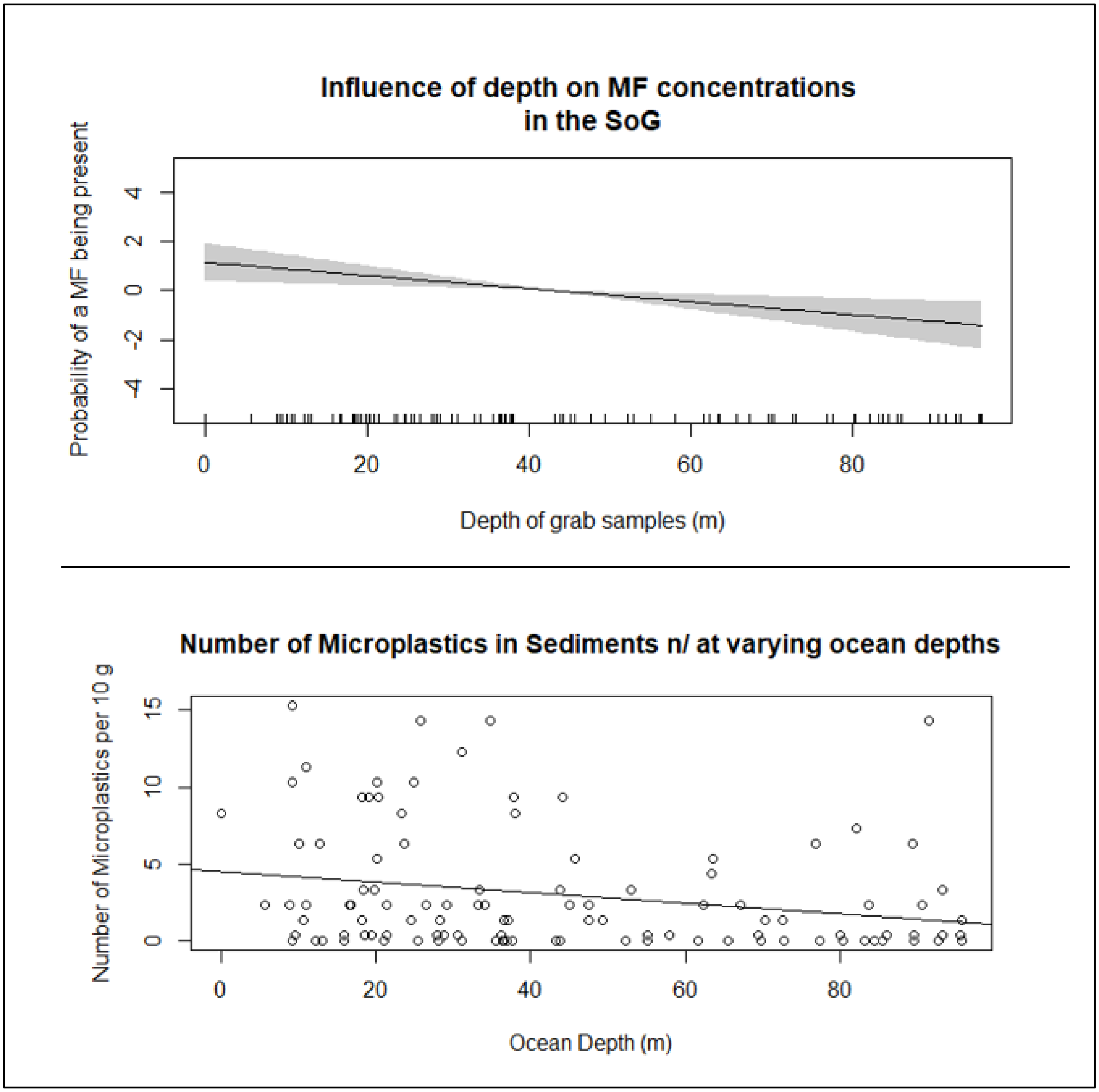

Figure 6. The top panel depicts of the influence depth has on MF concentrations. X-axis indicates depth at which the grab samples were located. Y-axis indicates probability of seafloor sediment being contaminated by MF, on a logit scale (0 represents a probability of 0.5). The dashed lines on the x-axis are the locations of the sampling sites. The solid black line is the relationship between MF and depth, with the grey area representing a standard error encompassing a 95% confidence interval.

The bottom panel depicts a scatter plot illustrating the relationship between MF concentrations (# of MF 10 $g^{-1}$) in each sediment sample and depth. A regression line was fitted to showcase the slope. The correlation coefficient for this relationship was determined using a spearman rank sum test and is $R^2$ = -0.24.

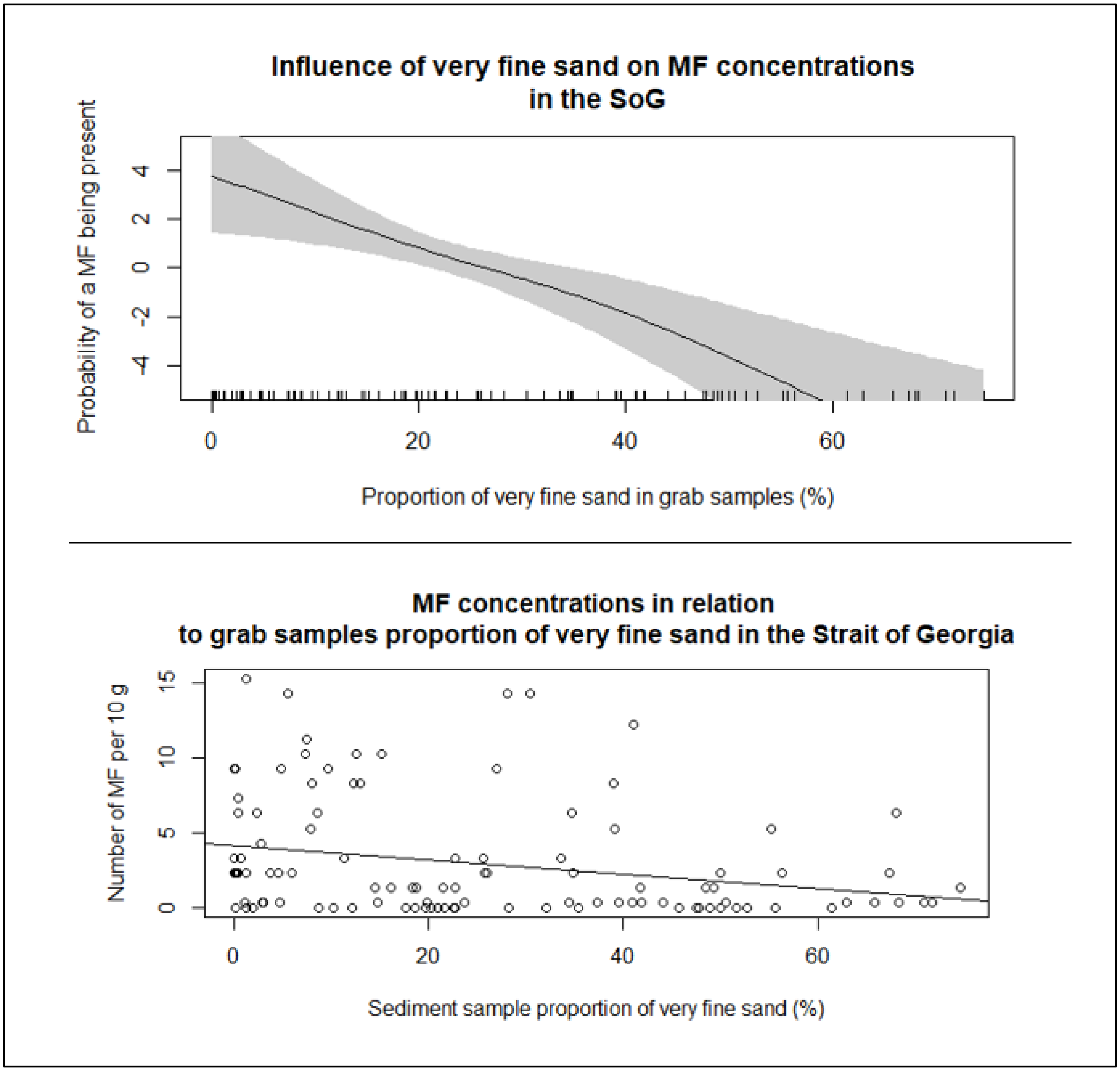

Figure 7. Top panel depicts the influence very fine sand has on MF concentrations in seafloor sediment. X-axis indicates proportion (%) of very fine sand in each grab sample. Y-axis indicates probability of seafloor sediment being contaminated by MF, on a logit scale (0 represents a probability of 0.5). The dashed lines on the x-axis are sediment grab samples. The solid black line is the relationship between MF and very fine sand, with the grey area representing a standard error encompassing a 95% confidence interval.

The bottom panel depicts a scatter plot illustrating the relationship between MF concentrations (# of MF 10 g$^{-1}$) in each sediment sample and the proportion of very fine sand. A regression line was fitted to showcase the slope. The correlation coefficient for this relationship was determined using a spearman rank sum test and is R$^2$ = -0.26.

A predictive map visualizing the final GAM model, based on the covariates distance from estuaries, distance from sewage outflow sites, and depth, illustrates the likelihood of MF concentrations in seafloor sediment in the SoG (figure 7). This map suggests high likelihoods of

MF concentrations in NSG sediments near the Comox regional district, Campbell River, and Powell River. The lowest likelihoods are associated with the middle of the SoG where the deepest waters are located.

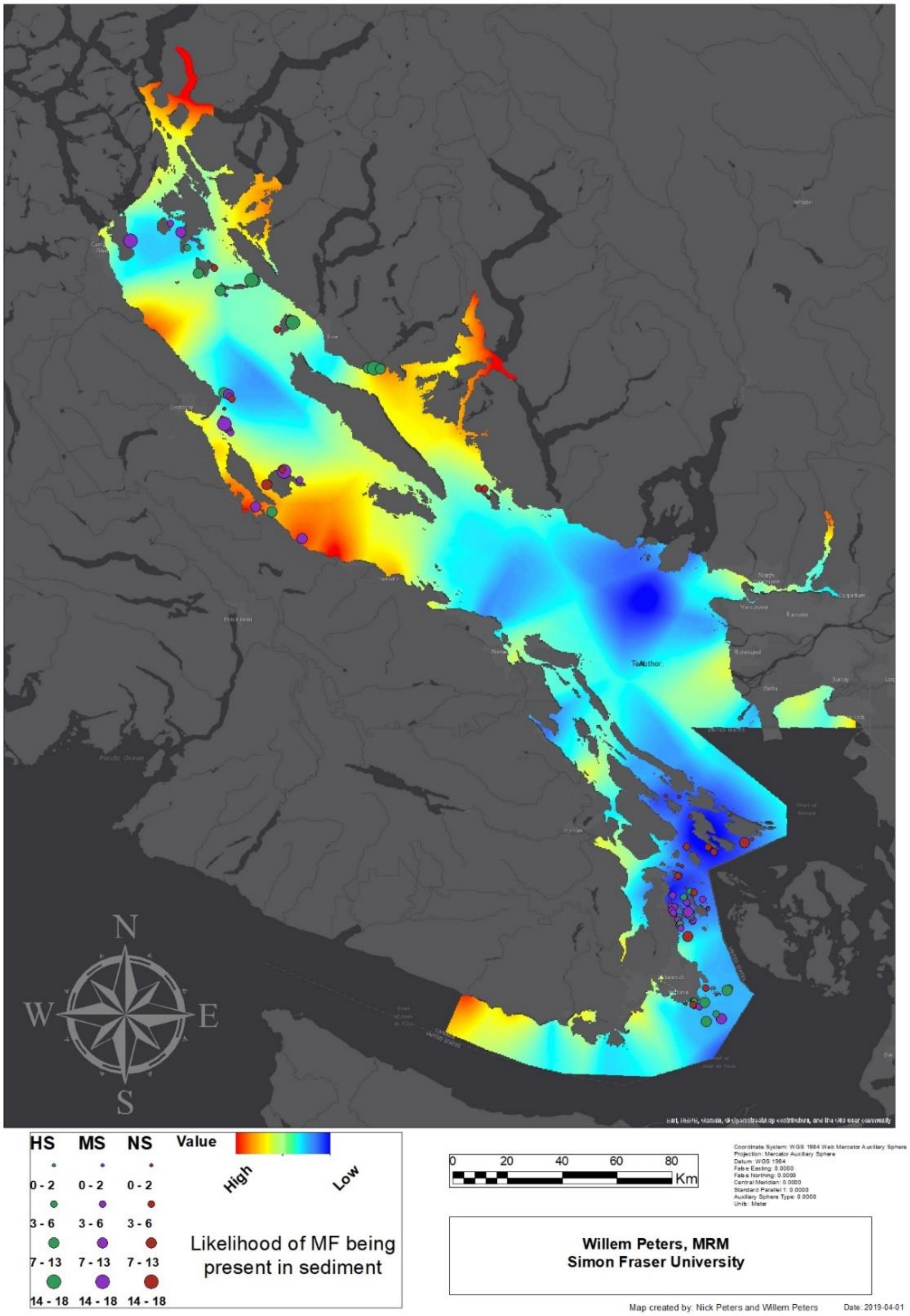

Figure 8. Map illustrating predicted MF concentration in the SoG produced by the GAM model, superimposed with the sediment grab samples. The warmer the colour, the higher the likelihood that MF are present in seafloor sediment.

Finally, our results indicated significantly higher MF concentrations in sediments from the northern (NSG) when compared with the southern parts of the SoG (SSG) (see Figure 1 for demarcation of NSG and SSG). A total of 51 grab samples were collected in the NSG with an average of 4.2 MF 10 $g^{-1}$. In contrast, 61 grab samples were collected in the SSG with an average of 1.9 MF 10 $g^{-1}$. Interestingly, the median concentration of MF was the same for both the SSG and NSG, at 1.3 MF 10 $g^{-1}$. Because the medians are the same, the average concentrations of MF in the SoG is clearly being influenced by a skewed distribution of MF, with more MF being in the NSG.

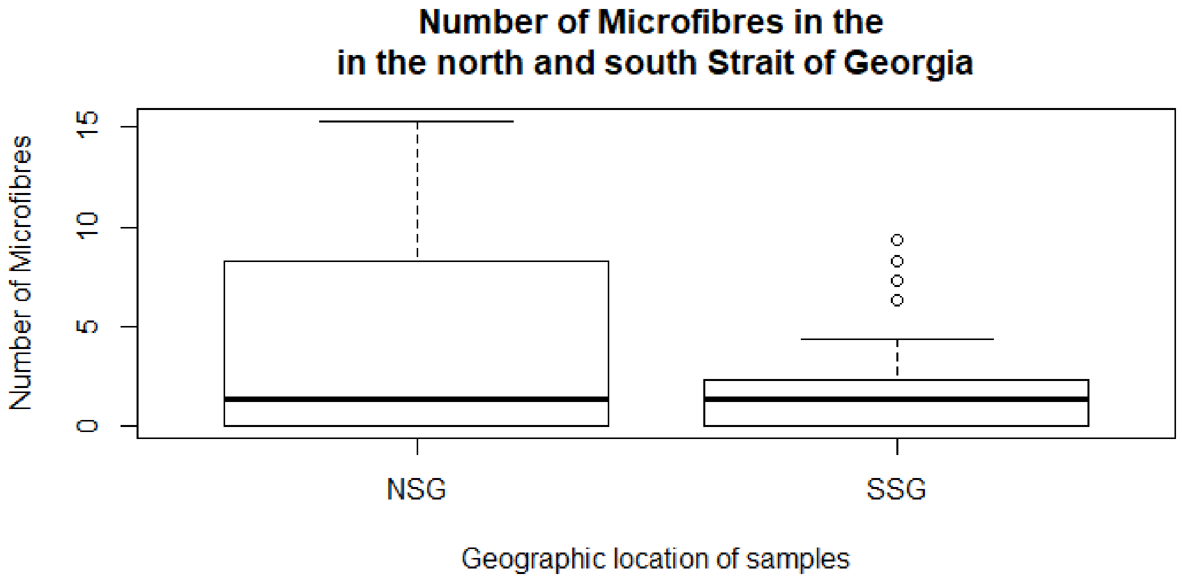

Figure 9. Box and Whisker plot illustrating MF concentrations between the northern SoG (NSG) and the southern SoG (SSG). The black line within the box represents the median MF concentration (# of MF 10 $g^{-1}$) found across all samples within their respective region. The upper and lower extents of each box represent 75th and 25th quantiles, respectively. Lines above and below each box represent maximum and minimum numbers (excluding outliers), respectively. Dots above the boxes represent data that fall at least 1.5 times outside of the interquartile range (the difference between 75th and 25th quantiles).

### *Discussion*

This study found MF in all three PSL burying habitat suitability classes and across all depths ranging from 1 to-100 meters. Importantly, higher concentrations of MF were found in sediments that were classified as highly suitable PSL habitat (average of 4.6 MF 10 $g^{-1}$ or 460 MF $kg^{-1}$) than in sediments classified as being not suitable (average of 1.2 MF 10 $g^{-1}$ or 120 MF $kg^{-1}$). Furthermore, more MF were found at depths of <40-50 m (average of 3.8 MF 10 $g^{-1}$ or 380 MF $kg^{-1}$) than at depths >40-50 m (average of 1.9 MF 10$g^{-1}$ or 190 MF $kg^{-1}$). The higher MF concentrations at shallower depths and in PSL habitat are important because PSL use their sediment habitat as refuge at night and during the day when they are not feeding in the water column (Robards et al. 1999, Haynes and Robinson 2011). Furthermore, PSL spend a large amount of their time buried in sediments in the late fall/early winter developing gonads (Haynes and Robinson 2011).

Our results suggest that MF concentrations are likely to be higher at shallower depths (Figure 3) and in sediments with low proportions of very fine sand sediment (Figure 7). This finding has also been observed in other sedimentary environments (Zobkov and Esiukova 2017) and goes against our original hypothesis that MF concentrations would be higher in sediment samples with high silt content taken at deeper depths. This finding is somewhat counter-intuitive if one assumes that MF, being small and lightweight, would show similar settling patterns as sediments of similar density, i.e. silts and clays. Silts and clays tend to settle in deeper waters than sand because they are generally lower energy environments with less wave and tidal energy (Goff et al 2004, Hill et al 2008). How MFs accumulate in low silt, medium coarse sand patches located in relatively shallow areas of greater wind, wave or current exposure is unclear, but we discuss a couple of possibilities below.

One possibility is that MF accumulate in coarse, sandy sediments because they enter the environment as sizes comparable to larger sediment grain sizes (e.g., 1 mm and 0.25 mm) and therefore have similar settling properties. Most MF are pieces of a much larger object; such as rope or an article of clothing. They could be entering the marine ecosystem in larger aggregates of similar size and density as sand sediments between the sizes of 0.25-0.5mm and thus settle in similar regions of the seafloor. These MF clumps would be unobservable through lab analysis because the analysis process breaks them into individual pieces.

Another possibility is that MF are being transported to the shallow, coarse sediment habitat by PSL. PSL would ingest them while feeding in the water column and excrete them in their preferred burying habitat while they are not feeding. This mechanism could explain the significant positive relationship between PSL habitat suitability and MF concentration and is also supported by evidence that the stomacP/HS of PSL retrieved from the SoG contain MF (Bertram *et al* 2016). Since PSL do not feed in their sediment habitats, they likely ingest the MF while feeding in the water column and bring them back to the sediment. Mallory (2008) also proposed this type of biologically-mediated MF transport in northern fulmars, an Arctic bird. The birds were found to transport plastics from their feeding grounds to their breeding grounds to feed their chicks. The highest concentrations of plastics were found to be at their breeding grounds.

### *GAM analysis*

The GAM was used in this study to identify potential influences that six possible sources of MF and three environmental factors might have on MF distributions in the SoG. The GAM was created using the concentrations of MF at specific distances from potential sources of MF, at certain depths, and in sediments with certain grain size compositions (the covariates of the model). Using this information, the GAM estimated the possible influence of each covariate on MF concentrations. The likelihood of finding MF in seafloor sediments based upon these criteria indicates the most probable areas where MF may settle in seafloor sediments. The GAM, which included all covariates except for distance from shellfish aquaculture and bathymetric tidal speed due to collinearity, had an $R^2$ value of 0.26 and explained 31% of the deviance in the data. The deviance explained in this study is very similar to that of Murase et al (2009), another study that used GAMs. Of the six potential sources considered, only distance from estuaries and sewage outflows exert statistically significant influences on MF concentrations in seafloor sediments. Of the two environmental factors considered (bathymetric tidal speed was excluded), both depth and proportion of very fine sand were significant environmental factors influencing MF concentrations in the SoG.

Distance to estuaries exhibited a positive relationship with MF concentrations, with probabilities of MF in seafloor sediments increasing as proximity to estuaries increased (Figure 4). Conversely, the GAM determined that distance to sewage outflows exhibited a negative relationship with MF concentration, decreasing in seafloor sediments as proximity to sewage outflows increased (Figure 5). The association between MF concentration, estuaries, and sewage outflow sites is an intriguing one. As direct conduits of human pollution, distance to sewage discharge sites were expected to be large sources of MF and thereforee have a positive relationship to MF concentrations in the SoG. Moreover, a major source of MF is synthetic fibres from clothing, which enters marine ecosystems such as the SoG primarily through wastewater effluent at sewage discharge sites (Cole et al 2011). However, the GAM indicated that, unlike estuaries, distance from sewage outflow sites exhibited a negative relationship with MF concentrations in sediments.

One possible explanation for this result could be that wastewater from sewage outflow sites is filtered at treatment plants while discharge from estuaries is not. Gies *et al* (2018) recently showed that up to 99% of MF can be retained by wastewater treatment plants, thus reducing the number of MF from entering the marine environment. However, this retention does not occur in water discharged by natural estuaries.

Another possibility for this relationship is the influence of ocean currents, mixing, dispersal and particle settling. The biggest individual sewage outflow site considered in this study was the Saanich Peninsula wastewater treatment site, which serves residents from Sidney to Victoria. It discharges into the Haro Strait, which is one of the most dynamic areas in the SoG, with powerful tides and currents year-round (Crean 1976; Thomson 1981; Foreman *et al.* 1995). Foreman *et al.* (1995) determined that 51% of volume transport between the Juan De Fuca strait and the SoG occurs through the Haro strait. This intense mixing and transport could disperse debris away from the sewage outflow effluent and inhibit settling of MFs near the entry point.

Conversely, an estuary is the end point for dozens of effluent discharges along its course, not all of which are necessarily filtered by a treatment plant. Gies et al (2018) determined that even with a 99% retention rate, sewage treatment plants can still release billions of MF into the marine environment. All of this effluent would then accumulate downstream at the estuary, meaning all excess MF not retained in upstream wastewater treatment plants would ultimately end up in the SoG from these estuaries. Hence, an estuary could be the end discharge point for multiple sewage outflow sites. This means that estuaries could be the release point for unfiltered wastewater containing MF pollution, thus resulting in estuaries having a positive relationship with MF concentrations.

The SoG GAM also indicated two environmental factors as possible significant influencers of MF concentration in the SoG as a whole. Both depth and very fine sand were shown to have a negative relationship with MF concentration, meaning that as depth (Figure 3) or the proportion of very fine sand (Figure 6) in sediment increases, the probability of finding MF decreases.

Depth was determined to exhibit a negative relationship with MF concentration by the SoG GAM, and this coincides with this paper's second finding that MF concentrations are higher in seafloor sediments at depths <40-50 m than at depths >40-50 m. Possible reasons for this were discussed in the *MF settling patterns* section of this paper. Likewise, the relationship between MF concentrations and very fine sand determined by the SoG GAM is reflected in the comparison between MF concentrations and PSL habitat suitability in Figure 2: sediments with high proportions of very fine sand (not suitable PSL habitat) have low concentrations of MF.

The northern and southern portions of the Strait of Georgia are oceanographically very different. To further understand this, two separate GAM analyses were conducted with the SSG and the NSG separated. In the SSG, estuaries and very fine sand were covariates indicated by the SSG GAM to possibly have a significant influence on MF concentrations in the SSG. Marinas were also significant, but they did not meet the GAM fit test for residual data as described in the *statistical analysis* section. Distance from finfish aquaculture and tidal speed were left out of the SSG GAM because they exhibited collinearity with each other and with shellfish aquaculture.

As the proportion of very fine sand in sediment increased, MF concentrations decreased (Appendix G, Figure 20). This result lends support to the theory that PSL are transporting MF to medium coarse seafloor sediments because of the relationship between PSL habitat suitability, MF concentration, and proportion of very fine sands. As seen in Figure 2, higher MF concentrations are found in highly suitable PSL habitat. Furthermore, PSL habitat suitability decreases as the proportion of very fine sand increases (Robinson *et al* 2013). Therefore, it can be inferred that MF concentrations decrease as proportion of very fine sand increases because MF are not being transported to this sediment by PSL due to the fact that is not suitable habitat for them to bury in.

Conversely, the NSG had only one covariate significantly influencing MF concentrations: depth. The relationship between depth and MF concentrations in the NSG shown by the GAM (Appendix G, Figure 21) supports our finding that MF concentrations are higher in shallower depths (figure 3) because as depth decreased, MF concentrations increased.

In the SSG GAM, estuaries were estimated to have a significant influence on MF concentrations (Appendix G Figure 19). However, they were not in the NSG GAM. This is intuitive because the SSG has much larger population centers than the NSG, and therefore the potential for MF pollution to be produced and released in the SoG from river estuaries in much greater.

However, in spite of this finding, greater concentrations of MF are found in the NSG. The average concentration of MF was higher in the NSG (4.2 MF 10 $g^{-1}$) than in the SSG (1.9 MF 10 $g^{-1}$). This geographic disparity in the number of MF found between the NSG and SSG is non-intuitive because higher human populations are in the SSG. MF concentrations in the SoG was also found to occur this way in the water column (Deforges et al 2014). Some possible reasons for this disparity are the oceanography and type of industrial activity in the NSG. Currents in the NSG create clockwise gyres that can trap debris (Crawford et al 1985; Freeland et al 1984). Thus, instead of being flushed into the Pacific, particles, such as MF, can be trapped in the NSG (Deforges et al 2014). Conversely, the SSG is being constantly flushed through the Juan De Fuca Strait, with the main driver of this flushing being the Fraser River (Thomson 1981; Foreman et al 1995). Thus, small particles, such as MF, are more likely to settle in the NSG where there is less flushing of water into the Pacific, than in the SSG.

Because of its influence on the SSG, this paper hypothesises that the Fraser River estuary is potentially having a disproportionally large influence on MF concentrations in the SSG.- The biggest estuary in the SoG is the Fraser River estuary, which is located in the south. The Fraser River estuary and its associated environmental stressors is on a massive scale. With a watershed of approximately 217,000 km$^2$, the Fraser River is the largest Canadian river that flows to the Pacific Ocean (Morrison *et al* 2002). Therefore, it is the main driver of water mixing in the SoG. Furthermore, the SoG is most strongly dominated by the Fraser River during spring montP/HS. The montP/HS of May and early June, when samples for this study were collected, is when the peak flows for the Fraser River occur (Cameron 1996). With an average annual flow of 3630 m$^3$ s$^{-1}$, the Fraser River is already the biggest discharge source into the SoG (Cameron 1996). During peak flow season, this influence would be even greater. The powerful currents produced at peak flow would inhibit MF from settling in much of the SSG. Only in the NSG, where the influence of the Fraser River is diminished, would MF be able to settle. Thus, more MF would be found in seafloor sediment in the NSG.

Unfortunately, the study area of this paper was constrained to the eastern coast of Vancouver Island and collecting seafloor sediment samples around the Fraser River estuary was beyond the scope of this paper. Future research on MF concentrations in the SoG should collect samples from around the Fraser River estuary to further explore the above hypothesis. Finding very low concentrations of MF near the Fraser River estuary during peak flow season would support this hypothesis because it means MF are unable to settle in sediment near the Fraser River estuary due to its discharge plume dispersing MF further into the SoG.

Another reason more MF were found in the NSG could be the type of industry in the area. The NSG has a number of aquaculture farms that use synthetic netting to create their enclosures (Davidson and Dudas 2016). MF from these nets could be the source of MF in the NSG. However, many aquaculture nets in the area are black in colour (Davidson and Dudas 2016), and although this study found black fibres (23% of recorded MF), many more blue fibres were found (56% of recorded MF). Furthermore, blue and black fibres were found in all sampling areas in the SoG. A possible source of these blue fibres is not aquaculture netting but clothing. These blue MF would therefore enter the SoG from estuaries and sewage outflow sites. Additionally, aquaculture was determined to not have a significant influence on MF concentrations by all three of the GAMs discussed in this study.

This geographic disparity has also likely influenced the map illustrating the GAM model (figure 8). This map visually represents the prediction made by the SoG GAM. This GAM predicted that the areas with the highest likelihood of MF being present in seafloor sediment is in waters around the Comox Valley Regional District (CVRD) and Campbell River in the west, and Powell River in the east. However, these northern waters have a much smaller population near them than in the SSG and therefore fewer sources of MF. One important limitation of the GAM is that it cannot isolate characteristics of individual estuaries beyond there depth, tidal speed, grain size compositions, and distance from potential sources of MF. Due to this limitation, the model does not take into account human population size near individual estuaries, or the currents in the SoG that could disperse MF to areas far from their source. Thus, the GAM assumes MF concentrations can occur in these northern waters because they are close to numerous estuaries (figure 12) and far from any sewage outflow sites (figure 13). Compound this limitation with the fact that most of the MF recorded in this study were located in the NSG, and the GAM therefore assumed that estuaries in the north are the most likely areas to find MF in seafloor sediment. And the estuaries that best match the criteria of the GAM were ones around the CVRD, Campbell River, and Powell River.

As stated above, this paper hypothesises that what is happening in the SoG is that currents driven by the Fraser River is flushing MF out of the SSG into the pacific, but MF in the NSG are being trapped by current gyres, thus leading to higher concentrations of MF in NSG sediments. Thus, a greater likelihood of MF concentrations occurring in the NSG is possible, as the SoG GAM suggests.

The SoG GAM is a rough model using one possible scenario to attempt to illustrate where high likelihoods of MF being concentrated in seafloor sediment in the SoG may be occurring. Unfortunately, the low values obtained for “Deviance explained” (Murase et al 2009), indicate that these GAMs need improvement before they can be applied successfully to predict MF concentrations in the SoG. The inclusion of different environmental covariates, such as current patterns and other oceanographic dynamics in the SoG, might improve the results. The SoG GAM results are not necessarily what will be seen in the real world, but it can be a useful tool to indicate areas of interest when studying MF concentrations in the SoG in the future. For example, the CVRD, Campbell River, and Powell River are all areas that could have higher concentrations of MF. It is near a population centre, close to estuaries, and in shallow depths. All factors that this paper identified as important factors for high likelihoods of MF being concentrated in seafloor sediments.

The above findings can be interpreted to indicate five main points: (1) estuaries, particularly in the NSG, potentially have the highest concentrations of MF in seafloor sediments in the SoG; (2) sewage outflows on the east coast of Vancouver Island may not be significant sources of MF, (3) the shallower seafloor sediments are located in the SoG, the more likely MF are present, (4) sediments with a high proportion of very fine sand content are less likely to be contaminated by MF than sediments with coarse sand in the SoG, and (5) the Fraser River is potentially having a large influence on MF concentrations in the SSG by inhibiting settling.

### *Future Research*

This study provides an initial step in quantifying previously unknown and possibly unexpected patterns to MF concentrations in marine sediments in the SoG. However, several areas of future work need to be pursued to identify the source(s) of MF in the SoG. For example, estuaries are shown to exhibit a significant influence on MF concentrations in the SoG. Using the sampling design of this study and centering it around major estuaries in the area, such as the Fraser River estuary, instead of sewage outflow sites, would help indicate which estuaries in the SoG have the biggest influences on MF concentrations in seafloor sediment. Furthermore, including more environmental factors in the GAM, such as current patterns and human population gradients, would help flush out the reasons for the regional disparity observed by this paper.

Other important future research would include more identification analysis on MF to determine their composition. This would ensure no non-plastic materials were misidentified as plastics. A popular method of plastic identification is to scan them with Fourier-transform infrared spectroscopy (FT-IR) (Crichton *et al* 2017). Studies that have compared microscopic identification to FT-IR scans found that the discrepancies between the two can be significant (Song *et al* 2015). For example, MF counts determined under a microscope have later been shown via FT-IR scans to include up to 35% organic materials, such as cotton (Lenz *et al* 2015). Refining identification and finding the sources of MF pollution in the SoG is essential to reducing MF concentrations in the marine ecosystem, including PSL habitat.

Finally, further examination of the PSL specimens in addition to their habitat would help create a more thorough understanding of MF concentrations in PSL habitat. If large quantities of MF are found in PSL stomacP/HS, which initial research is indicating there is (Bertram et al 2016), a definitive conclusion that PSL are in fact transporting MF to highly suitable PSL habitat may be able to be made.

*Limitations in the study*

Laboratory contamination is always a limitation for all microplastic studies (Woodall et al 2015, Stolte et al 2015, Davidson & Dudas 2016, Zobkov & Esiukova 2017). As of yet, there are no methodological standards for microplastic analysis (Zobkov & Esiukova 2017) and as a result many different techniques for dealing with contamination both in the field and lab are present (Mathalon & Hill 2014, Stolte et al 2015, Davidson & Dudas 2016, Zobkov & Esiukova 2017). The development of a standardized technique for microplastic analysis would limit discrepancies in microplastic research.

Furthermore, identification precision was a limitation. The methods followed and equipment used can change both the number of MF retained in extraction and the number correctly identified when analyzed (Zobkov and Esiukova 2017). The use of an FT-IR spectroscopy scanner can improve the accuracy of MF identification and can either increase the number of MF recorded by picking up on MF missed by the human eye or decrease the number recorded by eliminating falsely identified MF (Song *et al* 2015). However, in spite of these possible disparities between suspected and confirmed MF, many studies, like this one, do not use FT-IR because it is expensive and time-consuming.

While limitations were present in this study, a good initial step was taken to showcase that there may be previously unknown and possibly unexpected patterns to MF concentrations in seafloor sediment in the SoG.

### *Implications and Conclusion*

The ingestion of MF by forage fish and their subsequent biomagnification in the coastal food web is a growing concern to scientists, government, fisheries, and the health sector. One key forage species in the SoG is the Pacific sand lance (*Ammodytes personatus*), which buries in low silt, medium coarse sand patches from chart datum to 100 m depth. This research assessed the level of MF concentrations in PSL burying habitats in the SoG. The main results indicate a significant positive correlation between highly suitable PSL burying habitat and higher MF concentrations. This relationship between MF concentrations and PSL habitat suitability is not intuitive because PSL habitat was found to be mostly located in shallow waters (<40), which are generally associated with high energy environments, where settling dynamics would suggest that fewer small particles would settle. Possible explanations include: (1) MF are entering the marine environment in larger sizes; (2) MF concentrations are being strongly influenced by potential sources of MF, such as estuaries; (3) PSL are transporting MF from the water column to their preferred habitat; and/or (4) some unexplored factors are controlling the MF distributions. Furthermore, a general additive model (GAM) analysis comparing MF concentrations with several potential sources and environmental factors revealed that both estuaries and sewage outflow sites have a significant influence on MF concentration in seafloor sediment, but in opposite ways. Estuaries exhibited a positive relationship with MF concentrations, while sewage outflows exhibited a negative one. Estuaries in the heavy urbanized regions of the southern SoG, both on the Saanich Peninsula in the west and the Fraser River estuary in the east, could be a major influence on MF pollution in the marine system. The Fraser River in particular could exert a strong influence on MF concentrations in seafloor sediments in the SSG due to its ability to flush and mix water in the SoG.

Overall, the presence of MF in PSL habitats indicates more research is required to understand the implication to higher trophic level species that feed upon PSL, such as chinook and coho salmon, seabirds such as the endangered marbled murrelet, and marine mammals such as the humpback whale.

# Appendices

## Appendix A

Table 1 1. Defining parameters of Pacific sand lance habitat suitability (Robinson et al 2013).

| Present (P) | PSL caught* |
|---|---|
| Highly Suitable Habitat (P/HS) | < 1% silt (grain sizes < 0.0625mm)<br>Very Fine Grains (2.0-4.0 mm) < 25%<br>Medium coarse sand (0.25mm-0.5mm+1.0mm): > 60%<br>Very fine sand (0.125mm-0.0625mm): < 11% |
| Moderately Suitable Habitat (MS) | < 1% silt (grain sizes < 0.0625mm)<br>Very Fine Grains (2.0-4.0 mm) < 25%<br>Medium coarse sand (0.25mm-0.5mm+1.0mm): < 60%<br>Very fine sand (0.125mm-0.0625mm): > 11% |
| Not Suitable Habitat (NS) | > 1% silt (grain sizes < 0.0625mm)<br>Very Fine Gravel(2.0-4.0 mm) < 25%<br>Medium coarse sand (0.25mm-0.5mm+1.0mm): < 60%<br>Very fine sand (0.125mm-0.0625mm): > 11% |

*The 39 grab samples with PSL contained proportionally by weight a maximum of < 1% silt (<0.0625mm), <10% very fine sand (0.0625mm), < 25% very fine gravel (2-4mm), and a minimum of >60% medium-coarse sand (0.125-1.00mm)

## Appendix B

The Marine Strategy Framework Directive (MSFD) Technical Subgroup on Marine Litter recommended the use of NaCl for the separation of microplastics by density flotation since it is an inexpensive, eco-friendly salt (Rocha-Santos & Duarte, 2015). However, the use of saturated solution of NaCl (1.2 g $cm^{-3}$) may lead to underestimation of the MP content in sediments because the solution density is too low to enable the flotation of all polymers, principally those containing additives (Rocha-Santos & Duarte, 2015). Instead, a 1.4 g cm-3 sodium polytungstate solution is enough to separate the polymers containing additives, so it is preferable to use this solution. These modifications result in an increased extraction efficiency for high-density microplastics such as polyvinylchloride (PVC, density 1.14 to 1.56 g cm-3) or polyethylene terephthalate (PET, density 1.32 to 1.41 g cm-3). As these high-density plastics make up over 17% of the global plastic demand (PlasticsEurope 2013), not including these types of microplastics can result in a considerable underestimation of MP concentrations in sediments, especially as these high-density plastics have a negative buoyancy and thus are much more likely to sink (GESAMP 2016).

## Appendix C

Davidson and Dudas (2016) used five procedural blanks on their complete digestion and vacuum filtration procedure for detecting microplastics in cultured and wild manila clams in the Georgia Straight. The average concentration of microfibres was 5.8 ± 2.2 particles/sample (range 3–8 particles/sample). Davidson and Dudas (2016) had an average of 11.3 ± 6.6 microplastic particles/cultured clam and 8.4 ± 8.5 MP particles/wild clam. This resulted in a proportion of contamination to every sample of 51 and 69 % respectively between the cultured and wild clams. Likewise, Mathalon and Hill (2014) reported a proportion of contamination to every sample at 56 and 79–94 % between cultured and wild mussels, respectively. In comparison, this study reports a proportion of contamination of 37%, well within the range of other studies on this topic.

## Appendix D

In place of a one-way Anova, the non-parametric Kruskal-Wallis test was used to determine whether two variables, one dependant and the other independent, differ in their distributions. This was a useful test for this study because it can indicate if the distribution of MF (the dependent variable) is different to an independent variable such as the PSL habitat classifications. In replace of the two-sided T-test, a Wilcox Rank Sum test was used to compare the medians of MP counts with ocean depth and distance from sewage outflows.  Last, a linear regression analysis and a spearman's rank correlation analysis were conducted to determine if a linear or monotonic, respectively, correlation existed between grain size and MF concentrations.

## Appendix E

Table 1 2 Results of the GAM for the entire Strait of Georgia with all covariates included. All covariates with a statistical significance of $p < 0.05$ are listed.

| | $R^2$ for GAM | Deviance explained | Distance from estuary p-value | Distance to marinas p-value | Dist. From sewage outflow site p-value | Depth p-value | Very Fine Sand p-value |
|---|---|---|---|---|---|---|---|
| SoG GAM | 0.26 | 31% | 0.03 | $1.1 \times 10^{-4}$ | 0.01 | $2.8 \times 10^{-3}$ | $1.9 \times 10^{-3}$ |

Table 1 3 Results of the GAM for the southern Strait of Georgia only. All covariates with a statistical significance of $p < 0.05$ are listed.

| | $R^2$ for GAM | Deviance explained | Distance from estuary p-value | Very fine sand p-value |
|---|---|---|---|---|
| SSG GAM | 0.20 | 40.2% | 0.04 | $8.4 \times 10^{-3}$ |

Table 1 4 Results of the GAM for the northern Strait of Georgia only. All covariates with a statistical significance of $p < 0.05$ are listed.

| | $R^2$ for GAM | Deviance explained | Depth p-value |
|---|---|---|---|
| NSG GAM | 0.37 | 54.3% | 0.02 |

Appendix F

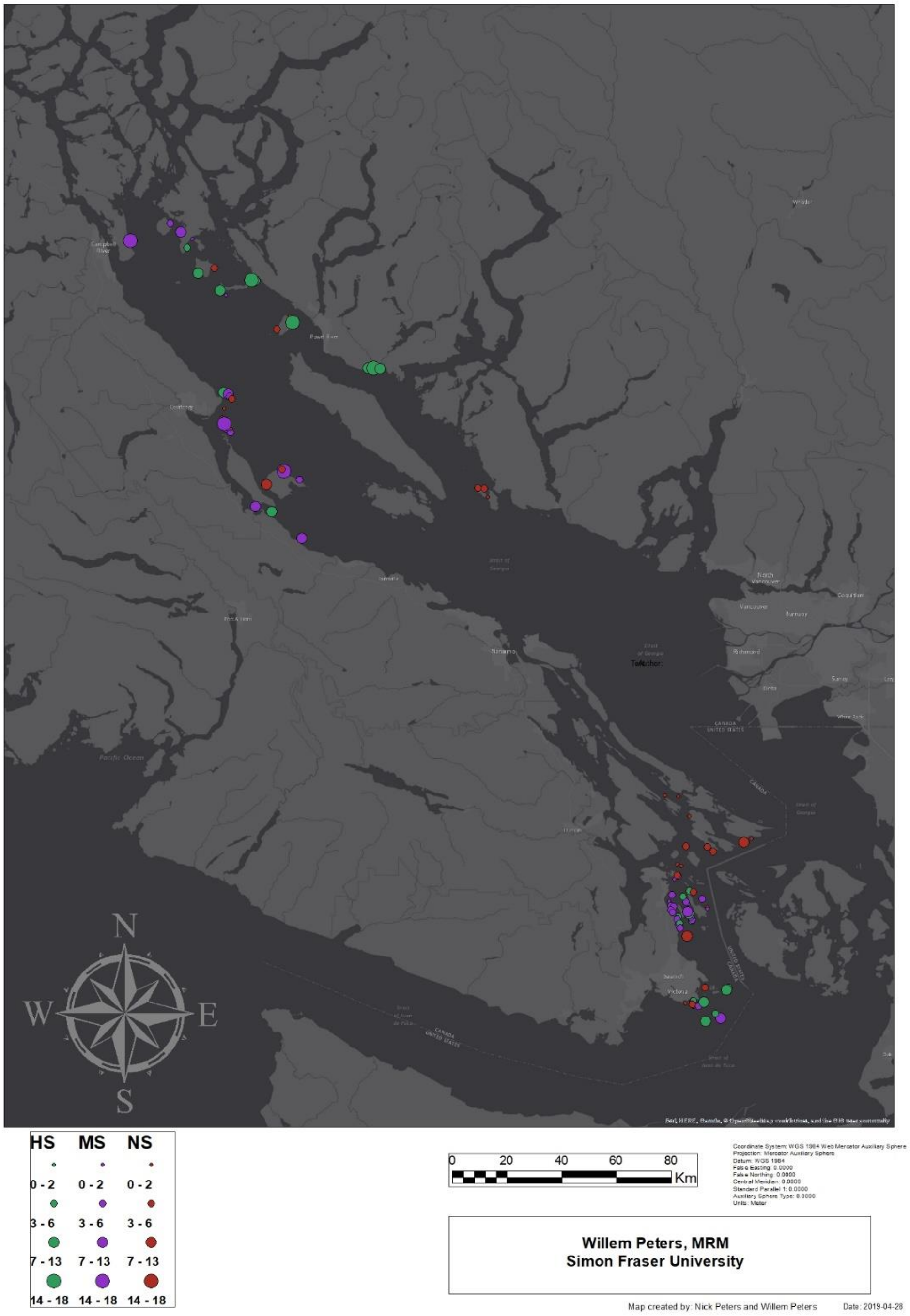

Figure 10. Map illustrating grab samples in the SoG.

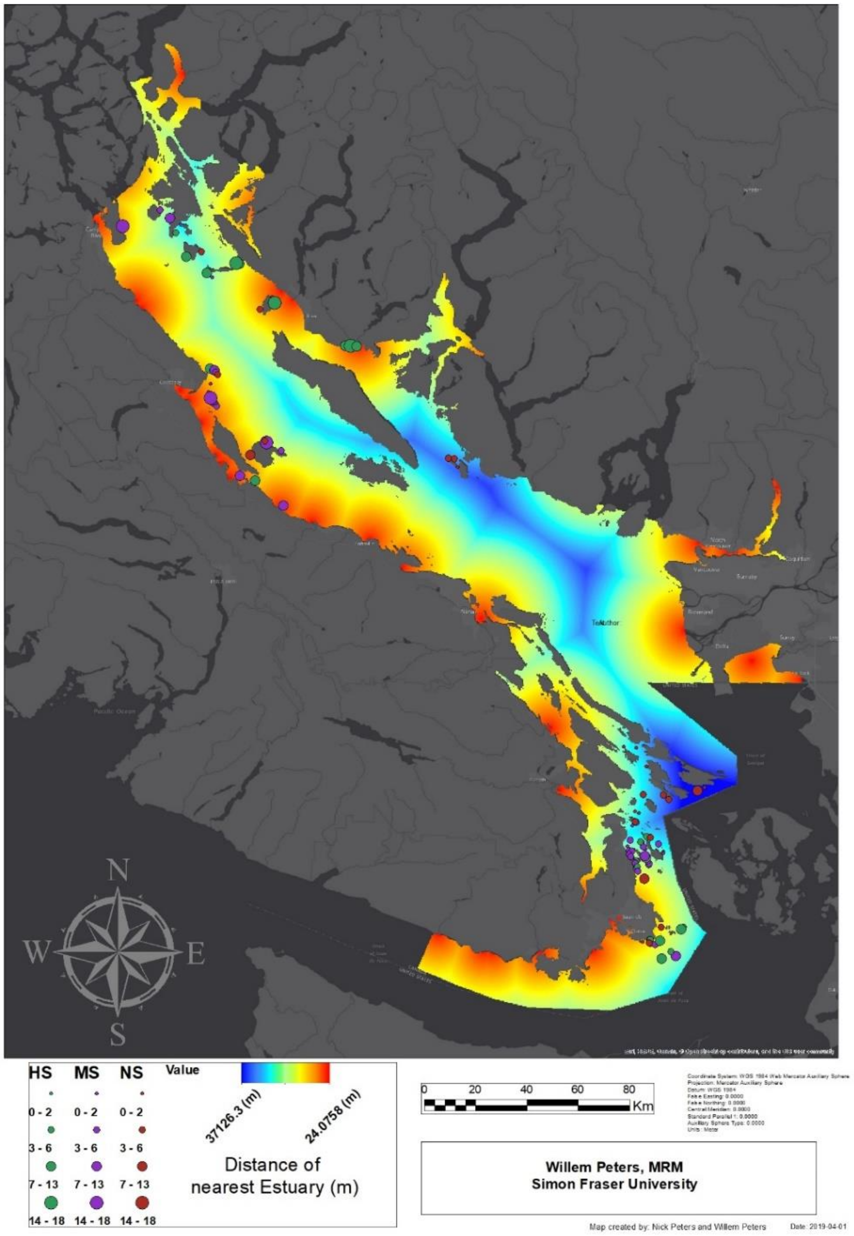

Figure 11. Map illustrating distance of grab samples from nearest estuary in the SoG. The warmer the colour, the closer an estuary (m).

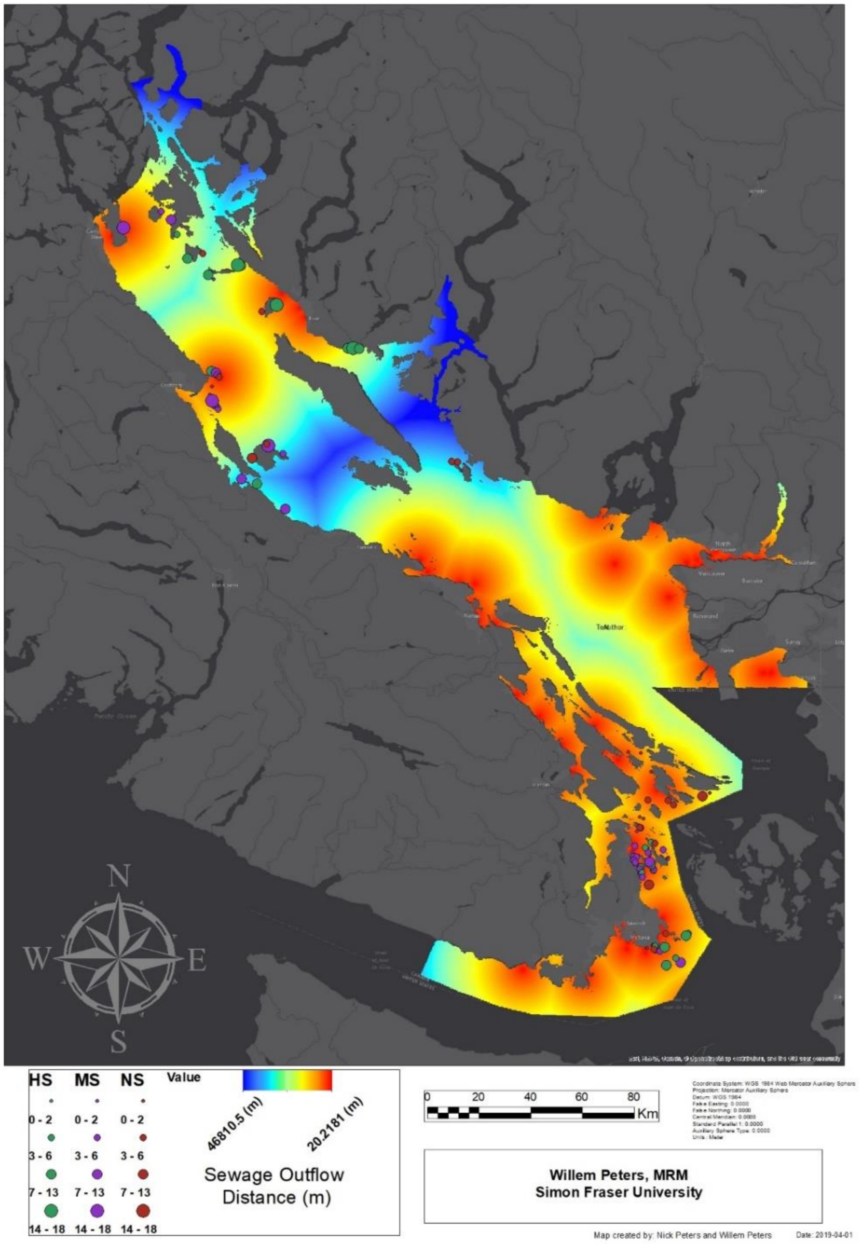

Figure 12. Map illustrating distance of grab samples from nearest sewage outflow site in the SoG. The warmer the colour, the closer that a sewage outflow site (m).

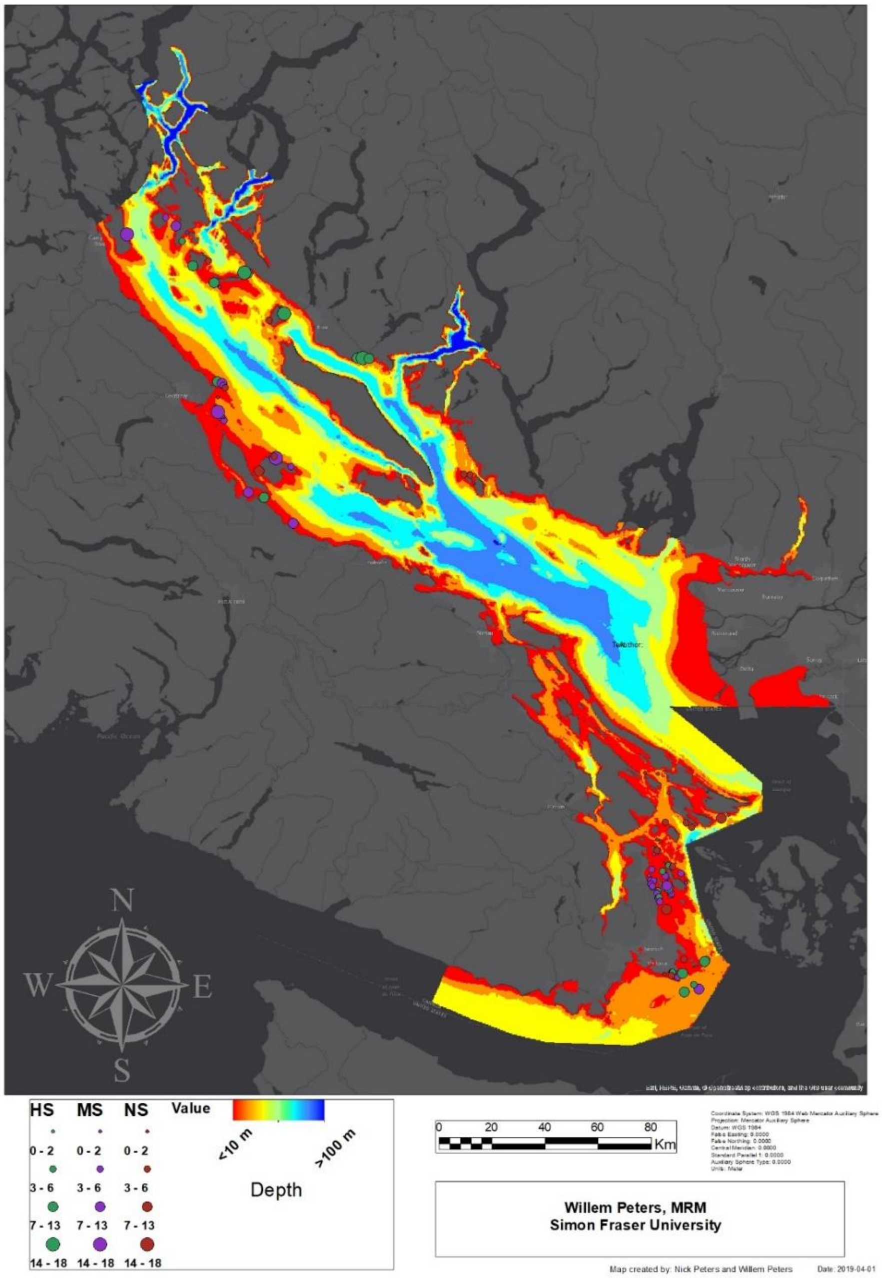

Figure 13 Map illustrating the depth of each grab samples in the SoG. The warmer the colour, the shallower the depth (m).

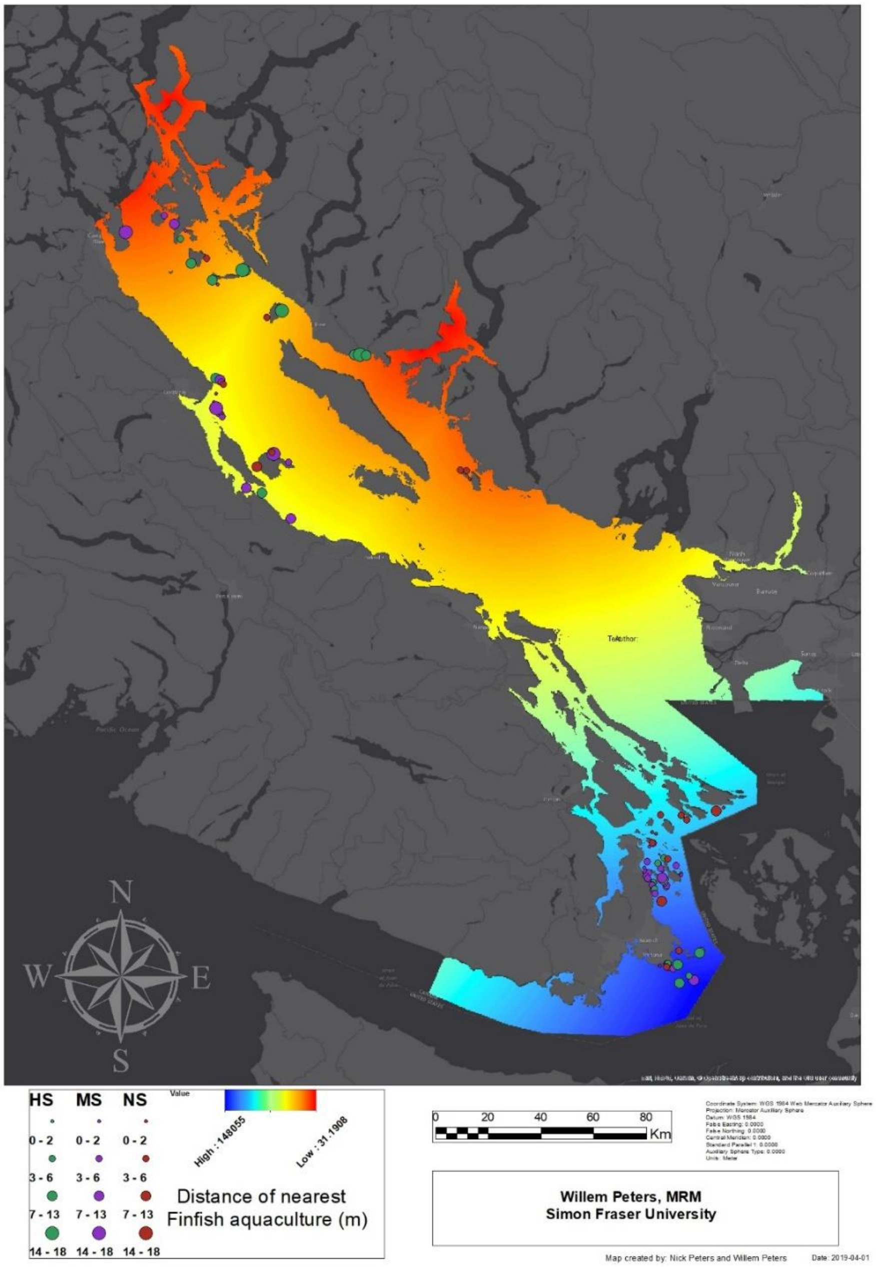

Figure14. Map illustrating the distance of each grab sample from the nearest finfish aquaculture site in the SoG. The warmer the colour, the closer a finfish aquaculture site (m).

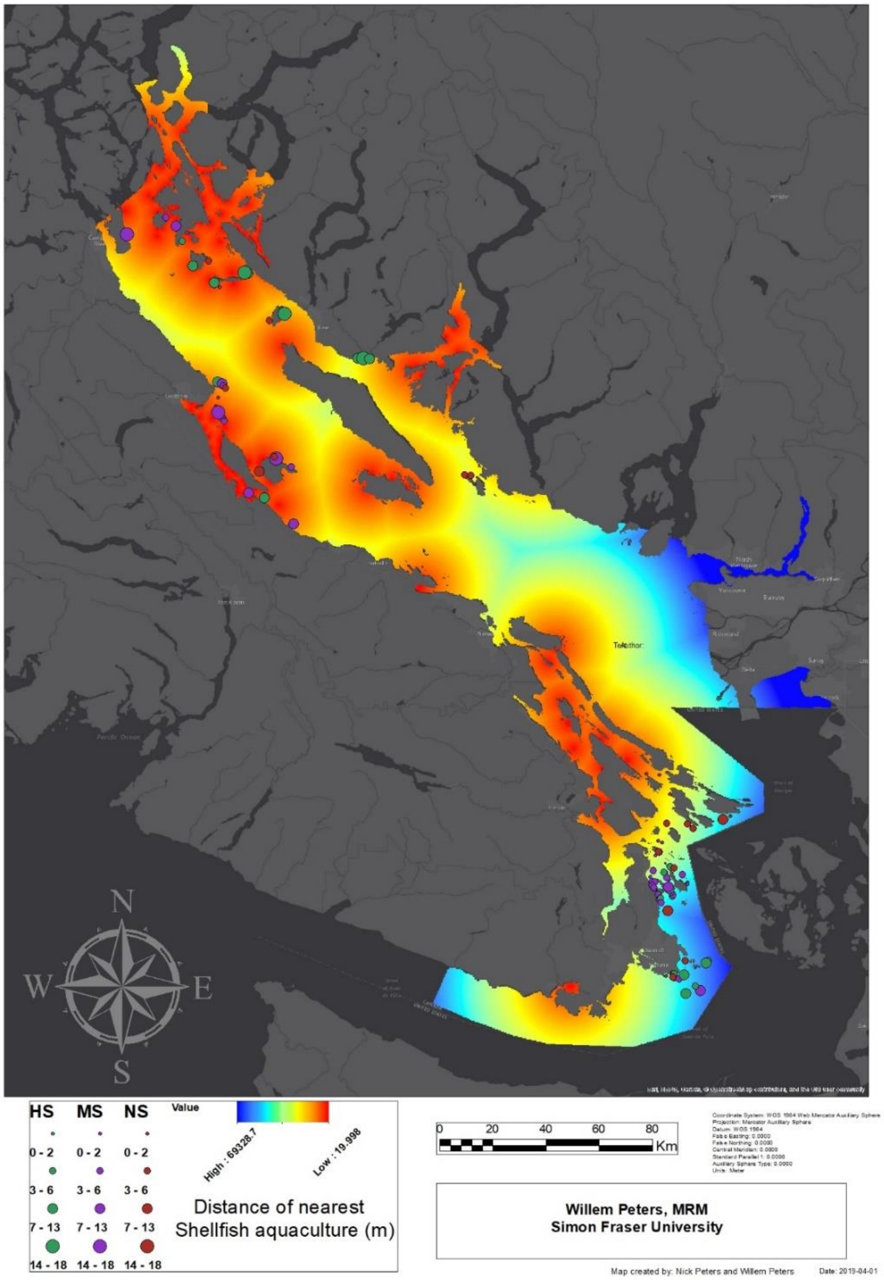

Figure 15. Map illustrating the distance of each grab sample from the nearest shellfish aquaculture site in the SoG. The warmer the colour, the closer a shellfish aquaculture site (m).

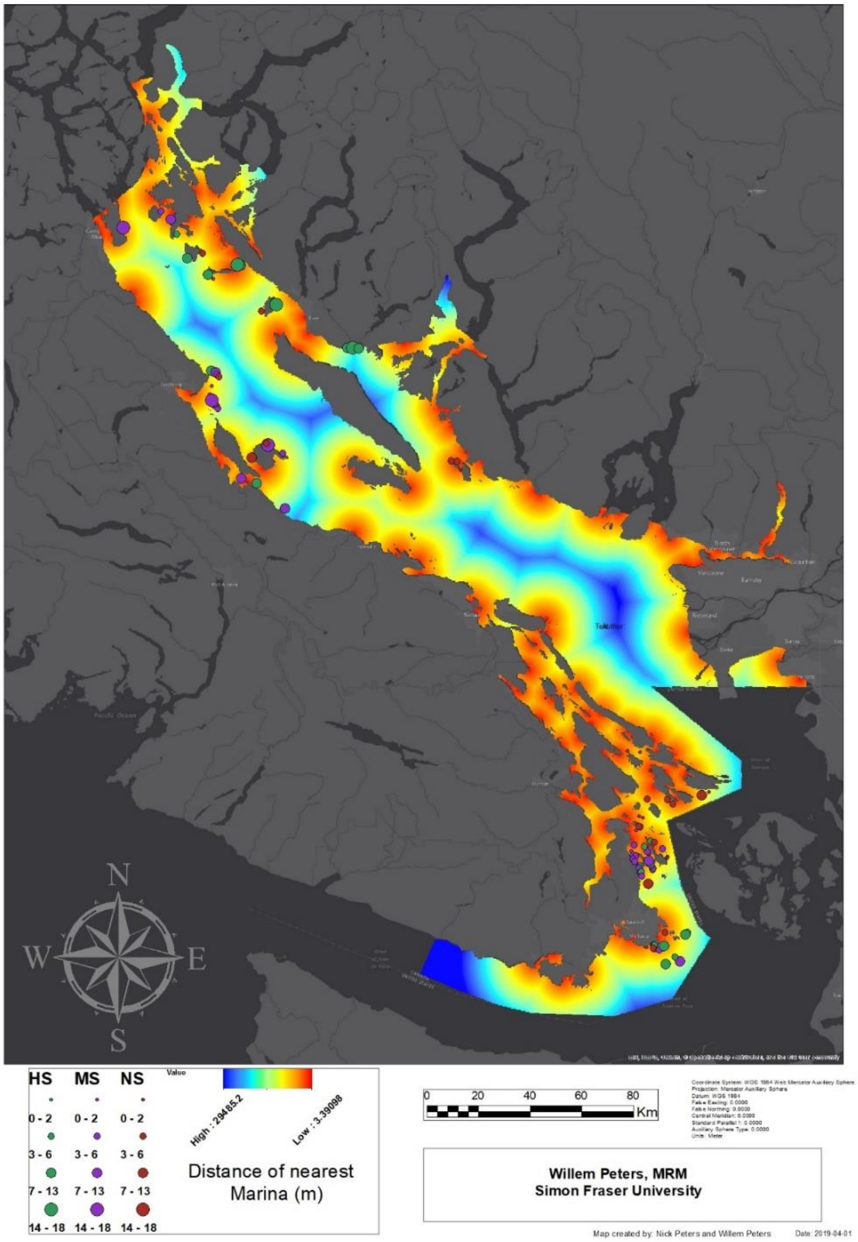

Figure 16. Map illustrating the distance of each grab sample from the nearest marina in the SoG. The warmer the colour, the closer a marina (m).

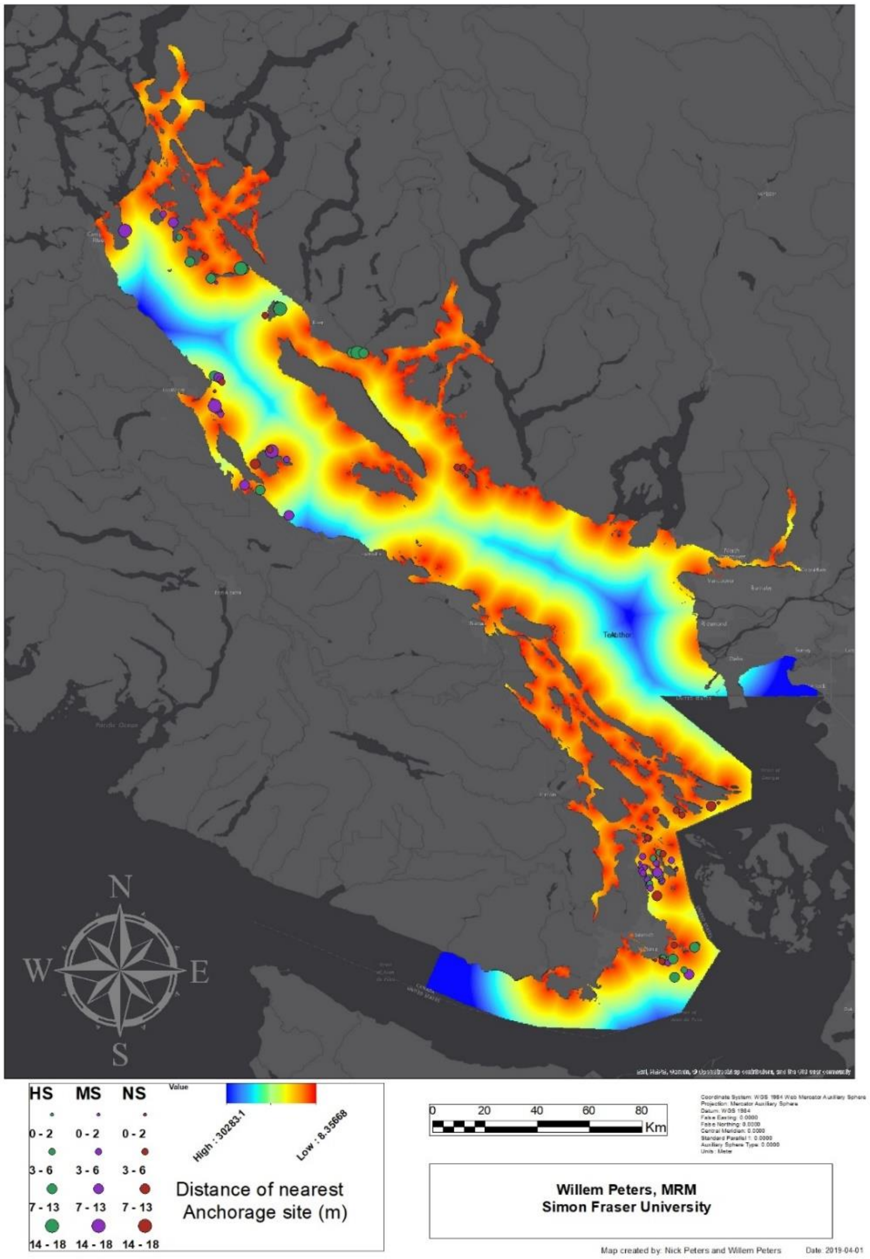

Figure 17 Map illustrating the distance of each grab sample from the nearest anchorage site in the SoG. The warmer the colour, the closer a anchorage site (m).

Appendix G

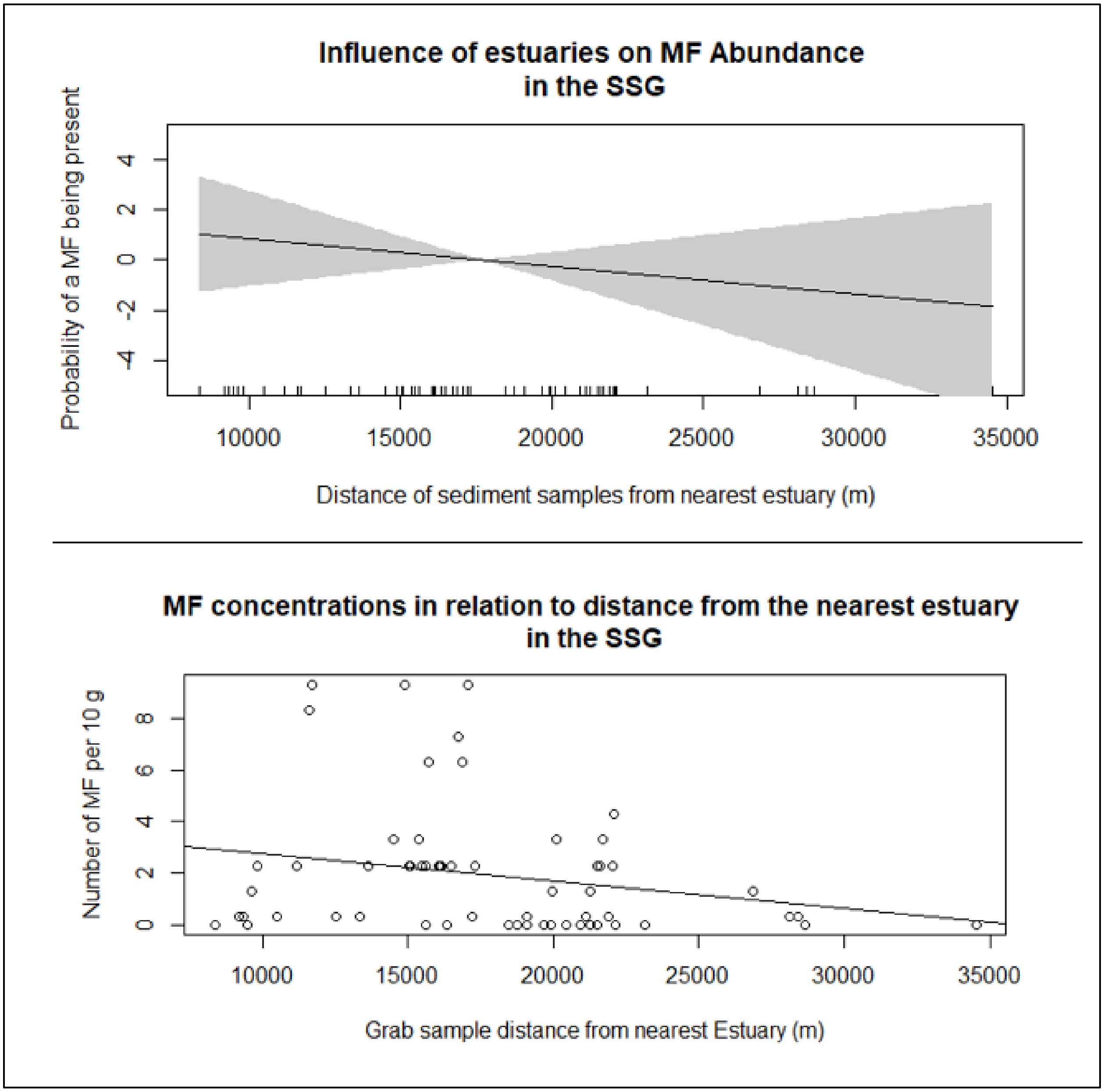

Figure 18 Top panel depicts the influence estuaries have on where MF settle in seafloor sediment in relation to proximity of estuaries in the SSG. X-axis indicates distance of sampling sites from estuaries. Y-axis indicates probability of seafloor sediment being contaminated by MF, on a logit scale (0 represents a probability of 0.5). The dashed lines on the x-axis are the locations of the sampling sites. The solid black line is the relationship between MF and estuary proximity, with the grey area representing a standard error encompassing a 95% confidence interval.

Bottom panel depicts a scatter plot illustrating the relationship between MF concentrations (# of MF 10 $g^{-1}$) in each sediment sample and the proximity to estuaries in the SSG. A regression line was fitted to showcase the slope.

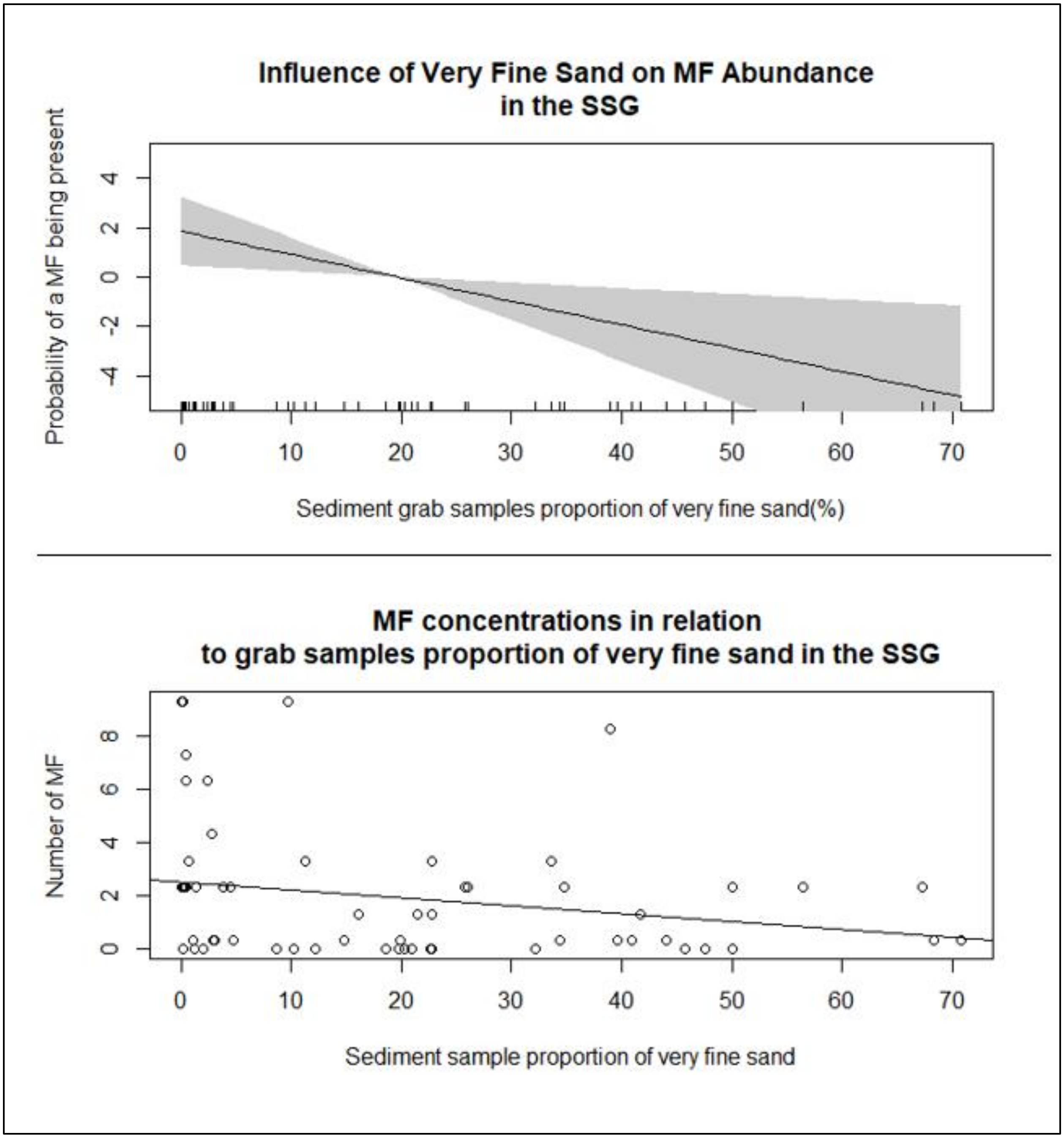

Figure 19 The top panel depicts the influence proportion of very fine sand has on MF concentrations. X-axis indicates proportion of very fine sand in a grab sample. Y-axis indicates probability of seafloor sediment being contaminated by MF, on a logit scale (0 represents a probability of 0.5). The dashed lines on the x-axis are the locations of the sampling sites. The solid black line is the relationship between MF and very fine sand, with the grey area representing a standard error encompassing a 95% confidence interval.

The bottom panel is a scatter plot illustrating the relationship between MF concentrations (# of MF 10 $g^{-1}$) in each sediment sample and the proportion of very fine sand per grab sample in the SSG. A regression line was fitted to showcase the slope.

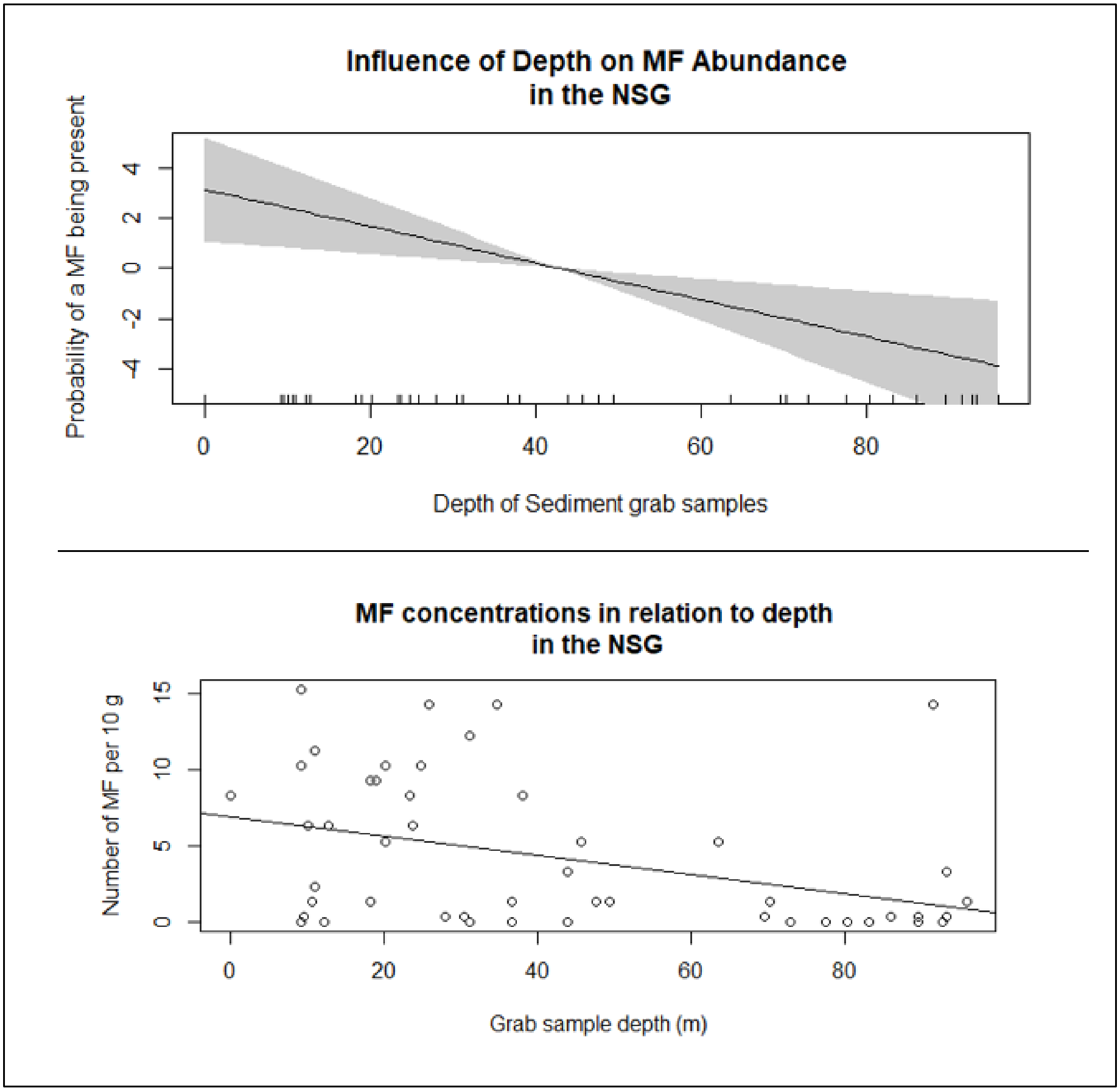

Figure 20. Top panel depicts the influence depth has on MF concentrations. X-axis indicates depth at which the grab samples were located. Y-axis indicates probability of seafloor sediment being contaminated by MF, on a logit scale (0 represents a probability of 0.5). The dashed lines on the x-axis are the locations of the sampling sites. The solid black line is the relationship between MF and depth, with the grey area representing a standard error encompassing a 95% confidence interval.

Bottom panel is a scatter plot illustrating the relationship between MF concentrations (# of MF 10 $g^{-1}$) in each sediment sample and depth in the NSG. A regression line was fitted to showcase the slope.

# Appendix H

Table 1 5. Pearson's correlation coefficient values for covariates included in the GAM. Correlations > 0.7 are highlighted.

| | MF concentration | Dist. From anchorage sites | Dist. From estuaries | Dist. From sewage outflow sites | Dist. From marinas | Dist. From finfish aquaculture | Dist. From shellfish aquaculture | Depth | Tidal speed | Fine gravel | Coarse sand | Fine sand | Very fine sand | Silt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MF concentration | 1 | 0.12 | -0.32 | 0.27 | 0.37 | -0.28 | -0.18 | -0.24 | -0.11 | 0.07 | 0.32 | 0.21 | -0.26 | -0.28 |
| Dist. From anchorage sites | 0.12 | 1 | 0.32 | -0.25 | -0.39 | 0.10 | 0.11 | 0.11 | 0 | -0.06 | 0.09 | -0.02 | 0.11 | -0.10 |
| Dist. From estuaries | -0.32 | 0.32 | 1 | 0.07 | -0.40 | 0.32 | 0.37 | -0.08 | 0.24 | 0.08 | 0.32 | -0.25 | 0.01 | 0.14 |
| Dist. From sewage outflow sites | 0.27 | -0.25 | -0.07 | 1 | 0.25 | -0.65 | -0.5 | 0.18 | -0.43 | -0.19 | -0.02 | -0.05 | 0.22 | 0.05 |
| Dist. From marinas | 0.37 | -0.39 | -0.40 | 0.25 | 1 | -0.29 | -0.14 | 0.02 | -0.19 | 0.10 | 0.32 | 0.01 | -0.16 | -0.09 |
| Dist. From finfish aquaculture | -0.28 | 0.10 | 0.32 | -0.65 | -0.29 | 1 | 0.89 | -0.18 | 0.84 | 0.15 | 0.11 | 0.01 | -0.28 | -0.10 |
| Dist. From shellfish aquaculture | -0.18 | 0.11 | 0.37 | -0.5 | -0.14 | 0.89 | 1 | -0.05 | 0.84 | 0.12 | 0.16 | 0.04 | -0.25 | -0.18 |
| Depth | -0.24 | 0.11 | -0.08 | 0.18 | 0.02 | -0.18 | -0.05 | 1 | -0.17 | -0.05 | 0.16 | 0.06 | 0.02 | -0.11 |
| Tidal speed | -0.11 | 0 | 0.24 | -0.43 | -0.19 | 0.84 | 0.84 | -0.17 | 1 | 0.18 | 0.28 | 0.08 | -0.38 | -0.20 |
| Fine gravel | 0.07 | -0.06 | 0.08 | -0.19 | 0.10 | 0.15 | 0.12 | -0.05 | 0.18 | 1 | 0.53 | -0.38 | -0.38 | -0.24 |
| Coarse sand | 0.32 | 0.09 | 0.32 | -0.02 | 0.32 | 0.11 | 0.16 | 0.16 | 0.28 | 0.53 | 1 | -0.08 | -0.63 | -0.43 |
| Fine sand | 0.21 | -0.02 | -0.25 | -0.05 | 0.01 | 0.01 | 0.04 | 0.06 | 0.08 | -0.38 | -0.08 | 1 | -0.4 | -0.6 |
| Very fine sand | -0.26 | 0.11 | 0.01 | 0.22 | -0.16 | -0.28 | -0.25 | 0.02 | -0.38 | -0.38 | -0.63 | -0.4 | 1 | 0.43 |
| silt | -0.28 | -0.10 | 0.14 | 0.05 | -0.09 | -0.10 | -0.18 | -0.11 | -0.20 | -0.24 | -0.43 | -0.6 | 0.43 | 1 |

Table 1 6 Pearson's correlation coefficient values for covariates included in the SSG GAM. Correlations > 0.7 are highlighted.

| | MF contamination | Depth | Fine gravel | Coarse sand | Fine sand | Very fine sand | Silt | Dist. from anchorage | Dist. from estuary | Dist. Finfish aquaculture | Dist. from marinas | Dist. from sewage outflow sites | Dist. Shellfish aquaculture | Tidal Speed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MF contamination | 1 | 0 | 0.09 | 0.29 | -0.04 | -0.24 | -0.06 | 0.35 | -0.23 | 0.36 | 0.4 | 0.05 | 0.37 | 0.45 |
| Depth | 0 | 1 | -0.02 | -0.08 | -0.05 | -0.07 | 0.16 | -0.28 | 0.11 | 0.08 | 0.15 | 0.18 | 0.12 | 0.12 |
| Fine Gravel | 0.09 | -0.02 | 1 | 0.57 | -0.49 | -0.42 | -0.23 | -0.03 | 0.21 | -0.03 | 0.19 | 0.25 | -0.12 | 0.09 |
| Coarse sand | 0.29 | -0.08 | 0.57 | 1 | -0.29 | -0.6 | -0.36 | 0.32 | -0.08 | 0.37 | 0.52 | 0.24 | 0.27 | 0.44 |
| Fine sand | -0.04 | -0.05 | -0.49 | -0.29 | 1 | -0.24 | -0.49 | -0.05 | -0.33 | 0.25 | -0.14 | -0.29 | 0.3 | 0.23 |
| Very fine sand | -0.24 | -0.07 | -0.42 | -0.6 | -0.24 | 1 | 0.32 | 0.11 | 0.04 | -0.2 | -0.3 | -0.16 | -0.14 | -0.4 |
| Silt | -0.06 | 0.16 | -0.23 | -0.36 | -0.49 | 0.32 | 1 | -0.3 | 0.21 | -0.38 | -0.12 | 0.1 | -0.44 | -0.37 |
| Dist from anchorage | 0.35 | -0.28 | -0.03 | 0.32 | -0.05 | 0.11 | -0.3 | 1 | -0.31 | 0.32 | 0.43 | -0.11 | 0.44 | 0.25 |
| Dist. from estuary | -0.23 | 0.11 | 0.21 | -0.08 | -0.33 | 0.04 | 0.21 | -0.31 | 1 | -0.78 | -0.09 | 0.58 | -0.46 | -0.44 |
| Dist. Finfish aquaculture | 0.36 | 0.08 | -0.03 | 0.37 | 0.25 | -0.2 | -0.38 | 0.32 | -0.78 | 1 | 0.46 | -0.16 | 0.79 | 0.73 |
| Dist. from marinas | 0.4 | 0.15 | 0.19 | 0.52 | -0.14 | -0.3 | -0.12 | 0.43 | -0.09 | 0.46 | 1 | 0.56 | 0.67 | 0.63 |
| Dist. from sewage outflow sites | 0.05 | 0.18 | 0.25 | 0.24 | -0.29 | -0.16 | 0.1 | -0.11 | 0.58 | -0.16 | 0.56 | 1 | 0.15 | 0.22 |
| Dist. Shellfish aquaculture | 0.37 | 0.12 | -0.12 | 0.27 | 0.3 | -0.14 | -0.44 | 0.44 | -0.46 | 0.79 | 0.67 | 0.15 | 1 | 0.72 |
| Tidalspeed | 0.45 | 0.12 | 0.09 | 0.44 | 0.23 | -0.4 | -0.37 | 0.25 | -0.44 | 0.73 | 0.63 | 0.22 | 0.72 | 1 |

Table 1 7 Pearson’s correlation coefficient values for covariates included in the NSG GAM.

| | MF contamination | Depth | Fine gravel | Coarse sand | Fine sand | Very fine sand | Silt | Dist. from anchorage | Dist. from estuary | Dist. Finfish aquaculture | Dist. from marinas | Dist. from sewage outflow sites | Dist. Shellfish aquaculture | Tidal Speed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MF contamination | 1 | -0.38 | 0.17 | 0.54 | 0.44 | -0.48 | -0.54 | 0.03 | -0.25 | -0.26 | 0.26 | 0.14 | -4 x $10^{-3}$ | -0.09 |
| Depth | -0.38 | 1 | 0.12 | -0.32 | -0.09 | 0.04 | 0.09 | 2 x $10^{-3}$ | 0.04 | 0.5 | -0.12 | -0.37 | -0.01 | -0.44 |
| Fine Gravel | 0.17 | 0.12 | 1 | 0.44 | -0.25 | -0.36 | -0.19 | -0.08 | -0.16 | -0.03 | 0.19 | -0.08 | 0.01 | 0.06 |
| Coarse sand | 0.54 | -0.32 | 0.44 | 1 | 0.28 | -0.71 | -0.55 | -0.13 | -0.15 | -0.33 | 0.31 | -0.02 | -0.09 | -0.15 |
| Fine sand | 0.44 | -0.09 | -0.25 | 0.28 | 1 | -0.61 | -0.75 | 6 x $10^{-3}$ | -0.26 | -0.23 | 0.16 | 0.04 | -0.24 | -0.21 |
| Very fine sand | -0.48 | 0.04 | -0.36 | -0.71 | -0.61 | 1 | 0.49 | 0.11 | 0.22 | 0.05 | -0.31 | 0.16 | 0.11 | -0.05 |
| Silt | -0.54 | 0.09 | -0.19 | -0.55 | -0.75 | 0.49 | 1 | 0.04 | 0.24 | 0.38 | -0.18 | -0.12 | 0.22 | 0.31 |
| Dist from anchorage | 0.03 | 2 x $10^{-3}$ | -0.08 | -0.13 | 6 x $10^{-3}$ | 0.11 | 0.04 | 1 | -0.31 | 0.27 | 0.41 | -0.41 | 0.12 | -0.26 |
| Dist. from estuary | -0.25 | 0.04 | -0.16 | -0.15 | -0.26 | 0.22 | 0.24 | -0.31 | 1 | 0.26 | -0.40 | 0.14 | 0.48 | 0.45 |
| Dist. Finfish aquaculture | -0.26 | 0.5 | -0.03 | -0.33 | -0.23 | 0.05 | 0.38 | 0.27 | 0.26 | 1 | -0.03 | -0.32 | 0.36 | 0.61 |
| Dist. from marinas | 0.26 | -0.12 | 0.19 | 0.31 | 0.16 | -0.31 | -0.18 | 0.41 | -0.40 | -0.03 | 1 | -0.20 | 0.20 | -0.47 |
| Dist. from sewage outflow sites | 0.14 | -0.37 | -0.08 | -0.02 | 0.04 | 0.16 | -0.12 | -0.41 | 0.14 | -0.32 | -0.20 | 1 | -0.07 | -0.07 |
| Dist. Shellfish aquaculture | -4 x $10^{-3}$ | -0.01 | 0.01 | -0.09 | -0.24 | 0.11 | 0.22 | 0.12 | 0.48 | 0.36 | 0.20 | -0.07 | 1 | 0.34 |
| Tidalspeed | -0.09 | -0.44 | 0.06 | -0.15 | -0.21 | -0.05 | 0.31 | -0.26 | 0.45 | 0.61 | -0.47 | -0.07 | 0.34 | 1 |

<u>Appendix I</u>

Table 1 8 Results of a GAM modeling MF concentrations with distance from anchorage sites as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Distance from Anchorage sites | 0.02 | 2% |

Table 1 9 Results of a GAM modeling MF concentrations with distance from estuaries as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Distance from Estuaries | 0.15 | 13% |

Table 1 10 Results of a GAM modeling MF concentrations with depth as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Depth | 0.04 | 2.2% |

Table 1 11 Results of a GAM modeling MF concentrations with distance from Finfish aquaculture as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Distance from Finfish Aquaculture | 0.07 | 3% |

Table 1 12 Results of a GAM modeling MF concentrations with distance from shellfish aquaculture as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Distance from Shellfish Aquaculture | 0.01 | 1.7% |

Table 1 13 Results of a GAM modeling MF concentrations with distance from marinas as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Distance from Marinas | 0.18 | 13.7% |

Table 1 14 Results of a GAM modeling MF concentrations with distance from sewage outflow sites as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Distance from sewage outflow sites | 0.1 | 7.2% |

Table 1 15 Results of a GAM modeling MF concentrations with tidal speed as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Tidal Speed | 0.03 | 3.7% |

Table 1 16 Results of a GAM modeling MF concentrations with fine gravel as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Fine Gravel | $4.9 \times 10^{-3}$ | 0.42% |

Table 1 17 Results of a GAM modeling MF concentrations with coarse sand as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Coarse Sand | 0.09 | 4.7% |

Table 1 18 Results of a GAM modeling MF concentrations with fine sand as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Fine Sand | 0.03 | 2% |

Table 1 19 Results of a GAM modeling MF concentrations with very fine sand as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Very Fine Sand | 0.05 | 3.8% |

Table 1 20 Results of a GAM modeling MF concentrations with silt as the only covariate included.

| Covariate | $R^2$ | Deviance explained |
|---|---|---|
| Silt | 0.07 | 4.6% |

Table 1 21 Results of a GAM modeling MF concentrations with only covariates that explained >10% deviance explained included.

| Covariates | $R^2$ | Deviance explained |
|---|---|---|
| Distance from Estuaries and Distance from Marinas | 0.18 | 16.2% |

Table 1 22 Results of a GAM modeling MF concentrations with only covariates that were determined to have a p-value of >0.5 by the SoG GAM included.

| Covariates | $R^2$ | Deviance explained |
|---|---|---|
| Distance from Sewage Outflow Sites, Distance from Estuaries, Distance from Marinas, Depth, and Proportion of Very Fine Sand | 0.3 | 22.7% |

## Appendix J

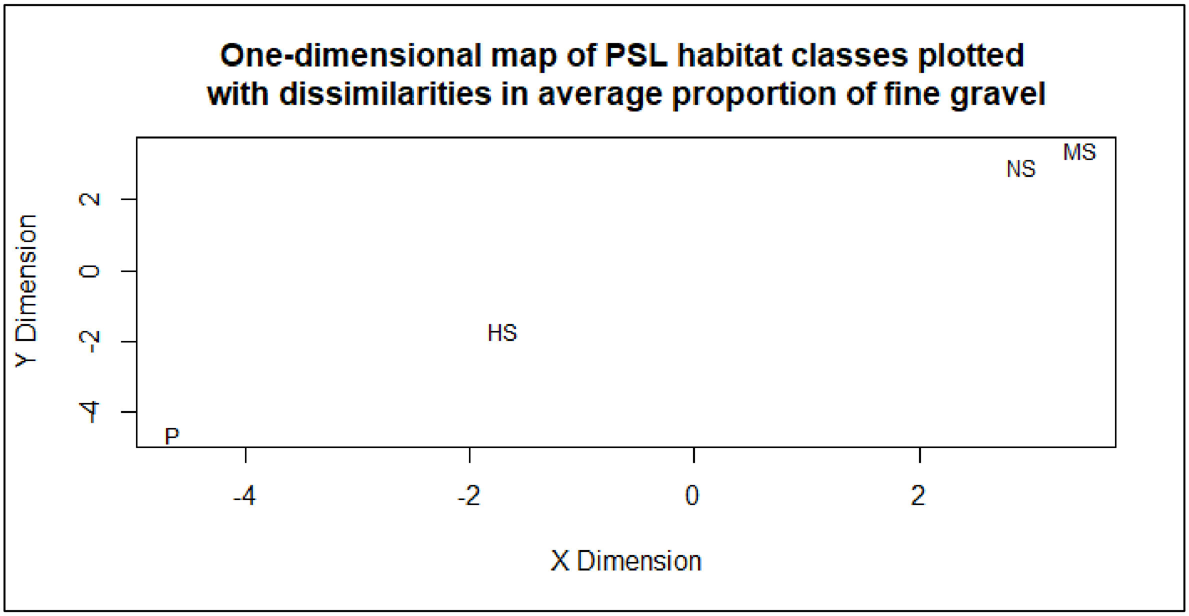

Figure 21. The differences in the average proportion of coarse sand for each PSL habitat suitability classification are plotted from a multi-dimensional scaling model. P/HS (highly suitable habitat) and P (PSL present in sample) are shown to be somewhat dissimilar, while MS (medium suitability habitat) and NS (not suitable habitat) are shown to have few dissimilarities between each other, but many dissimilarities with P/HS and especially P.

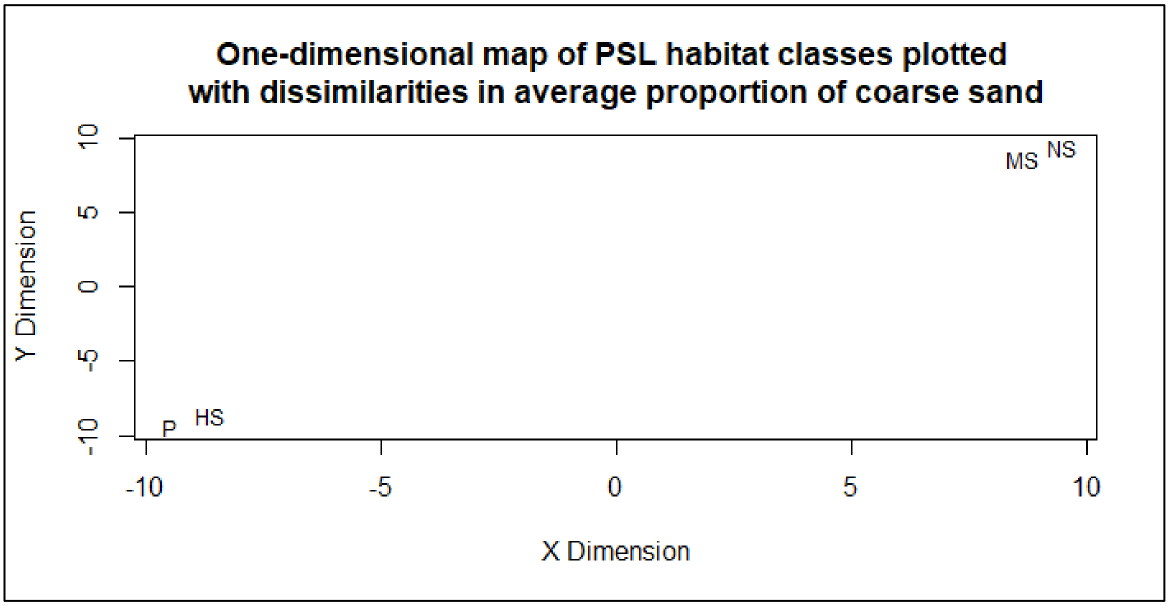

Figure 22. The differences in the average proportion of coarse sand for each PSL habitat suitability classification are plotted from a multi-dimensional scaling model. P/HS (highly suitable habitat) and P (PSL present in sample) are shown to have very little dissimilarities, *while MS (medium suitability habitat) and NS (not suitable* habitat) are shown to have few dissimilarities between each other, but many dissimilarities with P/HS and P.

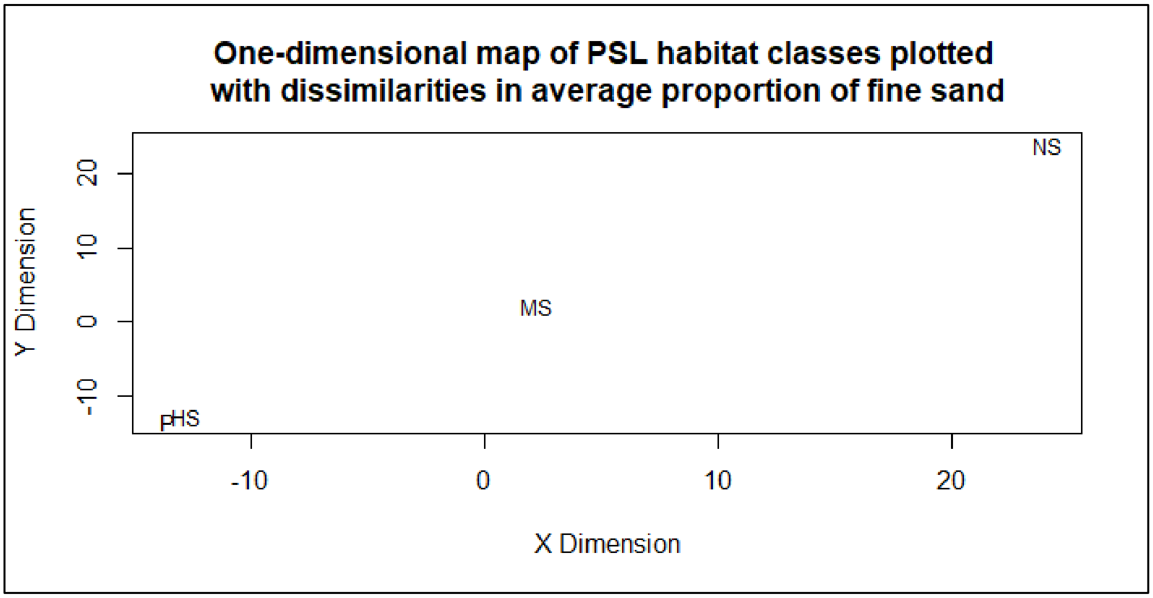

Figure 23. The differences in the average proportion of coarse sand for each PSL habitat suitability classification are plotted from a multi-dimensional scaling model. P/HS (highly suitable habitat) and P (PSL present in sample) are shown to have almost no dissimilarities, while MS (medium suitability habitat) and NS (not suitable habitat) are shown to be dissimilar between each other. MS is also dissimilar with P/HS and P, while NS is very dissimilar with P/HS and P.

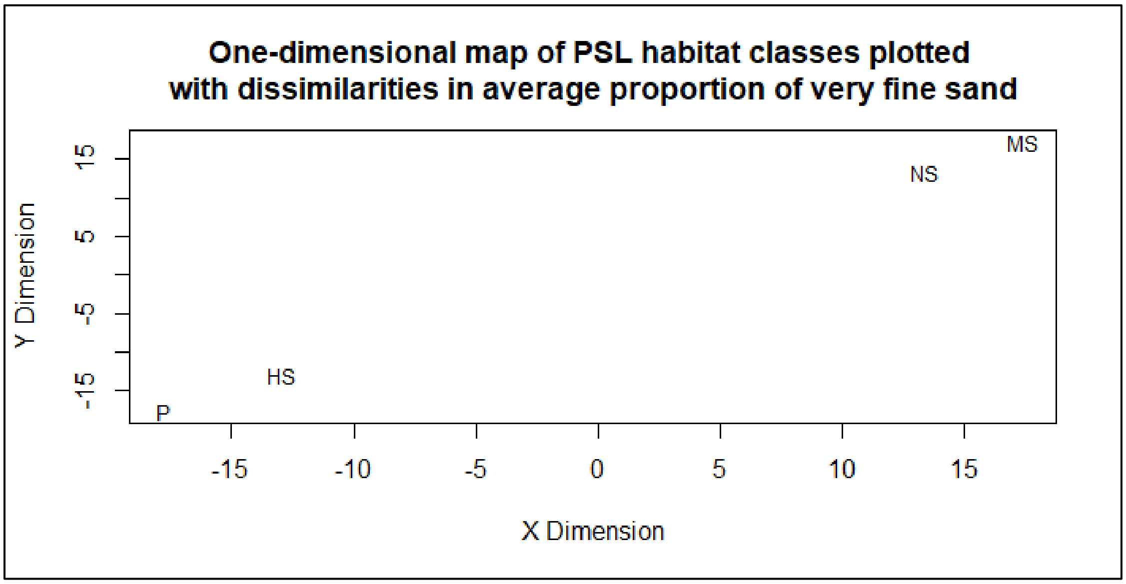

Figure 24. The differences in the average proportion of very fine sand for each PSL habitat suitability classification are plotted from a multi-dimensional scaling model. P/HS (highly suitable habitat) and P (PSL present in sample) are shown to have very little dissimilarities, while MS (medium suitability habitat) and NS (not suitable habitat) are shown to have few dissimilarities between each other, but many dissimilarities with P/HS and P.

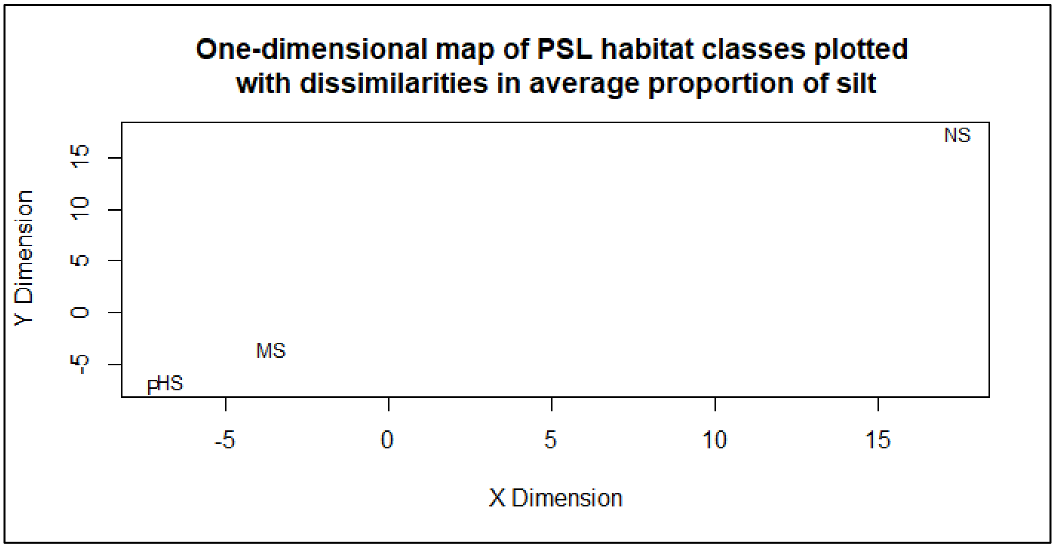

Figure 25. The differences in the average proportion of silt for each PSL habitat suitability classification are plotted from a multi-dimensional scaling model. P/HS (highly suitable habitat) and P (PSL present in sample) are shown to have almost no dissimilarities, while MS (medium suitability habitat) is shown to have very few dissimilarities between with P/HS and P. NS (not suitable PSL habitat) is very dissimilar with P/HS, P, and MS.

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