Quantification and identification of potential microplastics relative to the size of juvenile soft-shell clams (*Mya arenaria*)

by

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Bachelor of Science with Honours in Marine Biology

Supervisor(s): Dr. Heather Hunt, Department of Biological Sciences, Department Chair

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ABSTRACT

Microplastics (MP) are a widespread pollutant in the marine environment. These contaminants are found in benthic organisms, including the soft-shelled clam (*Mya arenaria*), an ecologically and economically important filter-feeding bivalve. This study aimed to quantify the relationship between the concentration of potential MP and clam size (mm and mean tissue weight). Since filtration rates are thought to affect MP ingestion in bivalves, it is hypothesized that smaller individuals will possess a higher concentration of MP per gram of soft tissue due to their greater filtration rate. I also examined whether MP type was independent of juvenile size. Juvenile and adult *M. arenaria* were collected from Kouchibouguac, NB and digested in KOH to extract potential MP, which was identified under a dissecting microscope. Potential MP concentration had a significant non-linear relationship to *M. arenaria* size, with the highest concentrations (per g of soft tissue or individual) in small juveniles. Potential MP type was independent of juvenile size. 90% of potential MPs identified in this study were fibres and their concentration shared the same non-linear relationship to size as total potential MPs. The results of this project suggest that juvenile *M. arenaria* may be more vulnerable to any negative effects of MP ingestion than larger individuals.

DEDICATION

I dedicate this work to my family, who provided me with the opportunity to pursue my dreams. Also, to those who motivated me in times of hardship and pushed me to do my very best, thank you.

ACKNOWLEDGEMENTS

I would firstly like to acknowledge my supervisor Dr. Heather Hunt. Throughout my project, Dr. Hunt helped me construct my project topic and provided me with guidance every step of the way. Dr. Hunt was extremely helpful as she suggested many collection sites for my thesis and provided general aid when needed through navigating my study. I would also like to acknowledge Dr. Hunt's Master's student Krista Beardy. Krista transported me to and from collection sites through the summer of 2021, helped me with species collection, and taught me all the safety protocols and lab procedures needed for my research. She also provided general feedback on my thesis and taught me how to identify MPs through microscope analysis. Both Krista and Dr. Hunt made my project what it is now, and I couldn't have asked to work with a better team.

STATEMENT OF RESEARCH CONTRIBUTION

This honours thesis is in part related to Krista Beardy's MSc thesis, sampling for both projects was conducted separately. Krista's project is exclusive to the Gulf of Saint Lawrence and is focused on a larger size class of *Mya arenaria* and other bivalves. I collected all juvenile *Mya arenaria* needed for my honours project from both the Bay of Fundy and Gulf of Saint Lawrence sites. I did all laboratory procedures including all solution preparations (10% KOH and filtered water), juvenile *M. arenaria* tissue extraction, measurements (weight of tissue and length of individuals), tissue digestion in KOH solution, suction filtration of all solutions, labelling petri plates and manual identification of MPs with the aid of Krista Beardy. Adult *M. arenaria* data used included in this thesis was provided by Krista Beardy to extend the size range when observing the relationship of potential MP to size.

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Introduction

The widespread use of plastics commenced in the early 1950s; many consumers and companies alike quickly gained a dependence on this material for its low cost, light-weight design and versatility (Andrady, 2011; Ding et al., 2021, Urban-Malinga et al., 2021). Plastic products were designed to replace other materials like glass, paper, and metal and implemented into consumer products designed for multiple or single-use while sustaining a pristine design like its predecessor materials (Andrady, 2011). With such a large market for plastic production, proper disposal of the material has been an ongoing issue and resulted in approximately 275 million tonnes of plastic waste being deposited into the marine environment in 2010 alone (Bour et al., 2018, Ding et al., 2021, Phuong et al., 2017). Commonly these plastics are made up of synthetic materials like polyethylene, acrylic and polypropylene (Bour et al., 2018, Thiele et al., 2019).

Over time larger pieces of plastic break down into millions of smaller microscopic pieces which quickly spreads throughout a given area (Li et al., 2015, Sendra et al., 2020). These microscopic plastic pieces are known as microplastics (MPs), defined as being <5 mm in any linear direction. (Andrady, 2011; Baechler et al., 2019; Bour et al., 2018, Li et al., 2015, Phuong et al., 2017, Sendra et al., 2020). There are two main groups of MPs, one which is manufactured for commercial use (Primary MPs) and one which weather and decay overtime off larger plastic pieces (Secondary MPs) (Urban-Malinga et al., 2020). Both groups of MPs are diverse in terms of appearance and composition. In terms of appearance, MPs can be in film, fragment, microbead and, most abundantly, fibre forms (Thiele et al., 2019, Ward et al., 2019).

MPs are found in every environment on earth; they infiltrate both land and aquatic ecosystems through wastewater, pollution, run-off, and atmospheric deposition (Baroja et al., 2021). However, marine environments are especially prone to MP infiltration due to the impact of anthropogenic activities, e.g., coastal landfills, coastal fisheries, wastewater treatment plants and industrial waste (Baechler et al., 2019, Baroja et al., 2021). Although MPs may be deposited from an initial location, abiotic factors such as wind, tides, bioturbation, and currents disperse the material to new environments (Ding et al., 2021). Once MPs infiltrate the marine environment, they either remain at the surface (low-density MPs) where they may be ingested by seabirds and fish, or they sink and reach the seafloor (high-density MPs) where crustaceans, fish larvae, zooplankton, and mollusks feed (Bour et al., 2018; Ding et al., 2021; Van Cauwenberghe et al., 2015). Although this is a relatively new topic of research, ingested MPs have been found to affect biological processes in some organisms (i.e., bivalves), from feeding behaviour and reproductive fitness to immune response and triggering chemical toxicity (Covernton et al., 2019). However, presently there are no studies that have observed mortality in bivalves directly induced by MPs (Sendra et al., 2021). Within the past decade, the widespread presence of MPs in the environment has been recognized and has quickly become one of the most researched topics in marine science for its potential effects on marine organisms and humans consuming such species (Andrady, 2011, Baechler et al., 2017, Baechler et al., 2020).

Many previous studies have focused on bivalves as bioindicators for MPs (Ward et al., 2019). This is because they are abundant, have a wide distribution, are filter feeders, and are a human food source (Phuong et al., 2017). The detection of MPs in bivalves has been established worldwide (e.g., France, China, US, Korea, and Germany, Zhang et al., 2019). Bivalves are

ecologically important, aiding ecosystem function through water filtration, bioturbation, and sediment irrigation (Urban-Malinga et al., 2020). This group of organisms is also highly susceptible to ingesting MPs since they are primarily filter feeders (Ding et al., 2021; Li et al., 2015). This means bivalves can capture an abundance of small particles in large volumes through filtration of the water and sediment around them with high efficiency (Sendra et al., 2020; Ward et al., 2019). Since bivalves are more susceptible to MP ingestion due to their feeding style, consumption of such species could pose potential problems to human health as it is unknown what the effect may be should MPs transfer from bivalve to human during consumption (Ding et al., 2021). A previous study by Li et al. (2015) examined 9 commercially important bivalve species from China and observed the frequent occurrence of MPs (2.1 to 10.5 items/g tissue, 4.3 to 57.2 items/ individual, depending on the size class and species). Based on the calculations from this study, it is estimated that consumers are exposed to 11,000 MPs per year (Li et al., 2015). However, the effects of said exposure are unknown and require future study

The accumulation of MPs within an organism may vary depending on size. A study done by Doucet et al., (2021), indicated a negative significant relationship between microfiber concentration and freshwater mussel (*M. margaritifera*) size in the Saint John River watershed. Smaller mussels had a higher concentration of microfibers per gram of tissue than the larger individuals collected (smallest < 10g of soft tissue and largest 80 g of soft tissue). A negative size relationship was also observed by Sathish (2020) in the wedge shell clam (*Donax cuneatus*) from India, in which smaller individuals (0.5-2 cm) had higher concentrations of MPs than medium (2-3 cm) and large individuals (3-4 cm). Another Atlantic Canada study found that adult (> 3.0 cm) soft-shelled clams (*Mya arenaria*) in the Bay of Fundy, also showed a negative

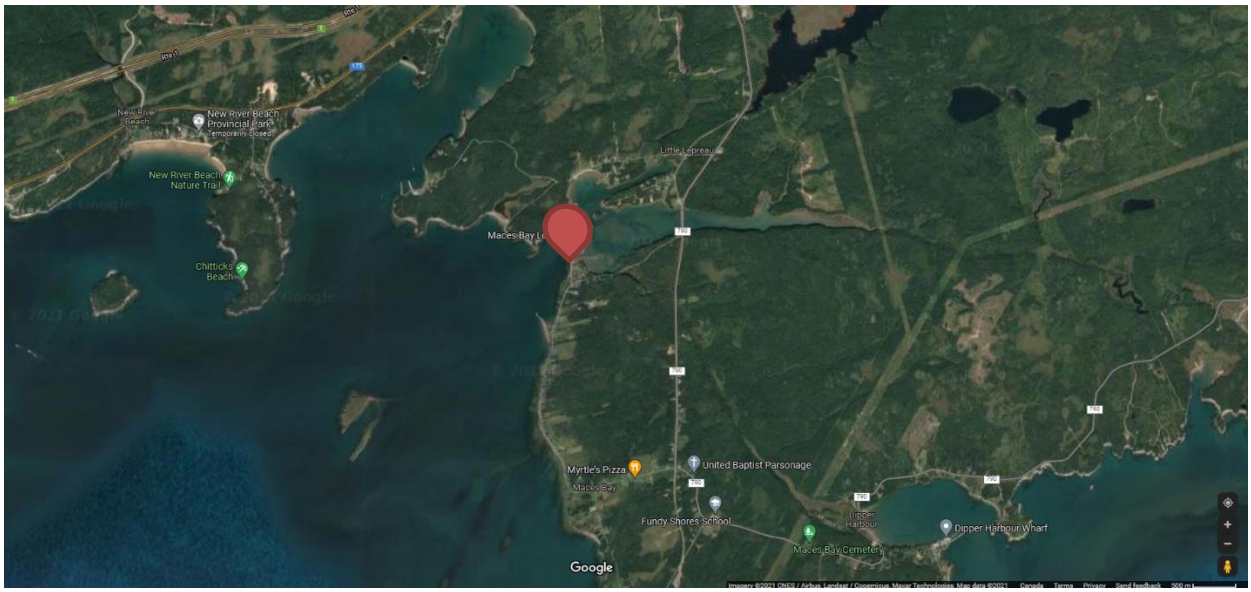
relationship between the concentration of potential MPs per g tissue and clam weight (Beardy, unpublished data). Although a relationship between clam size and MP concentration has been established in a few studies, the mechanism causing this relationship is currently unknown. However, a higher filtration rate per gram of tissue in smaller individuals than larger individuals may contribute to this correlation (Doucet et al., 2021). Another possible reason for this relationship of MP concentration to bivalve size is the size and type of MP ingested by the bivalve.

The objective of this study is to quantify the relationship between MP concentration and *M. arenaria* size. In particular, the focus was to examine size dependence of MP concentration in juvenile clams as previous sampling has focused on adult individuals. It is hypothesized that *M. arenaria* size influences the filtration rate for a given individual and in turn, the concentration of MPs ingested. Therefore, smaller *M. arenaria* would accumulate a higher concentration of MPs (per g of tissue or individual) due to a higher feeding activity than larger individuals. This study will also examine whether the types of MPs found in juvenile *M. arenaria* are independent of size. This study will determine whether potential MPs are present in juvenile *M. arenaria* at sites in New Brunswick in the Gulf of Saint Lawrence and Bay of Fundy as well as provide some insight into whether a relationship between MP concentration and bivalve size is observed in juvenile *Mya arenaria*. Knowledge about size dependence of MP concentration is needed to understand which sizes are most vulnerable to any negative effects due to MP ingestion.

Methods

Sample Collection

Juvenile bivalves were sampled at two sites, one on the southern (Bay of Fundy) and one on the eastern coast (Gulf of Saint Lawrence) of New Brunswick from June-August 2021. The sites were in Maces Bay (45.0992° N, 66.4708° W) and Kouchibouguac New Brunswick (46.7958° N, 65.0559° W) (Figure 1). At both sites, juvenile bivalves were collected using a shovel or a trowel at the location of an identified siphon hole along the sediment surface; individuals were then sieved through a 1.0 or 2.0-mm sieve due to their small sizes to isolate bivalves from sediment. Once bivalves were found, they were manually extracted from the sediment carefully and placed in a pre-rinsed mason jar. A total of 272 juveniles were collected from Kouchibouguac and 28 individuals from Maces Bay. Sizes ranged from 3-29.9 mm as anything larger than 29.9 mm was deemed an adult *M. arenaria*. Bivalve samples were then kept in a cooler until being placed in a freezer at the laboratory for further processing. Nine adult clams were also sampled from Kouchibouguac New Brunswick during August 2021. Adults were collected using shovels by digging at the location of a larger siphon hole along the sediment surface. Individuals were placed in a pre-rinsed jar for transport back to the lab.



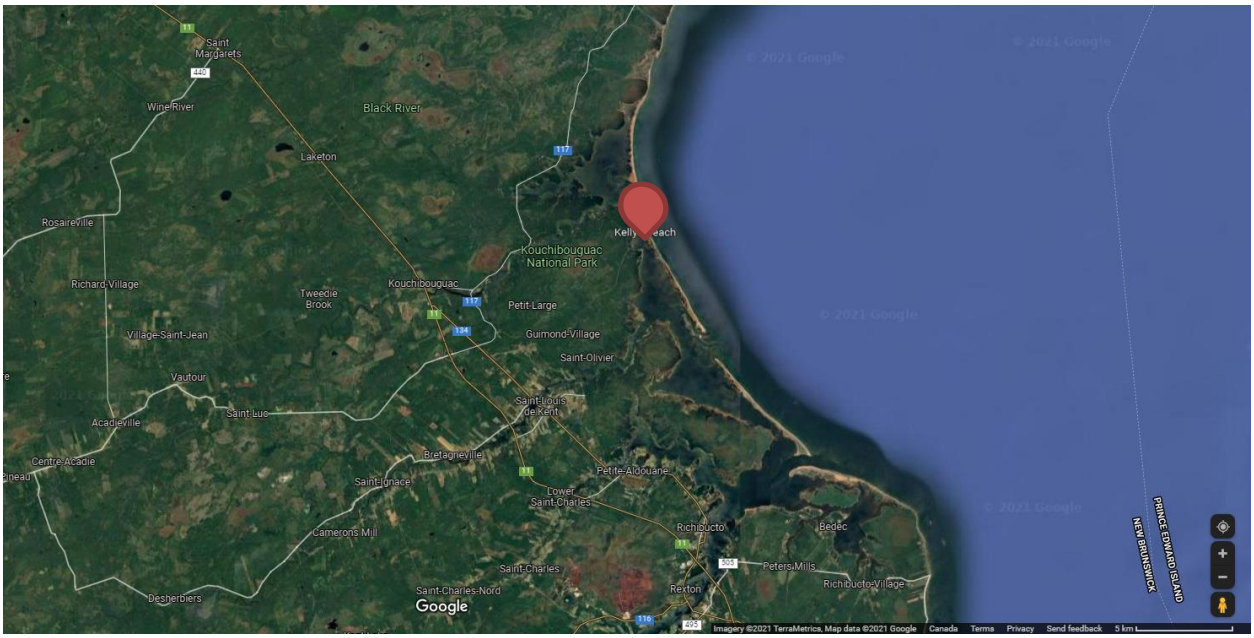


Figure 1: Maps of sites at A) Maces Bay, New Brunswick (Top), B) Kouchibouguac, New Brunswick (Bottom) where *Mya arenaria* were collected. Red indicators are representative of collection sites.

Contamination Reduction

To ensure quality control, specific precautions and protocols were carried out in the field and laboratory setting to reduce the potential of contaminating our samples. Deionized water filtered through a 0.22-micron membrane was used to rinse containers and tools to remove any impurities and contaminants (potential MPs) wherever needed. This included glassware and jars used during laboratory and field work as they were triple rinsed with pure water filtered to remove any impurities and contaminants (potential MPs) before use during the experiment. Latex gloves were worn and 100% cotton clothing including but not limited to lab coats and shirts. Any aluminum foil sheets, or plates used to store, measure, or carry samples, were rinsed three times with filtered water and then baked in an oven at ~350°F to destroy potential residue and contaminants. Any other materials such as tweezers, spatulas, or scissors were triple rinsed with filtered water before use in both the lab and field.

MP Isolation and Identification

Bivalve Digestion and Filtration

Soft-shelled clams were removed from the freezer and left to thaw for ~ 30 minutes before tissue extraction. Once the individuals were thawed, measurements were made with a calliper to determine the individual length (mm) from the siphon edge to the furthest point on the opposite side of the shell. Juveniles were divided into size classes: 0-4.9 mm, 5-9.9 mm, 10-14.9 mm, 15-19.9 mm and 20-29.9 mm. Within each size class individuals were pooled to create samples with sufficient tissue weight (as close to 5g as possible) for sample analysis. For Kouchibouguac, within the 20.0-29.9 mm size class there were 29 individuals per pooled sample

for a total of 5 pooled samples. Within the 15-19.9 mm size class there were 32 individuals per pooled sample for a total of 3 pooled samples within this size class. Within the 10-14.9mm class were 11 individuals per pooled sample for a total of 2 pooled samples for this size class. The smallest size classes each were represented by one pooled sample of 7 individuals for 5-9.9mm and 3 individuals for 0-4.9mm. For Maces Bay, within the 20.0-29.9 mm size class there were 13 individuals total for one pooled sample within that class. The 15-19.9 mm size class was pooled as one sample of 11 individuals. The 10-14.9mm class was one pooled sample of 4 individuals and the two smallest classes (5-9.9 and 0-4.9mm) contained no individuals. For adult individuals, pooled samples weren't necessary as they possessed enough tissue weight on their own for KOH digestion. The adult weight was measured (g) and then the tissue was extracted using pre-rinsed spatulas.

Extracted tissue for each replicate sample was placed in a small pre-rinsed mason jar with 10% KOH solution that had been pre-filtered through a 0.22-micron membrane to remove any impurities. The amount of KOH added was determined by the total tissue weight per group (150 ml for 10-15g, 100 ml for 5-10g and 50ml for <5g). Once all tissues were submerged in the appropriate volume of KOH, samples were placed in an incubator set to ~55º for 24-48 hours.

After 24-48 hours, the samples were checked to distinguish full digestion of tissues; the sample was considered fully digested if only the exterior of the siphon was seen, and no other organic matter remained. If fully digested, the samples were then removed from incubation and brought over to the biosafety cabinet where filtration of the solution occurred. The biosafety cabinet filters inflow and outflow air, ensuring little to no impurities deposit onto samples. The

upper KOH layer was removed from the sample jar into a separate pre-rinsed mason jar using a 5 mL glass pipette

Each sample was then filtered through a series of filters of different sizes (.5-micron, GFC, .45-micron and .22-micron) using a Welch Dryfast 2014 vacuum system. Filtration began with the use of a .5-µm membrane until the solution was easily able to filter through. Once the solution was filtered completely through the membrane (or until the filtration was slow and no more solution could pass through), the used filters of varying sizes were each placed on a petri plate and labelled (Clam ID, Site, Plate ID, Date) before being moved to the biosafety cabinet with the lid slightly removed from the plate to dry. This process occurred using each filter size until the solution was as clear as possible and was able to pass through the .22-micron membrane with ease (quick filtration, no bubbling or foaming).

For each sample batch, a blank filtration was conducted to account for the environmental contaminants in the lab. Procedures mimicked that of a bivalve solution; however, only the .22-micron membrane was used as there was no actual digested tissue in the solution.

Potential MP Observation

Using a dissecting microscope, each filter was examined for any potential MPs. The filters were viewed from 1.0x-10.0x magnification. Once a probable MP was observed (based on irregular shape and colour), characteristics such as colour and MP type were noted. MP types consisted of fragments, fibres, spheres, and films. Types were identified based on distinct characteristics provided by Krista Beardy. Any MP observed during this study is denoted as ‘potential’. This is because the identification as a plastic polymer has not been confirmed by analysis such as FTIR (Fourier Transform Infrared Spectroscopy).

Statistical Analysis

All statistical analyses were done with RStudio v 1.4.1717. Data collected from Maces Bay; New Brunswick were excluded from statistical analyses due to the insufficient sample size. For data for juvenile clams from Kouchibouguac, non-linear regressions were carried out on MP concentration (per g tissue or individual) in relation to mean length (mm) and mean weight (g) of juvenile *M. arenaria* in pooled samples. Three types of regressions were compared: linear, logarithmic, and polynomial regressions of order 2,3,4 and 5. The best non-linear regression was selected based on AIC values. For juveniles the best fit for the concentration data vs length was a 5th order polynomial regression while the best models for juvenile concentration vs mean weight (g) were logarithmic regressions. For micro-fibres, the most abundant type of potential MP, a separate analysis of microfiber concentration (per g tissue) was done against *M. arenaria* juvenile length (mm) and a 3rd order polynomial regression was selected as the best model. To determine whether potential MP type (fibre or non-fibre) was independent of juvenile size (0.0-19.9mm and 20.0-29.9mm), a chi-square test of independence was run. The final analysis done was on potential MP concentration (per g tissue or individual) against *M. arenaria* weight (g) with the inclusion of adult *M. arenaria* samples (n=9). It is to be noted that the weight is a mean value for juvenile individuals and is the actual weight for adults since there were no pooled samples with adults. Logarithmic regression was selected as the best model using AIC for both MP concentration per g and per individual.

Results

Juveniles

Relationship of Potential MP Concentration to Juvenile *M. arenaria* Size

Potential MPs were present in juvenile *M. arenaria* in all 12 pooled samples from Kouchibouguac, and the 3 pooled samples collected from Maces Bay. The size range of juvenile *Mya arenaria* collected from both locations ranged from 3-29.5 mm in length and 0.0015-1.562 g in wet tissue weight. For Kouchibouguac, the highest concentration of potential MPs were found in individuals < 5 mm in length (21.34 MPs/g; 2.3 potential MPs/ individual, 3 individuals in pooled sample) and the smallest concentration from individuals between 20-29.9mm (0.21 MPs/g; 0.07 potential MPs/ individual, 5 pooled samples of 29 individuals) (Fig. 2). A significant non-linear relationship was observed between MP concentration/ g of tissue and juvenile *M. arenaria* length (mm) (Polynomial regression: R^2=0.9954, $F_{5,6}$=259.5, $p < 0.01$) (Fig. 2A) and mean weight (g) (Logarithmic regression: R^2=0.6386, $F_{1,10}$=17.67, $p< 0.001$) (Fig. 2B). Extremely small individuals show a high concentration of MPs/ g of tissue and could be skewing the relationship. However, using concentration per individual to standardize for this possible error still resulted in a non-linear relationship (Fig. 2C, D). A significant non-linear relationship was observed between MP concentration/ individual and juvenile *M. arenaria* length (mm) (Polynomial regression: R^2=0.9673, $F_{3,8}$=78.89, $p < 0.01$) (Fig. 2C) and mean weight (g) (Logarithmic regression: R^2=0.5587, $F_{1,10}$=12.66, $p< 0.01$) (Fig. 2D). Although statistical analyses weren't carried out on the Maces Bay data, a similar non-linear relationship can be observed with an increased potential MP concentration in smaller juveniles (Fig. 2).

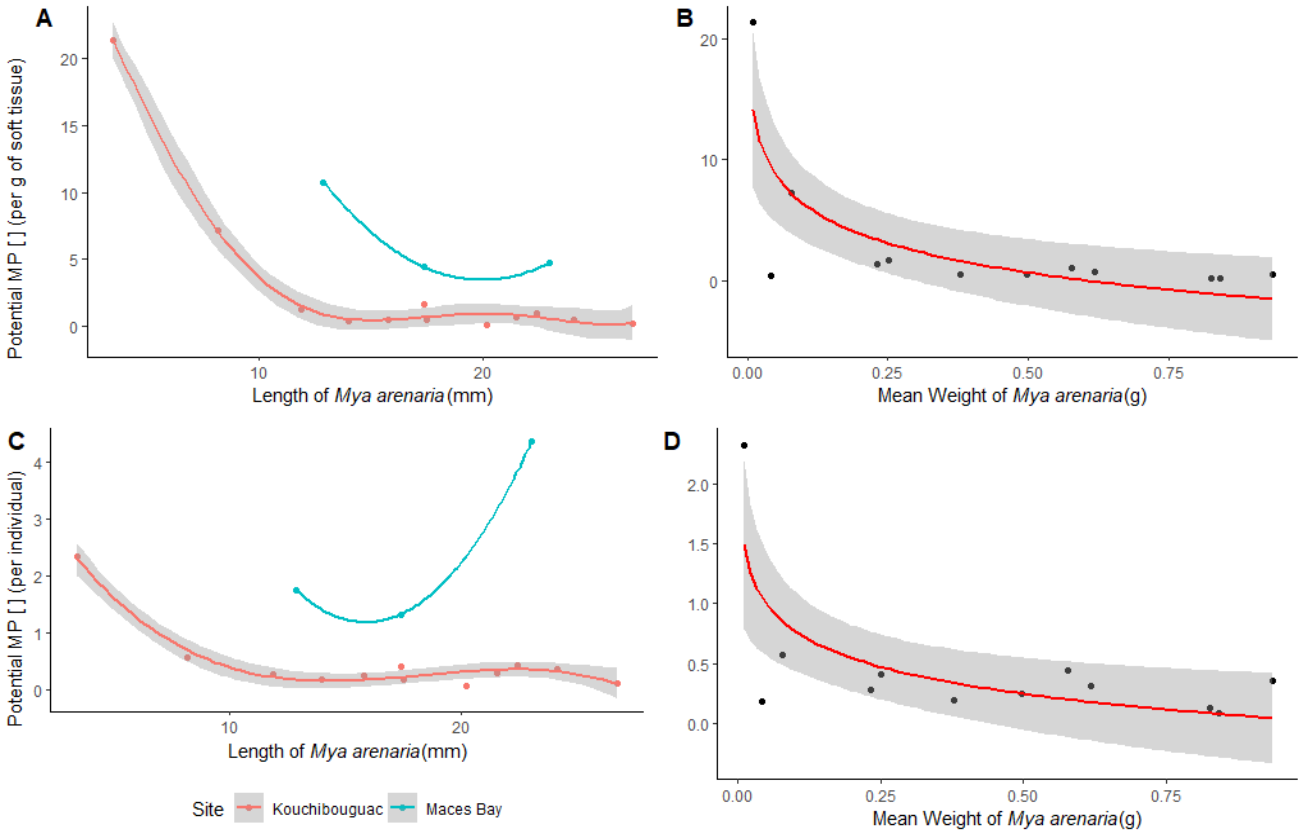


Figure 2: Potential MP concentration in juvenile *M. arenaria* expressed as A) potential MPs/g tissue in relation to length (mm), B) potential MPs/g tissue in relation to weight (g), C) potential MP concentration (per individual) in relation to length (mm), and D) potential MP concentration (per individual) in relation to mean weight (g). Samples collected from Kouchibouguac are shown as red points (n=12 pooled samples) and Maces Bay samples are shown as blue points (n=3 pooled samples.) Non-linear regression trend lines are shown for Kouchibouguac samples.

Potential MP Types

The types of potential MP found in juvenile *M. arenaria* were microfibers, fragments, and films (Fig. 3). The most abundant type found across all samples was fibres (n=79), which made up 80% of all potential MPs identified. The remaining 20% of non-fibre potential MP (n=15) was comprised of fragments and films. There was no presence of microbeads in the

samples collected during this study. The relative frequency of potential MP types was similar in larger (20-29.9mm, 52 potential MPs) and smaller juvenile clams (0-19.9mm, 43 potential MPs) (Fig. 3). A chi-square test of independence indicated that the type of potential MP was independent of juvenile *M. arenaria* size class (Chi-square: X^2=1.11, df=1, p=0.2926).

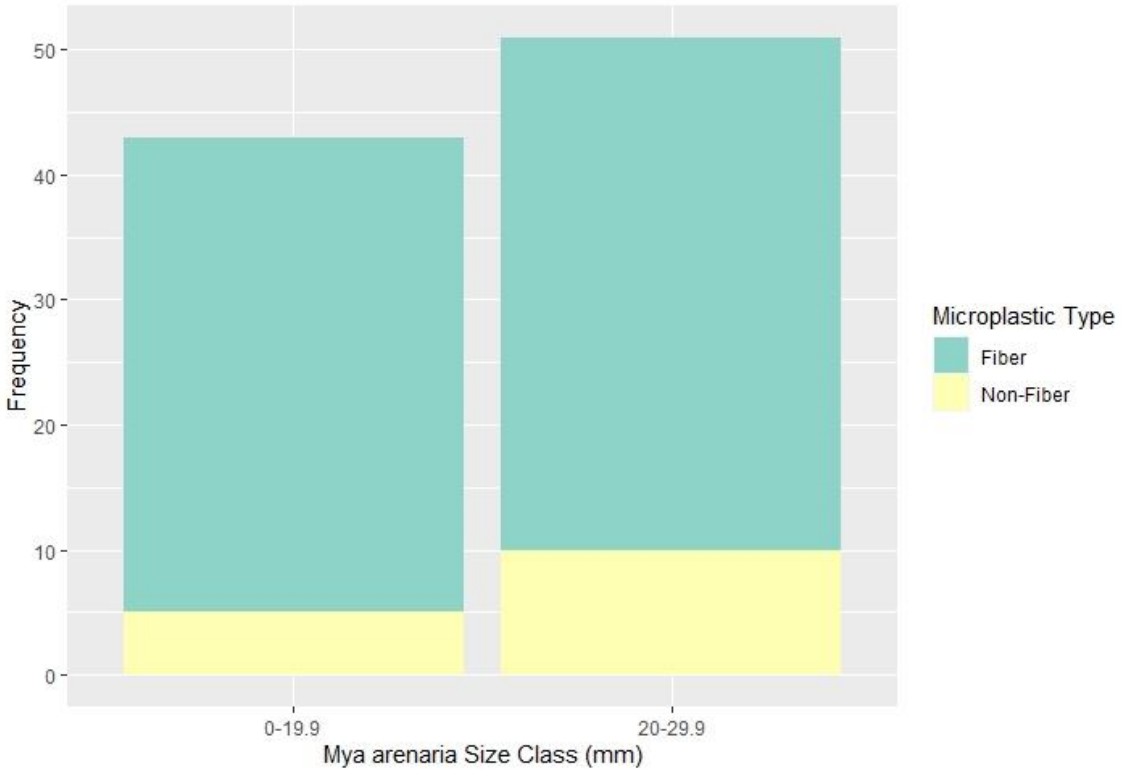


Figure 3: Relative frequency of fibrous potential MPs and non-fibre potential MPs with the size of juvenile *M. arenaria* (mm).

Since microfibers were the most abundant potential MPs found in juvenile clams, regression analysis was repeated for microfibers alone. The observed pattern was similar to that seen for total potential MP. The highest concentration of fibres from Kouchibouguac was in individuals < 5mm in length (15.24 fibres per gram of tissue) and the lowest concentrations were in individuals 20.0-29.9mm (0.21 fibres per gram of tissue) (Fig. 4). There was a significant non-

linear relationship between microfiber concentration/g of tissue and juvenile *M. arenaria* length (mm) from Kouchibouguac (Polynomial regression: R^2=0.9922, $F_{4,7}$=223.3, p< 0.001) (Fig. 4).

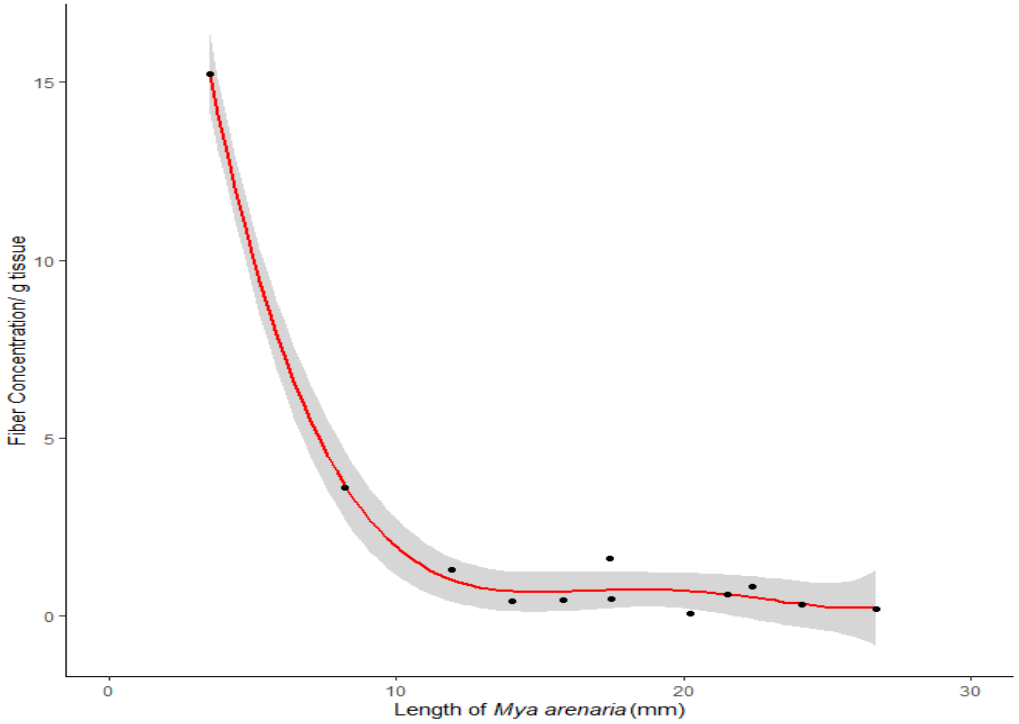


Figure 4: Potential microfiber concentration (fibers/g tissue) and juvenile *M. arenaria* length (mm) for all samples collected from Kouchibouguac (n=12).

Juvenile and Adult *M. arenaria*

To extend the relationship of concentration of potential MP to a larger range of *M. arenaria* sizes, the data was reanalyzed with the addition of data from adult individuals (≥ 30 mm in length) collected from Kouchibouguac on the same date for another project. Adult individuals were measured only for the weight (g) and thus this regression analysis was

conducted concerning juvenile and adult *M. arenaria* weight (g). Adult *M. arenaria* had a maximum concentration of 3.40 potential MPs/g of tissue, lower than the smallest juveniles, but a higher maximum concentration of MPs/individual at 16 MPs/individual. A significant non-linear relationship was found between potential MP concentration/ g of tissue and *M. arenaria* juvenile and adult weight (g of soft tissue) (Logarithmic regression, R^2=0.6845, $F_{1,19}$=7.6, p=0.01278), with potential MP concentration per g decreasing with weight (Fig. 5A). A significant non-linear relationship was also found between potential MP concentration/ individual and *M. arenaria* juvenile and adult weight (g of soft tissue) (Logarithmic regression, R^2=0.3029, $F_{1,19}$=8.255, p< 0.01), but the relationship was in the opposite direction, with concentration per individual increasing with clam weight.

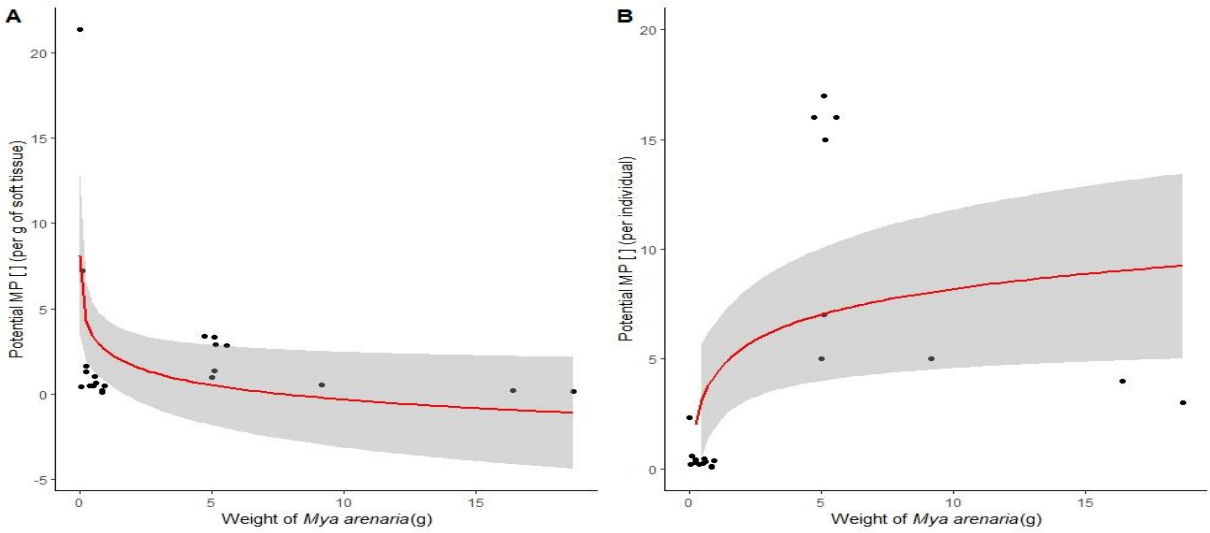


Figure 5: Potential MP concentration expressed as A) (MPs/g tissue) and B) (MPs/individual) in relation to weight (g) for adult (n=9 individuals) and juvenile (n=12 pooled samples) *M. arenaria* collected from Kouchibouguac. Logarithmic trend lines are representative of red lines with 95% confidence intervals.

Discussion

The main objectives of this study were to quantify the relationship between potential MP concentration and *M. arenaria* size and to observe the types of MPs found in juvenile *M. arenaria* at two sites in New Brunswick (Kouchibouguac and Maces Bay). Due to the small sample size collected from Maces Bay, statistical analyses were only carried out using data from Kouchibouguac individuals. The results of this study identify the presence of potential MPs in juvenile *M. arenaria* at both sites. The most abundant potential MP type observed in the tissues of juvenile *M. arenaria* were microfibers (80% of observed potential MPs). This is similar to studies that identified that 99% of potential MPs are from the gut tissue of manilla clams (*Venerupis philippinarum*, Davidson & Dudas 2016*)* and razor clams (*Siliqua patula*, Baechler et al. 2020) were microfibers. In my study, it was found that the frequency of MP type was independent of juvenile clam size. The relationship between microplastic type and clam size has not been assessed previously and should be examined in future studies with other species as well as additional sampling of *M. arenaria* to confirm these results.

It is important to note that micro-FTIR or other spectroscopic analysis was not used to confirm whether the potential MPs identified during this study were indeed plastic polymers. A dissecting microscope was used to visually identify potential MPs due to lack of laboratory infrastructure for and the cost of micro-FTIR analysis; however, this visual method introduces error to counts of MPs (Covernton et al., 2019). Visual identification can mistake clear or white material for microfibers and any MPs < 1mm are difficult to identify with confidence under a microscope (Song et al., 2015). In a study by Baechler et al., (2020) microplastics were identified using manual observations through a microscope in razor clams from the west coast of the

United States. 10% (52 MPs) of the total MPs identified were analyzed spectroscopically, of which 81% were confirmed as synthetic or semi-synthetic materials. Although we do not yet have spectroscopic analysis confirming the identity of MPs in *M. arenaria* in the Maritimes, the results of the Baechler et al. (2020) study suggest that most of the material identified as potential MPs in my study likely is of synthetic or semi-synthetic material. To confirm potential MPs sampled from juvenile *M. arenaria* in the future, the use of micro-FTIR would be recommended.

There was a significant relationship found between potential MP concentration (per g tissue and individual) and *M. arenaria* size (length and weight) at Kouchibouguac for both juveniles and adults. The observed relationship is non-linear and demonstrated a larger concentration per g of tissue for smaller individuals (i.e., individuals 0-15 mm in length, 0-0.49 g of soft tissue). Since this study included *M. arenaria* that were extremely small (< 10 mm in length or 0.0109 mean soft tissue weight), a high MP concentration per g of tissue is produced and potentially influences the observed relationship patterns. To standardize for this potential bias, the relationship between the concentration of potential MP per individual and *M arenaria* length (mm) or mean weight (g) was also assessed. The second analysis allowed a different view of the relationship of potential MP concentration against clam size but showed the same general pattern to that per g tissue for juvenile clams. Smaller juveniles in terms of mean weight (g) and length (mm) demonstrated a greater concentration of potential MPs than larger juveniles. The addition of adult samples did show a different pattern, with the highest concentration of MP per individual in small adult clams and a positive relationship between MP concentration per individual and clam weight. The reason for the difference in observed relationships to size for

juveniles and adults is unclear. Exploring the filtration rates and particle selection mechanisms of *M. arenaria* over the size range could further the understanding of this non-linear relationship.

A negative linear relationship of potential MP concentration/ g of tissue to the weight of bivalves has also been observed in adult *M. arenaria* (individuals ≥ 30mm in length) from sites in the Bay of Fundy (Beardy, pers. comm). A negative relationship to size has also been observed by Doucet et al., (2021) who used freshwater mussels (*Margaritifera margaritifera L*) as bioindicators of microfiber content in the Saint John River, New Brunswick. The non-linear relationship between the concentration of potential MP per g of tissue observed in my study as well as others suggests either an increased uptake of potential MPs in smaller individuals or an increased ability in larger individuals to selectively ingest or clear particles compared to smaller individuals. Studies have observed a higher filtration rate in smaller bivalves that could potentially explain this negative relationship (Jørgensen, 1943; Sathish, 2020). Since smaller individuals grow at a faster rate than large bivalves, the greater filtration rate of small individuals would increase the potential of ingesting more MPs (Doucet et al.,2021). There is also evidence that some bivalves (mussels, oysters, and scallops) can reject particles prior to ingestion when feeding (Ward et al., 2019). Although the mechanism of such a process is still unresolved, it is known that the particle shape, size, and composition influence whether it is rejected at the gill ventral tract as pseudo feces or ingested. In a review of MP ingestion and selection in bivalves, Ward et al., (2019) explained how a greater distance in the folds of the gills prevents the ingestion of larger particles. In smaller individuals, this ridge distance in the gill filaments is likely smaller and therefore small juveniles may be unable to reject larger particles. Further

studies on *M. arenaria* and other bivalve gill surface area and ridge distance are needed to further explore this hypothesis.

The presence of potential MPs in soft shell clams from the Bay of Fundy and the Gulf of Saint Lawrence raises concerns for marine coastal ecosystems and food webs. MP toxicity and potential risks posed to the marine environment require further study as the effect of MPs on organisms in nature is not yet clear. This study serves as an important addition to MP analysis done in the Southern Gulf of Saint Lawrence, New Brunswick and further supports the evidence identifying a significant relationship between bivalve size and MP concentration per g of tissue and individual. A limitation of the study is that the non-linear relationship detected was driven mostly by a few data points. The addition of more samples of clams <10 mm in size during this study could significantly change the estimate of the shape of the relationship between potential MP concentration (per g tissue or individual) and clam size because there were fewer data points for these individuals compared to the larger juveniles. It would also be ideal to have the relationships observed in my study confirmed in different sites around the Gulf of Saint Lawrence and the Bay of Fundy. Another limitation of this study is that my data does not provide evidence for the mechanism for the relationship between potential MP concentration and clam size. Should filtration rates of *M. arenaria* be measured in future studies, the hypothesized cause for such observed relationships could be either supported or refuted. If smaller individuals have an increased filtration rate compared to larger individuals, this would support the hypothesis that greater filtration is a cause of the observed greater concentration of potential MPs per g of tissue in smaller individuals.

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