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An Evaluation of Microplastics in Lac Hertel Sediment Over Time

Abstract

Background: Microplastics, defined as plastic smaller than 5 mm, are pervasive in both marine and freshwater ecosystems. Humans, zooplankton, and fish have been shown to ingest microplastics, which could have detrimental health impacts. Consequently, this project investigated the question: are there microplastics in the sediment of Lac Hertel, located in the Mont Saint Hilaire Biosphere Reserve in Quebec, and if so, how has the amount of microplastics changed over time?

Methods: One sediment core was obtained from the centre of the lake and one was obtained from the edge near the mesocosm dock. Next, one section from the top, middle, and bottom of each core was collected. Afterwards, the microplastics were extracted from the sediments, counted with a dissecting microscope under regular light, and a subset of fragments were tested with a hot needle to confirm that they were plastic.

Results: A generalized linear model indicated that the number of microplastics in our samples increased significantly over time and that the sediment samples from the mesocosm dock had significantly fewer microplastics than the lake's centre. Similarly, a Pearson correlation test revealed that an increasing sediment depth had a significantly negative relationship with the number of microplastics at the lake's centre. However, another Pearson correlation test determined that this trend was not reflected at the mesocosm dock, potentially because of sediment focusing.

Limitations: Due to resource and time constraints, we had a small sample size, only analyzed microplastics larger than 250 μm, and counted microplastics instead of weighing them.

Conclusion: Our results suggest that there has been a significant increase in microplastics in Lac Hertel sediment over time. Ultimately, our results emphasize the need to mitigate plastic pollution.

Introduction

Plastic is ubiquitous in modern society. The cheap cost, light weight, and durable structure of plastic are just some of the factors that created a global annual demand of about 245 million tonnes in 2011 (1). Aquatic biologists have only recently discovered that tiny particles of plastic are also consumed by organisms of a much wider size range than initially thought, allowing high concentrations of plastics to circulate food webs much more easily (1). Studies have now shown that microplastics, defined as plastic particles smaller than 5 mm, can be ingested by and pose risks to the health of organisms such as zooplankton, fish, and potentially humans via the consumption of seafood (2–4). Yet there is little evidence that the problem is slowing; since the 1960s, demand for plastics has been increasing exponentially, likely leading to an increased accumulation of microplastics in aquatic ecosystems (2).

The Gault Nature Reserve is located on Mont Saint Hilaire and has been designated as an International Biosphere Reserve by the United Nations (5). Situated roughly 32 km east of Montreal, the reserve consists of seven low peaks that form a ring around Lac Hertel (5), a glacially formed depression that has a maximum depth of 9 m (6). The lake is fed by three streams and a fourth flows toward the Richelieu River. Swimming, fishing, and boating are not permitted because Lac Hertel is a secondary reservoir of drinking water in the region (6). Although the reserve has experienced little known human disturbance relative to the surrounding area (7), it is vital that the lake is monitored for pollution that could negatively affect the community of species within it and its use as a research site. As a result, our research question was the following: are there microplastics in Lac Hertel's sediment and if so, how has the amount of microplastics changed over time?

Despite the regulations in place at the Gault Nature Reserve to preserve the ecosystem, like prohibiting fishing, swimming, and boating at Lac Hertel, the accumulation of plastic pollution remains a potential threat. One possible source of plastic pollution could be atmospheric deposition of microplastics (8–10). In a study quantifying atmospheric deposition in the remote French Pyrenees, Allen et al. found that microplastic fibers up to ~750 μm long and fragments smaller than 300 μm were being deposited over distances of up to 95 km (8). Similarly, Dris et al. recorded a rate of atmospheric fiber fallout between 2 and 355 particles/m2/day, 29% of which was estimated to be synthetic (9). Moreover, the UV photo-degradation (11), wave deterioration (1,11), and weather degradation (1) of larger plastic items originating from litter in or around Lac Hertel could be another source. Research has been conducted at the reserve since the 1950s, potentially also contributing to the accumulation of microplastics in the area (12). Since the creation of the Gault Nature Reserve, the number of annual visitors to the lake and use of plastics by society has been increasing (13). Specifically, the number of annual visitors surpassed 63 000 in 2017 (14) and plastic production has exponentially increased over the last ~65 years, with 8300 million metric tons having been produced by 2017 (15). Such an increase in visitation and plastic consumption has likely tested the efficacy of the reserve's visitor use regulations, potentially still allowing for plastic litter or fragments from visitors and researchers to be degraded into microplastics and contaminate the sediment of Lac Hertel.

Understanding the scope of microplastic pollution in this protected area is crucial to recognizing the extent to which its 'protection' has actually been effective. Past research has highlighted that many protected areas are protected in name only. For example, though the establishment of Marine Protected Areas (MPAs) theoretically aimed to limit anthropogenic pollution, pollution status is unknown "in most MPAs worldwide" (16). Though the lack of current pollution data is itself alarming, without long term records, it can be even more difficult to ascertain the causes of pollution. Historical records may also assist in predicting future trends in pollution (17), which can inform what kind of restrictions are necessary for protected areas to limit future risks. Unfortunately, such long-term records are even more scarce than current data on pollution, especially because certain inconspicuous pollutants like microplastics have only been recognized as a risk in recent years (1,2). Consequently, paleoenvironmental methods provide an extremely valuable mechanism of tracking how changes to ecosystems, such as pollution, have accrued over time (17).

In this vein, this study aimed to discover: to what extent is Lac Hertel's sediment polluted with microplastics and how has this pollution changed over the years? We hypothesized that there has been a significant increase in microplastics in Lac Hertel sediment over time. To test this hypothesis, we analyzed sediment cores from two areas of the lake for microplastic pollution. We then compared the microplastic abundance of the top, middle, and bottom of the cores, representing sediment from recent years and from the past several decades, in order to evaluate these historical trends.

Methods

The first step of our methodology was completed in November 2019 and involved retrieving columns of sediment using a piston corer, a widely-used instrument that results in minimal sediment disturbance (18). We took one sediment core at the mesocosm dock, depicted in Fig. 1, which was installed in 2011. We analyzed sediment from the mesocosm dock, the site of the reserve's partially enclosed outdoor limnological experiments (19), because large plastic bags are used as part of the set-up. Since larger pieces of plastic debris are known to fragment into microplastics over time (11,20), we wanted to test whether this was potentially contributing microplastics to the sediment underneath it. Next, we obtained another sediment core from the centre, i.e. deepest part, of Lac Hertel. Sampling from the centre of a lake is standard practice in limnology (21), since it has the highest probability of representing the whole-lake assemblage (22,23). The centre of a lake is more representative due to sediment focusing, which refers to how sediments redistribute from shallow to deep areas of a lake because of waves and currents (23–25).

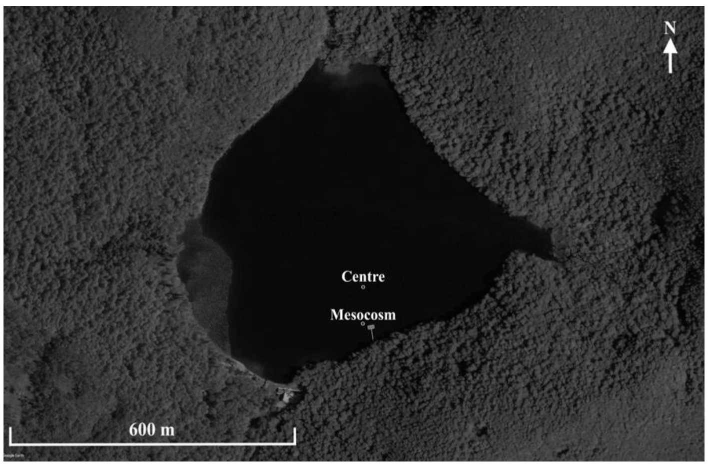

Figure 1. Lac Hertel, Quebec, Canada, surrounded by the mature temperate forest of the Gault Nature Reserve. We retrieved our sediment cores from the centre, i.e. deepest part, of the lake and the mesocosm dock. (26)

After retrieving the cores, we used an extruding device and spatula to take incremental 3 cm samples of the sediment. In each sediment core, we obtained the sediment that was 0-3 cm and 15-18 cm from the top. We also obtained the sediment that was 3 cm from the bottom of each core, although the total depth of each core was different. Accordingly, the bottom sediment obtained from the mesocosm dock was 30-33 cm from the top of the core, while the bottom sediment from the lake's centre was 35-38 cm from the top. Gélinas, Lucotte & Schmit (2000) concluded that the sediment accumulation rate in Lac Hertel is 0.55 cm/year (27). Based on this rate, the top sections contained sediment that was ~0 to 5.5 years old (2019-2013) and the middle sections contained sediment that was ~27.3 to 32.7 years old (1991-1986). The bottom section from the mesocosm dock contained sediment that was ~54.5 to 60 years old (1964-1959) and the bottom section from the centre of the lake contained sediment that was 63.6 to 69.1 years (1955-1950). As a result, we were able to determine whether microplastics in Lac Hertel's sediment have increased over at least the past 60-69 years. However, the bottom sections are likely older than 60-69 years because sediment tends to compact as it accumulates (16,17,28).

We observed several of the precautions outlined in Crew et al. (2020) to minimize the potential contamination of the sediment samples (29). To avoid contamination in the field, we rinsed all our tools with water between sample extrusions. In addition, the samples were stored in glass containers and covered with aluminum foil. Both in the field and in the lab, we avoided wearing clothes made out of synthetic material and wore cotton lab coats while handling the samples. Moreover, lab surfaces were regularly wiped down with 70% ethanol and glassware was washed between samples with 4% non-foaming detergent and distilled water. Next, petri dishes with wet borosilicate filters were placed beside all our workstations to serve as procedural blanks. The filters were later analyzed under a microscope to assess whether airborne particles had contaminated our samples. Lastly, we implemented a positive control by spiking an extra sediment sample with a known number of easily identifiable microplastics and putting it through our lab procedure. We also analyzed this sample under a microscope to evaluate the proportion of microplastics that were recovered. This provided us with a recovery rate that we used to account for the microplastics we lost throughout our lab procedure.

Our lab procedure was adapted from Crew et al. (2020) and consisted of the following steps (27). Firstly, the samples were transferred to Erlenmeyer flasks and 10 mL of 10% KOH was added to each flask. Next, the samples were deflocculated for 25 minutes in a hot water bath set to 60 °C in order to digest the organic material in the samples. Afterwards, each sample was placed on a 250 µm sieve and rinsed with distilled water to remove the digested organic matter and KOH. What remained on the mesh sieve was then transferred to a petri dish lined with aluminum foil and dried in an oven set to 60 °C for 12 hours. Since the melting points of most plastics are above, or just below, 100 °C (30–32), the oven and water bath would not have melted any of the potential plastic in the samples. Once the samples were dried, an oil extraction procedure adapted from Crichton et al. (2017) was performed to isolate the microplastics (33). This method was chosen because it is quick and cost-effective. The extraction procedure involved adding 2.5 mL of canola oil and 50 mL of distilled water to each Erlenmeyer flask, swirling the flasks for 30 seconds, and leaving the mixtures to settle for approximately two minutes. Since plastic is oleophilic, this traps microplastics in the oil layer (33). Next, the contents of a flask were poured into a 250 mL separatory funnel and several rinsing, swirling, and settling steps ensued, with the water layers being discarded. Finally, the oil mixture was dispensed onto a funnel equipped with a polycarbonate membrane filter and dried using vacuum filtration. In between each sample, 13 mL of 4% non-foaming detergent was added to the separatory funnel and swirled vigorously, then poured onto the polycarbonate filter. Following this, the funnel was rinsed 3 times with distilled water.

Our next step involved identifying and counting the microplastics under a dissecting microscope at 40X magnification. Microscopy is a widely used identification method for microplastics in the hundreds of micrometer range, meaning 100-999 µm (29), like the particles we were investigating. Two individuals counted each sample and then the average of the two counts was rounded to the nearest whole number. We used the identification schemes that were created by Crew et al. (29) to differente between microplastics and other oleophilic particles under regular light and then applied the hot needle test to a subset of what we observed. The hot needle test has been used to verify particles as plastic in a number of studies (3,34–36) and consists of touching the suspected microplastic particles with a hot needle. The hot needle test can help distinguish plastics due to their melting points; many plastics have relatively wide temperature ranges as melting points, creating a zone between the solid and liquid states where their physical properties shift (37). When heated to temperatures in this intermediate zone, plastic is semi-solid and flexible. Thus, if the particles are plastic, the hot needle will be able to make the particle sticky and leave a mark, or the particle will curl. If a particle is non-plastic, it will either fragment into smaller pieces or not react at all.

We analyzed our data using a Generalized Linear Model (GLM) in RStudio Cloud version 3.6.0 (38). GLMs are based on an assumed relationship between the mean of the response variable and the explanatory variables. In GLMs, data can be assumed to have a variety of probability distributions, like normal or Poisson (39). As a result, GLMs are considered more flexible and better suited for analyzing ecological relationships, which do not always fit a normal distribution We employed a GLM to help us determine: 1- whether sediment from the centre of the lake or the mesocosm dock had more plastic, 2- whether the number of microplastics changed over time, i.e. sediment depth, and 3- whether the relationship between sediment depth and number of microplastics was different at the centre of the lake versus the mesocosm dock. Accordingly, the response variable in the GLM was the average number of microplastics in each sample, adjusted by our recovery rate, which we determined to be %65, and rounded to the nearest whole number. The explanatory variables were area, i.e. centre of the lake versus mesocosm dock, and sediment depth. Furthermore, the interaction between the explanatory variables was also evaluated in order to answer the third aforementioned question. Our GLM equation was thus "average number of microplastics ~ depth*area". Microplastic abundance is an example of count data, which frequently follows a Poisson distribution (39). To check if our data fit the Poisson distribution, we applied a chi-square goodness-of-fit test. The resulting p value was 0.4050313, which indicated that our data was not significantly different from the Poisson model. Consequently, the GLM was implemented with a Poisson distribution.

Although the GLM could help answer questions 1 to 3, it could not answer our fourth question: was the relationship between sediment depth and number of microplastics stronger at the centre of the lake or the mesocosm dock? As a result, we also analyzed our data using Pearson correlation tests in RStudio Cloud version 3.6.0 (38). The response variable in the two correlation tests was the average number of microplastics found in the sediment at the centre of the lake and the mesocosm dock, respectively, rounded to the nearest whole number and adjusted to incorporate our recovery rate. The explanatory variable of the two tests was the depth of the sediment. Although Pearson's correlation coefficient is not as robust in the presence of non-normality, it can still be used if data is approximately normal (40). To determine if this was the case, we applied a Shapiro-Wilk test to the average number of microplastics found at the centre of the lake and at the mesocosm. The resulting p values were respectively 0.9449 and 1, suggesting that the distribution of our data was not significantly different from the normal distribution. This seems contradictory to the results of the chi-square goodness-of-fit test but may be explained by our small sample size. Nevertheless, given that the variables in question are examples of ratio data, that the relationships appeared to be linear when graphed (see Fig. 2), and that the Shapiro-Wilk test implied that the normal distribution fit our data, the Pearson correlation test was used.

Results

We found between 23-82 microplastics in every sample we retrieved, as is depicted in Table 1. Examples of the microplastics we found include fibers, clear fragments, and microbeads. When we touched a hot needle to a subset of the pieces that we suspected to be plastic, the pieces curled or melted, confirming that they were plastic. We also confirmed that the debris we excluded from our counts were not plastic, as these items broke when they came into contact with the hot needle. Fig. 3 demonstrates some examples of microplastics and non-plastic debris that we tested with a hot needle. When we looked at our samples under a microscope, we noticed that there was an abundance of synthetic-looking fibers. In addition, we found an abundance of very similar fibers on the procedural blanks that we left beside our workstations. Consequently, we suspected that these fibers may have largely been the result of contamination and we deducted all the fibers from the average number of microplastics to be conservative. After counting the number of microplastics we retrieved in the spiked sediment sample, we calculated that our recovery rate was 65%. In comparison, Crew et al. (2020) reported their recovery rate for fibers was 67% ± 2.3 (SE), 63% ± 3.5 (SE) for microbeads, and 61% ± 2.2 (SE) for fragments. Since our recovery rate was similar to those of Crew et al. (29), we adjusted our average number of microplastics to reflect how 33% of the microplastics had been lost throughout the lab process.

Analyzing the data using a GLM revealed three things, summarized in Table 2. Firstly, when the GLM analyzed the overall change in microplastics over sediment depth, i.e. time, meaning without distinguishing whether samples came from the centre or mesocosm, the GLM indicated that the

Sample	Average number of fibers	Average number of clear fragments	Average number of other microplastics
Top/Centre	34	43	5
Middle/Centre	28	33	3
Bottom/Centre	15	22	0
Top/Mesocosm	20	13	12
Middle/Mesocosm	10	15	2
Bottom/Mesocosm	22	20	1

Table 1. Summary of the types of microplastics that were found in the sediment samples obtained from Lac Hertel. Each column reflects the average of two counts, rounded to the nearest whole number but not adjusted by our 65% recovery rate. *Centre* denotes the samples from the centre of the lake and mesocosm denotes the samples from the mesocosm dock. Moreover, *top*, *middle*, and *bottom* refer to the section of the sediment core that the samples were from. Examples of microplastics that fell under *other* include microbeads and coloured fragments.

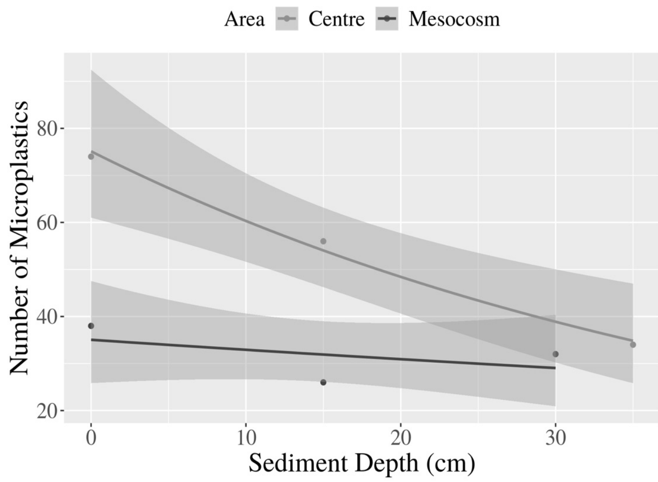

Figure 2. The average number of microplastics that we found in our sediment samples from the centre of Lac Hertel and the mesocosm dock. The average number of microplastics was minus the fibers we observed, adjusted to account for our 65% recovery rate, and rounded to the nearest whole number. The figure was fitted using a Poisson distribution and the shaded areas represent the standard error of the mean.

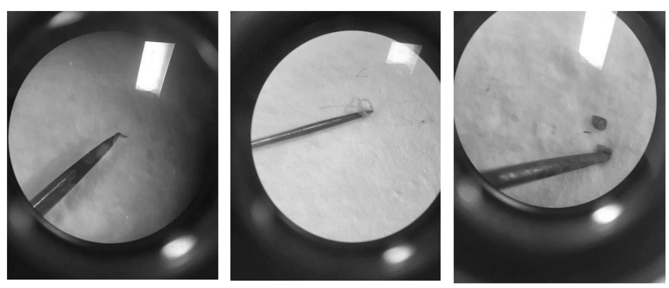

Figure 3. Test Statistic map before and after adding to the model a source for PSR J1930+1852. Unexpected black pixels are likely due to the optimizer not converging in that pixel. If the model is an excellent fit to the data, one would expect the TS map to be close to featureless.

abundance of microplastics increased significantly over time. Secondly, when the GLM analyzed the number of microplastics at the centre of the lake versus the mesocosm dock, it determined that the mesocosm dock had significantly fewer microplastics than the centre of the lake. Third, the GLM showed there was not a significant interaction between the two explanatory variables, depth and area, meaning that the effect of depth on the number of microplastics was not significantly different at the centre of the lake versus the mesocosm dock. Next, the Pearson correlation tests, summarized in Table 3, revealed one more thing. The first test indicated that at the centre of the lake, there was a significantly negative relationship between the depth of sediment and the number of microplastics in our samples. Conversely, a second Pearson correlation test indicated that at the mesocosm dock, there was not a significant relationship between the sediment depth and the number of microplastics in our samples.

	Estimate	*p*	Standard error	*z*
Explanatory Variable #1: Depth (cm)	-0.021952	0.000154	0.005802	-3.784
Explanatory variable #2: Area (mesocosm)	-0.762377	$5.16*10^{-5}$	0.188321	-4.048
Interaction between variable #1 and #2:	-0.015693	0.122774	0.010169	1.543

Table 2. Results of the generalized linear model (GLM) in which the explanatory variables were the depth of the sediment samples in cm and the area that the samples came from, i.e. centre of Lac Hertel versus the mesocosm dock. The response variable was the average number of microplastics that we identified in the samples minus the fibers we observed, adjusted to account for our 65% recovery rate, and rounded to the nearest whole number. The interaction between the explanatory variables was also evaluated.

	Pearson Correlation Coefficient	*p*	*t*	Degrees of freedom
Test #1: Number of microplastics at the lake's centre vs sediment depth	-0.9985728	0.0340 2	-18.697	1
Test #2: Number of microplastics at the mesocosm dock vs sediment depth	-0.5	0.6667	-0.57735	1

Table 3. The results of two Pearson correlation tests. Test #1 and #2 analyzed the relationship between sediment depth and the average number of microplastics at the centre of Lac Hertel and at the mesocosm dock, respectively. Fibers were removed from the average number of microplastics used in these analyses and the data was also adjusted for our 65% recovery rate.

Discussion

The results of the GLM and Pearson correlation tests revealed novel information about Lac Hertel. Based on the lake's sedimentation rate (27), the oldest samples from the mesocosm dock and lake centre were at least 54.4-60 years old and 63.6-69.1, respectively. The GLM demonstrated that overall, the number of microplastics increased significantly with time. This was supported by the first Pearson correlation test, which indicated that there was a significantly negative correlation between sediment depth and abundance of microplastics. However, the second Pearson correlation test revealed that this trend was not present at the mesocosm dock. Since the mesocosm dock is located on a steep slope and sediment is particularly susceptible to redistribution along steep slopes (25), it is likely that the sediment and microplastics are being redistributed towards the centre of the lake. This may partially account for the lack of a significant correlation between sediment depth and number of microplastics at the mesocosm dock. The GLM also determined that the samples from the mesocosm dock had significantly fewer microplastics. To be clear, the area row in Table 2 depicts the effect of area on the number of microplastics. The GLM output specified that these values refer to the mesocosm dock, and since the p-value was significant and the estimate was negative, we can conclude that the sediment taken from the mesocosm dock had significantly fewer microplastics than the centre of the lake. In short, there are microplastics in Lac Hertel sediment and the amount of microplastic pollution has increased significantly over at least the last 60-69 years.

Our findings are notable because the literature documenting microplastic contamination in protected lakes is scarce; yet, our results are consistent with the limited literature concerning microplastic pollution in freshwater. For instance, microplastics have been found in Lake Tahoe, a freshwater lake that does not experience wastewater dumping and that is surrounded by National Forest, a classification of protected lands in the United States (41). However, Lac Hertel has the additional protection of being closed-off to visitor usage, like boating and swimming, as well as having a catchment that is encapsulated by the biosphere reserve. Microplastics have also been found to be abundant in other freshwater environments (2,41–44), similar to how microplastics have been found in Lac Hertel. Furthermore, plastic debris has been found along the beach of the remote Biosphere Reserve of Lanzarote in the Canary Islands (45), near the Pelagos Sanctuary in the Mediterranean Sea (46), as well as in a Marine Protected Area in Croatia (47). Microplastic pollution has even reached the Arctic (48,49). Our results support the findings that microplastic contamination is an increasing environmental problem that has spread to even the most protected habitats, and caution against viewing protected areas as pristine havens from anthropogenic pollution.

Our results add to the growing collection of research documenting microplastics' presence and increase over time using sediment coring. Matsuguma et al. found that the abundance of microplastics in sediment cores from Japan, Thailand, Malaysia, and South Africa increased toward the top layer of sediment, demonstrating an increase of microplastic pollution over time on a global scale (50). Specifically, the cores from Japan demonstrated that microplastic pollution started in the 1950s and then increased considerably as the sediment depth decreased. These results are consistent with our data, as the bottom sections of our cores reflect the presence of microplastics in Lac Hertel as early as 1950, though microplastics were far less abundant in this section. Research by Turner et al. that examined microplastics in the sediment record of an urban lake in London similarly found an increase in microplastic accumulation over time, starting in the 1950s (51). Furthermore, sediment cores taken from an estuary in Tasmania were found by Willis et al. to have microplastics present in every layer sampled, dating from 1744 to 2004, and increasing in abundance closer to the surface (52). The authors found that deeper sediment layers pre-dating the proliferation of plastic in society mostly contained fibers, leading them to suspect that fibers were a major form of contamination, which is consistent with what we discovered in our results. Still, there is a relative dearth of studies that utilize sediment coring in areas less obviously affected by anthropogenic influence, further underlying the relevance of our results to knowledge in the field.

Given that Lac Hertel has a small catchment and is not open to the public, our results lead us to ask: where are the microplastics coming from? One possible culprit could be atmospheric deposition, which is an important pathway for microplastic pollution (8–10). Atmospheric deposition, however, is likely to deposit microplastics too small to identify under 40x magnification; one study found that the majority of deposited microplastics were ≤50 μm (8). Typically, atmospheric deposition also mainly deposits microplastic fibers (10,11). Thus, atmospheric deposition may not be as substantial of a contributing source to the microplastics we observed, which were greater than 250 μm and mostly fragments after we accounted for the contamination from our own clothing. Moreover, microplastics could stem from littering on Lac Hertel's beach, which is open to the public, as well as the use of the lake for research purposes.

Researchers have been doing limnological experiments at the mesocosm dock on Lac Hertel since it was built in 2011, which often uses large plastic bags that can become torn. We observed that this had occurred when we were carrying out our experiments. These activities could result in large plastic fragments polluting the environment that could then be further broken down (1,11), contributing to the microplastics found in our top sediment samples. Though we controlled for plastic contamination in the petri dishes and glass jars that the sediment was stored and transported in, the one relatively unavoidable source of potential microplastic contamination was the plastic coring tube itself. Though concerns have been raised about the potential for coring tubes to be scratched by sediment particles and contaminate samples (53), there is limited research to show that such contamination is substantial in practice. However, the presence of plastic in sediment coring is not limited to this one study; sediment coring with plastic corers is employed regularly by researchers at Lac Hertel, along with the long-term use of other plastic equipment like sampling bottles, buoys, Secchi disks, life vests, and ropes. Even the simple presence of researchers on the lake could enable their clothing to pollute the lake with microplastics if it is made of synthetic fibers, which could eventually settle into the sediment. Little to no data exists to document the microplastic footprint of limnological research, which should be a focus for future investigation. The Gault Nature Reserve has been used for research, teaching, and public recreation dating back to the 1950s and 1960s (12). As such, it is probable that non-point sources like atmospheric deposition, littering, and research activities may have contributed to the microplastics that we detected in all of our samples.

A few factors limit the scope of our results. Due to time constraints, we were not able to weigh the amount of microplastics that we found in each sample. Resource constraints prevented us from using Nile Red dye and fluorescent microscopy, thus we could only evaluate microplastics larger than 250 μm. Since our cores were not radiometrically dated, the dates were approximated from sedimentation rates of the lake, which does not account for compaction. Consequently, our bottom layers are likely older than our calculations, resulting in some ambiguity about exactly how far back microplastic pollution extends in Lac Hertel's sediment. Another notable limitation was our small sample size, since we only had three data points per core. We did not finely partition our core as this would have multiplied the number of oil extractions we had to perform, which was not feasible within our timeframe. Still, a more finely partitioned core would allow for a better understanding of the relationship between microplastic abundance and time. Additionally, we chose to leave the procedural blanks out for the entire time that we were conducting experiments to get a better idea of the kind of contamination that could be coming from our surroundings. However, this meant that the amount of contamination on the blanks was likely greater than what any single sample would have been exposed to, since they were only exposed for brief periods of time. As a result, we could not draw direct conclusions about the quantity of contamination in the samples from the procedural blanks, but the blanks still enabled us to observe what kinds of microplastics may have been the result of contamination.

Future research would benefit from the following adaptations to our methodology: weighing microplastics, using additional methods to analyze even smaller microplastics, obtaining more sections from each sediment core, dating the sediment cores, and only setting procedural blanks out when samples are exposed. The primary focus of future research could be to determine the origins of the microplastics at Lac Hertel, specifically focusing on two potential sources: the beach, which is used by the public, and the mesocosm dock, which is used by researchers. The beach and dock areas should additionally be monitored for macroplastic pollution, e.g. torn mesocosm bags and incidents of littering, so that original sources can be tracked prior to degradation into microplastics. Further research could also explore whether these microplastics are present in tissues of biota of the lake, as well as the biological impacts of microplastics on these organisms. This is especially important given the use of this lake as an experimental system for researchers, where unaccounted microplastic pollution may confound experiment results. For example, studies interested in the diets or life history of the lake's biota may not account for the consumption of microplastic and the possible effects on development and reproduction (54,55). More protected lakes could be studied to see if our hypothesis is supported in other protected areas as well. Most importantly, further regulations to limit non-essential plastic use by researchers at the Gault Nature Reserve should be considered where possible, at least until the source(s) of the plastic are identified.

Conclusion

Microplastics have become a major pollutant that poses a risk to the health of numerous organisms. Today, microplastics can be found in even the most remote corners of the world. Despite an increasing amount of research on freshwater microplastic pollution, there is still very little research about how microplastics impact protected areas. As a result, we evaluated whether there are microplastics in the sediment at Lac Hertel, which is part of a UN biosphere reserve, and if yes, whether microplastics have increased over time. We hypothesized that this was the case. To be conservative, all fibers were excluded from our counts, as we suspected they were likely the result of contamination. We also factored in our 65% microplastic recovery rate to account for the plastic lost during our lab procedure. Despite our conservative microplastic counts, we found microplastics in all our samples and determined that microplastics significantly increased in abundance over time. It is important to note that we only analyzed microplastics that were larger than 250 μm; further analysis of smaller particles would likely reveal a greater extent of contamination. Nevertheless, our results support our hypothesis. Ultimately, our findings are consistent with other research, which concludes that microplastics are a pervasive, growing issue that warrants more research and action.

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