I would like to dedicate this paper to Dr. Tammy Windfelder, who helped foster my passion for biology and ecology, conservation, and inspired me to do the research for this project. Without her continuing support of my work here, none of it would have been possible.

Thank you to my family and friends who supported and encouraged me along the way.

Drew University

College of Liberal Arts

# Assessing the Impact of Agriculture on the Introduction of Microplastics in New Jersey's Ponds and Lakes

A Thesis in Biology

by

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ABSTRACT

Microplastics, by definition, are particles of plastics that measure less than 5 millimeters in diameter. While for many previous decades the concept of microplastics has been relatively unheard of, it has been catching more public and scientific attention in recent years. Microplastics can be introduced into the environment in a wide variety of ways, one of which includes agricultural methods. In this study, I attempt to find if there is any significant difference in the presence of microplastics being introduced into freshwater ponds and lakes in New Jersey as a result of agriculture.

Sites were chosen based on their potential geographic proximity to agricultural areas, and water samples were then collected, filtered, and observed for microplastic presence. Specific qualities that were accounted for were color, shape, and size. At the end of this study and after consideration of statistical analysis, several results were found. A total of 71 microplastics were found. There was not any significant correlation between agricultural impact and size of microplastics. The vast majority of microplastics found happened to be line shaped, with only one appearing to be film shaped. For color, a variety of black, blue, green, purple, and clear microplastics were identified. Black was the most common color of microplastic comprising 73.2% of all microplastics collected, though the only significant difference in color between sites impacted by agriculture and not impacted by agriculture was the prominence of blue colored microplastics, with agricultural sites having a statistically significant higher amount than sites not impacted by agriculture. Surprisingly though, microplastics were found in almost all sites; regardless of the correlation with agriculture, it's interesting to see such a high presence of them.

Overall, the results show there to be at least a small difference in quality of microplastics in agricultural versus nonagricultural sites based on the variation of blue microplastics, and the

results of this particular study somewhat line up with the trend in many other works of scientific literature and studies in this area that agriculture, to some extent, can introduce microplastics into freshwater environments, though the extent to which it occurs in respect to this study requires further work. While there were some differences between sites in terms of data, though above all else microplastics were ubiquitous and found in almost all of the ponds and lakes sampled.

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# INTRODUCTION

In recent times, microplastics are a topic that seem to be discussed more than ever, with some papers garnering larger attention such as the one involving microplastics being found in human blood (Leslie et al. 2022). Their presence in the media and news outlets bordered to the point of sensationalism, and accordingly have been a source of concern for many as their mentions are not for good reasons – rightfully so. There have been many studies in the past decades that reveal the harmful effects of microplastics, many of which will be discussed throughout this paper both to provide context into the general harm of microplastics on an ecological and environmental level as well as on human health, and these studies have led to an increasingly weary public perception of microplastics (Catarino 2021, Al Mamun 2023). Speaking from a personal level, it wasn't uncommon in the past years and even to this day to see the occasional appearance of a topic regarding microplastics circulating on social media, and many people I know have said to me they have seen similar articles floating around as well. Even prior to expressing interest in studying microplastics, it's always something that's come to my attention from time to time for one reason or another. It's not just me who's had an increased exposure to information regarding microplastics; in the past 5 years, many have gone from not even knowing about the term "microplastic" itself to having a heightened sense of awareness of them (Catarino 2021). This is likely due to how since 2011, scientific publications in microplastics have been ever increasing and in the past years, and monthly news articles regarding microplastics are also steadily increasing, further exposing the public to microplastics as a whole (Henderson & Green 2020). I did a search for microplastic* in Web of Science and found an exponential increase in the number of papers published on this topic over time (Fig. 1).

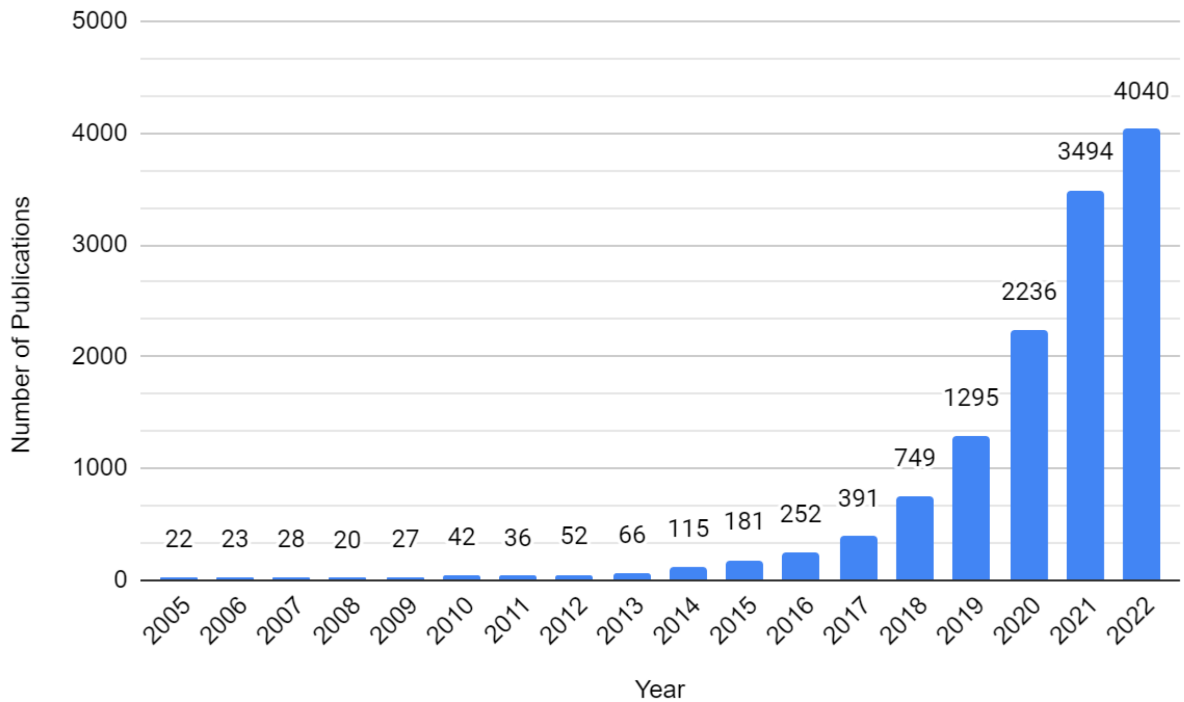


Figure 1: Number of publications on the subject of microplastics over time, as determined by a search in Web of Science

Despite this, however, there hasn't been much of a broader discussion of how *exactly* microplastics are harmful, and literature overall discussing the potential solutions to removing microplastics from the environment are still mostly discussing experimental methods, and this is probably due to how the concept is perceived as new to many along with there being a general uncertainty as to what they are and how they affect us outside of the occasional article one may stumble upon (Henderson & Green 2020, Na 2021). I myself was certainly under the impression that microplastics were something obscure upon initially learning about them, not even paying that much attention to them, but it wasn't until I got involved with them directly that they piqued my interest. In my thesis, I aim to address some of the misconceptions surrounding microplastics, describe exactly what they are, how they not only impact humans but also the environment, and then discuss my study and the results of it regarding the extent to which agriculture introduces microplastics into freshwater bodies in New Jersey.

*My Interest in Studying Microplastics*

My interest in wanting to research microplastics arose around March of 2022. Prior to this, I was aware of the presence of microplastics, but I didn't have a full understanding of what they were and never even considered studying them during my time at Drew University. Given that I had been involved in studies related to ecology for quite some time at that point, I figured that I would eventually be discussing them to some degree in one of my classes. It wasn't until I took the Tropical Marine Ecology course that I directly studied microplastics – rather unexpectedly too. During this course, our class spent our spring break in South Water Caye in Belize performing various labs and studies, including collecting data to see the impacts of island biogeography and observing what occurs during twilight changeover in coral reefs to name a couple. One other study that we did, of course, was to collect data on the presence of microplastics in Belize. Initially, I was under the impression that we wouldn't find any microplastics whatsoever regardless of what methods we tried – the waters surrounding the coral reefs in Belize seemed so clear, pristine, and clean, that I thought it would be unrealistic to find any (Fig. 2).

Figure 2: An picture I took while in South Water Caye in Belize – who would ever expect to find microplastics here?

Additionally, this was the second time that the course ran a lab on microplastics, so with this in mind my expectations weren't substantially high. At the time I also thought it would have been extraordinarily difficult to even identify any microplastics if we found them – given that this was an undergraduate study where we weren't using any advanced equipment such as using mass spectrometry to identify specific types of plastics.

The methodology and equipment we used were fairly simple; our class used two different ways to find microplastics, one through sieving and one through looking at cut turtle grasses

*Thalassia testudinum* under a microscope. I was part of the group that examined turtle grasses. For this we simply cut samples of turtle grass right off of South Water Caye. We looked at each blade we cut under a microscope to see if there were any microplastics embedded in them, with the age of each blade considered. Our goal for this specific lab was to see if there was any correlation between the rates of herbivory on grasses that had microplastics versus ones that had none. The results of this study were rather surprising to me. We found that blades of turtle grass with microplastics had a statistically significantly higher rate of herbivory, and it was genuinely surprising to me that not only did we find microplastics, but they were also fairly abundant. I was completely shocked at the fact that we were even able to find microplastics at all, afterall, how could a place that seemed so isolated and clean have such a noticeable presence of microplastics? What was even more surprising to me is that we were able to find so many using the fairly simple methods that we used. This was my initial exposure to research into microplastics, and following this research I would see more and more articles discussing microplastics and their negative impacts both on the environment, various animal species, and even human health. Over time I became increasingly more interested and invested in microplastics research, and eventually I wanted to conduct my own study on the presence of microplastics in New Jersey's waters. If the waters in Belize contained microplastics to the degree I saw, then I considered that the presence of microplastics in New Jersey would be far greater.

*Freshwater Microplastics and Bioaccumulation*

Methods to go about studying microplastics proved to be surprisingly diverse, and pinpointing exactly how I should go about conducting this study came down to reviewing specific methodologies people have used in the past to collect microplastics in freshwater systems. I chose freshwater specifically since in terms of distance to Drew University, it was the most accessible option. Initially, I wanted to study the impacts of microplastics on fish species in freshwater bodies – specifically ponds and lakes. It has been found that many fish populations in freshwater bodies around the world have microplastics present within them to varying levels (Silva-Cavalcanti et al. 2016). The ways that this occurs can vary, whether it be direct ingestion of microplastics or through bioaccumulation (Silva-Cavalcanti et al. 2016, Grigorakis et al. 2017). When specifically considering bioaccumulation, this can be a larger problem considering how contaminants and pollutants can travel through a food web and accumulate in increasing concentrations at higher trophic levels, eventually making their way to humans in addition to various other species that rely on the food webs in question (Jaafar 2021). This doesn't just occur in terms of consumption by fish, it has also been shown that microplastics have been found in the gills of fish as well (Jaafar 2021). Considering the world's increasing consumption of fish, the presence of microplastics in fish is certainly an area of concern (Jaafar 2021). Having reviewed some of the studies regarding this, I wanted to try studying the presence of microplastics in fish species in New Jersey as I was not able to find much research on fish species as a whole in the freshwater systems near Drew University. Though there were a lot of surveys published by the New Jersey Department of Environmental Protection (NJDEP) regarding trout (https://dep.nj.gov/njfw/fishing/freshwater/bureau-of-freshwater-fisheries-highlights/). However, there were some ethical concerns that would have needed to be considered for this study, specifically how generally studies that look at microplastics in fish use gut tract samples, meaning that fish would need to be trapped and dissected for this study to move forward (Silva-Cavalcanti et al.2016). While attempting to find the presence of microplastics in fish would have certainly been an interesting study, because of this concern I decided to shift my study in a different direction.

*Human Health*

Before continuing with the semantics of what my study would finally come to, I think it's important to discuss some of the harmful effects of microplastics to humans that have been found recently, as I feel it's one of the main concerns of microplastics in conjunction with their negative impact on the environment. One impact on human health that I typically see get discussed in the media is the presence of microplastics in human blood (Leslie et al. 2022). In a study performed in 2022, participants who were in good health had blood drawn and analyzed for microplastics, and it was found that microplastics were present in human blood to some extent, with the average in this study among all participants being 1.6 micrograms per milliliter (Leslie et al. 2022). I remember first seeing an article discussing this study and was quite shocked at first, and it wasn't long before I stumbled upon another scholarly study, though it was produced at a prior date to this study, regarding the presence of microplastics in human breast milk (Ragusa et al. 2022). In this study, it was found that in the 34 samples of human breast milk analyzed, microplastic contamination ranging in shape, size, and color appeared in 26 of them (Ragusa et al. 2022). While this by itself is concerning, this matter becomes even more problematic upon consideration that this is being ingested by human infants (Ragusa et al. 2022). Of course, it is acknowledged that the full impacts of microplastic contamination aren't completely understood, and that the implications of this require more research (Leslie 2022 et al.). Still, microplastic contamination remains a concern to human health as microplastics become increasingly common, and it has been suggested that there may be risks to organs in the form of toxicity, potential immunity issues, and even a potential for microplastics to be carcinogenic to some extent (Al Mamun 2023). Additionally, plastic fragments have been observed to carry harmful bacteria such as E. Coli (Rodrigues et al. 2019). As previously mentioned, the exact consequences that microplastics can have on human health still need to be researched to a larger extent, though microplastics are unfortunately very much present in humans as studies have revealed.

*Impact of Agriculture*

I then considered what other factors could be introducing microplastics into freshwater bodies in the first place, and after reviewing more primary literature on microplastics, I turned my focus more on the potential impact of plastics used in agricultural settings on the introduction of microplastics into freshwater bodies in New Jersey. Agricultural practices, while not always ubiquitously, sometimes implement the usage of plastics in farming methods (Lwanga et al. 2022). Runoff from agricultural sites has always been a focal point of concern when it comes to toxicology for a variety of reasons, though recently the introduction of microplastics as a result of agricultural runoff into freshwater systems has been an area of attention in microplastics research (Lwanga et al. 2022). Some of the elements present in agriculture that may introduce microplastics into the environment as a whole include but are not limited to mulching films, twine, fertilizers, pesticides, pipes, manure, improper irrigation, and even a general mismanagement of stored equipment (Lwanga et al. 2022). For New Jersey specifically, the use of plastic mulches may be an area that could be looked into, as they've become an area of concern as a whole for freshwater microplastic pollution (Horton 2017, Lwanga et al. 2022). While of course the introduction of microplastics as a result of agricultural activity itself may be harmful due to how microplastics will end up bioaccumulating in whichever environment they end up in, there is also a potential for microplastics to introduce both harmful chemicals and potentially pathogens into the environments they end up being introduced to in addition to the agricultural runoff that gets added into said environment (Lwanga et al. 2022). The exact implications of this are not yet well researched, however I feel it's important to identify the potential sources of microplastic contamination in order to be able to better pinpoint what contaminants are also being introduced into the environment. Northern New Jersey, specifically in the area surrounding Drew University, doesn't see very much agricultural activity as other parts of the state might such as with Southwestern New Jersey, however, agricultural practices are still very much present and I thought it would be interesting to see if there was any correlation between bodies of water that might be impacted by agriculture versus those that might not be.

Of course, the concern over microplastics is certainly something that has been under a scholarly lens for quite some time now. Research into this area has become so extensive that some even smaller microplastics that span as small as nanometers have been categorized as nanoplastics which sees their own respective research, though microplastics as a whole remains a big focus (Shen et al. 2019). In the field of toxicology for example, microplastics are an area of ever expanding research to try to find the true extent to which microplastics are harmful to humans as well as the environment as a whole.

*Microplastic Definition*

Firstly, it is important to not only identify what exactly a microplastic is, but also what their implications on the environment are, and for the purpose of this study their impact on freshwater ecosystems. Though the exact parameters and limits of microplastics have been debated by many and continue to be debated, it is generally agreed upon that a microplastic is a particle of plastic which is less than 5 millimeters in diameter total (Frias & Nash 2019). In addition to this, there are considered to be two kinds of microplastics in terms of origin, primary microplastics and secondary microplastics respectively (Frias & Nash 2019). A primary microplastic is any microplastic that was purposefully designed and produced under 5 millimeters, and a secondary microplastic is any microplastic that was created due to a larger plastic either degrading or having bits broken off from it to the point where it falls under the parameters of a microplastic (Frias & Nash 2019). While there is no firmly established limit to how small a microplastic can be, it is generally agreed upon that microplastics under a certain diameter become considered nanoplastics, however the definition for this greatly varies among researchers with ranges varying from less than 100 nanometers to less than 1 micrometer (Mendoza et al. 2017). For the purposes of this paper, however, microplastics will be the sole focus due to there being different environmental implications between microplastics and nanoplastics (Mendoza et al. 2017). It is thought that microplastics in the environment, accordingly, did not exist before the advent and subsequent spread of plastics from the 19th and 20ths centuries and onwards, and is a fairly recent issue due to this (Mendoza et al. 2017). Plastics in the environment remain where they are unless physically removed – this is inherently problematic when considering microplastics as they typically cannot be seen as they are so small (Mendoza et al. 2017).

Seeing these developments and the overall increase in microplastics research, I felt prompted to investigate this issue myself. Having lived in New Jersey my entire life and consistently being engaged with the varying freshwater bodies that are near me, I felt it was only right to conduct studies on the prevalence of microplastics. While initially I thought it would simply be interesting to see whether or not there are microplastics at all in these bodies of water, I eventually came to realize that these microplastics must stem from somewhere, and after doing some more research I found that it could be possible that some microplastics could stem from agricultural runoff (Lwanga et al. 2022). Accordingly, when I began my research to see the extent of the presence and impact of microplastics on freshwater bodies in Morris County, New Jersey as a result of being introduced through agriculture, I wasn't able to find much information or research on it. There is a great abundance of research regarding water quality in New Jersey including the impact of agricultural practices, though microplastics were something that tended to be left out (Westendorf et al. 2013). Considering how crucial of a topic microplastics have proven to be, I thought it would be important to investigate such a matter and to determine the extent to which there are microplastics in freshwater bodies in Morris County. By doing so, I hoped to witness any of the implications of any current agricultural practices on the introduction of microplastics into freshwater bodies.

*Hypothesis and Goal of Study*

The primary goal of this study was to determine if there was a substantial difference in the quantities, types, and sizes of microplastics present in bodies of water that are influenced by agriculture and in those that aren't. This was performed by sampling water from various different sites within Morris County, New Jersey based on their proximity to areas that see agricultural activities. All collected samples were then passed through filter paper, and all of the filtered materials were examined under a microscope. Any material that matched the criteria for being a microplastic was labeled as such and was categorized accordingly. The initial hypothesis for this study was that there would be a significantly greater density of microplastics in samples collected from water bodies likely impacted by agriculture, and that these microplastics would reveal which specific agricultural practices and elements are contributing the most microplastics. Additionally, I expected to see a larger array of colors and shapes of microplastics from agricultural sources, which could stem from many facets related to agricultural pollution.

# METHODS

The ultimate goal of this study was to determine if there were any differences between the quantity and type of microplastics in freshwater bodies that potentially receive fertilizer runoff and freshwater bodies that don't. Lakes and ponds that were chosen for this study were selected based on their proximity to any area that receives fertilizer; any lake or pond within one mile of these areas were considered to be directly impacted by agriculture for the purpose of consistency. Additionally, any necessary permissions to sample at any of the chosen sites were obtained before sampling took place.

Sites considered impacted by agriculture were as follows: Crystal Lake, Lake Hopatcong, Lake Kinnelon, Speedwell Lake, Kay Lake, and Lake George. All other sites, being Kitchell Pond, Cook's Pond, Lake Valhalla, Long Pond, Round Pond, Old Troy Pond, and Sunrise Lake, were considered to not be impacted by fertilizers (Fig. 3).

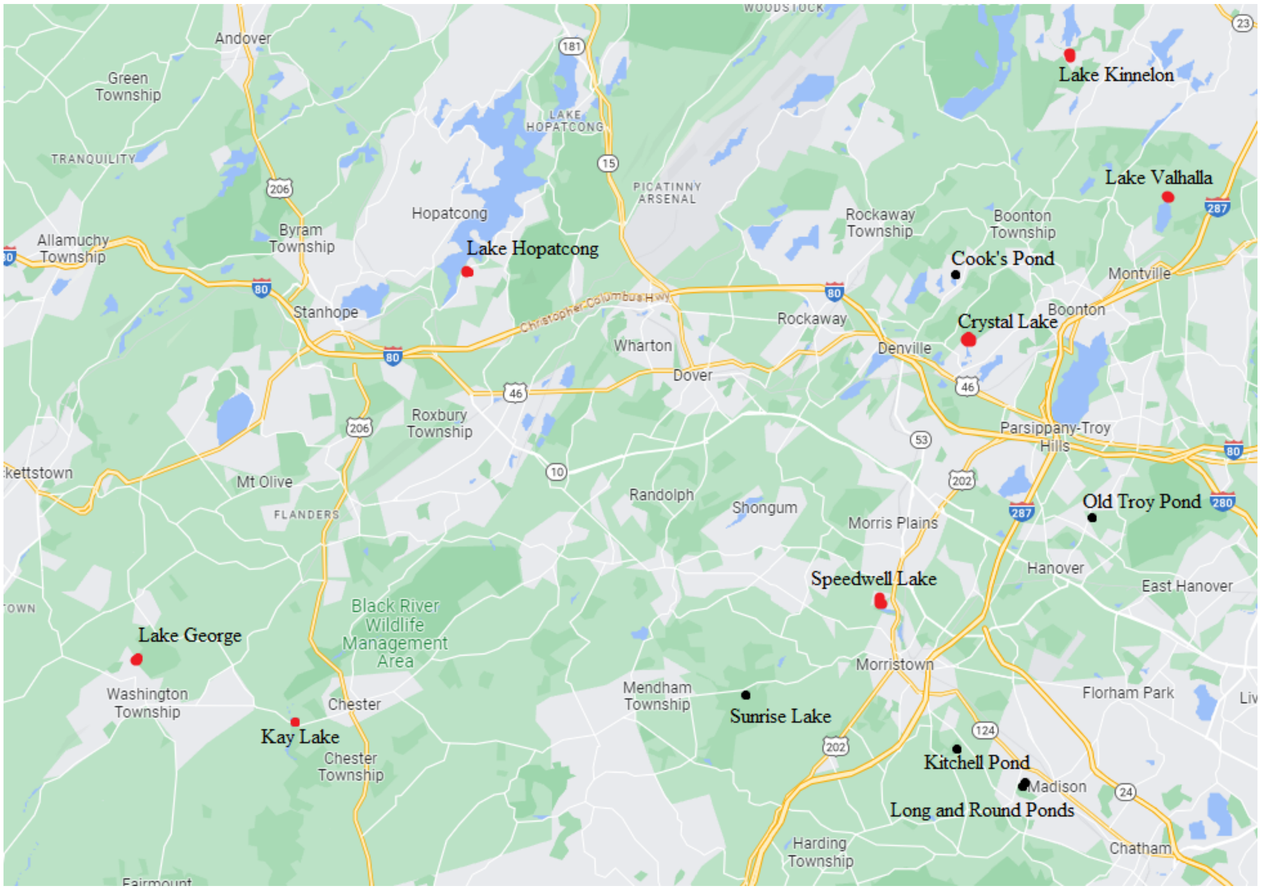


Figure 3: Map showing the location of sites sampled. Sites dotted in red were deemed impacted by agriculture, and sites dotted in black were not impacted by agriculture. Map provided by Google Maps

*Samples Collection*

At lakes and ponds, water samples were collected by placing two 50 mL tubes in the surface of the water until the tube was completely filled with as little excess debris or sediment as possible for a total of 100 mL of water collected.

Samples were brought back to the laboratory to be filtered and further examined. The setup for the filtration involved the use of Whatman GF/C Filter Paper with a diameter of 47 mm and 1.2 µm pores placed over a handheld funnel filter matching the diameter of the filter paper, which was then attached to a flask designed to be used to create a vacuum. All samples were individually poured through the filter and then vacuum filtered until no water remained. Any remaining water in the flask was disposed of. Any materials that remained on the filter paper were then examined under a microscope at 40x power, though higher powers up to 100x were also used in order to identify finer details if needed. Microplastics were categorized based on size, shape, and color. The points of reference used to identify the specific shapes of the microplastics followed similar protocols established by de Carvalho (2021); the specific shapes that were looked for were lines, films, fragments, pellets, and spheres. Microplastics sizes were categorized from under 1 mm, between 1 mm and 2 mm, and 2-5 mm. Exact lengths were taken when possible, however due to the logistical difficulty of recording the exact lengths due to risk of a microplastic either breaking or being lost in the process of attempting to measure it, these categories were established. Colors for these microplastics were recorded by direct visual observation, and were labeled under the closest corresponding general color. Additionally, due to not being able to use spectrometry, any object that appeared to be a microplastic was additionally observed under higher magnification up to 100x in order to verify that the observed particle was a microplastic and not an organic compound or any other potential material with consideration of the content in the previously mentioned study (de Carvalho 2021).

RESULTS

A total of 13 ponds and lakes were sampled, with 6 being not impacted by agriculture and 7 not being impacted by agriculture. A total of 71 microplastics were found, with 33 found in agricultural sites and 38 being found in agricultural sites. Of the total 1300 mL of water sampled, this would mean an average of around 1 microplastic for 18 mL of water; 1 for every 18 mL in agricultural sites and 1 for every 18 mL of water in non agricultural sites accordingly. Only two ponds contained no microplastics, those being Kitchell Pond and Lake Kinnelon – all other sites contained microplastics (Fig. 4).

Figure 4: The first microplastic I found – it was a purple line microplastics from Long Pond

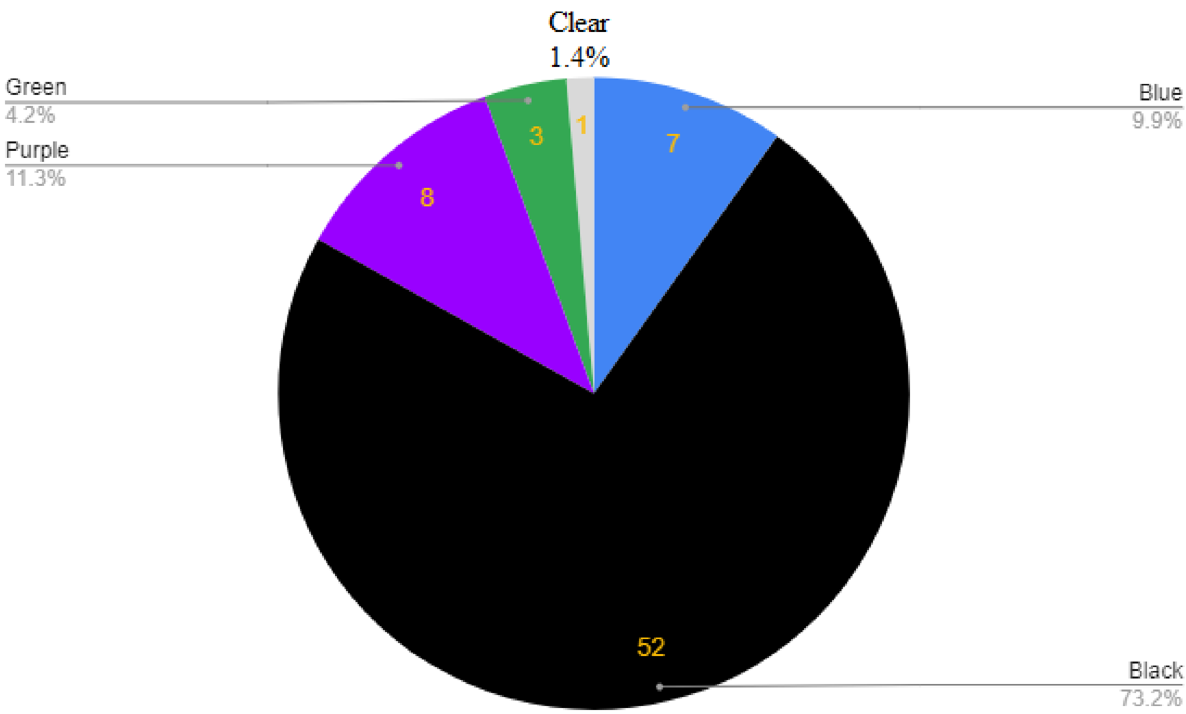


Figure 5: The total quantities of each color microplastic found among all ponds and lakes.

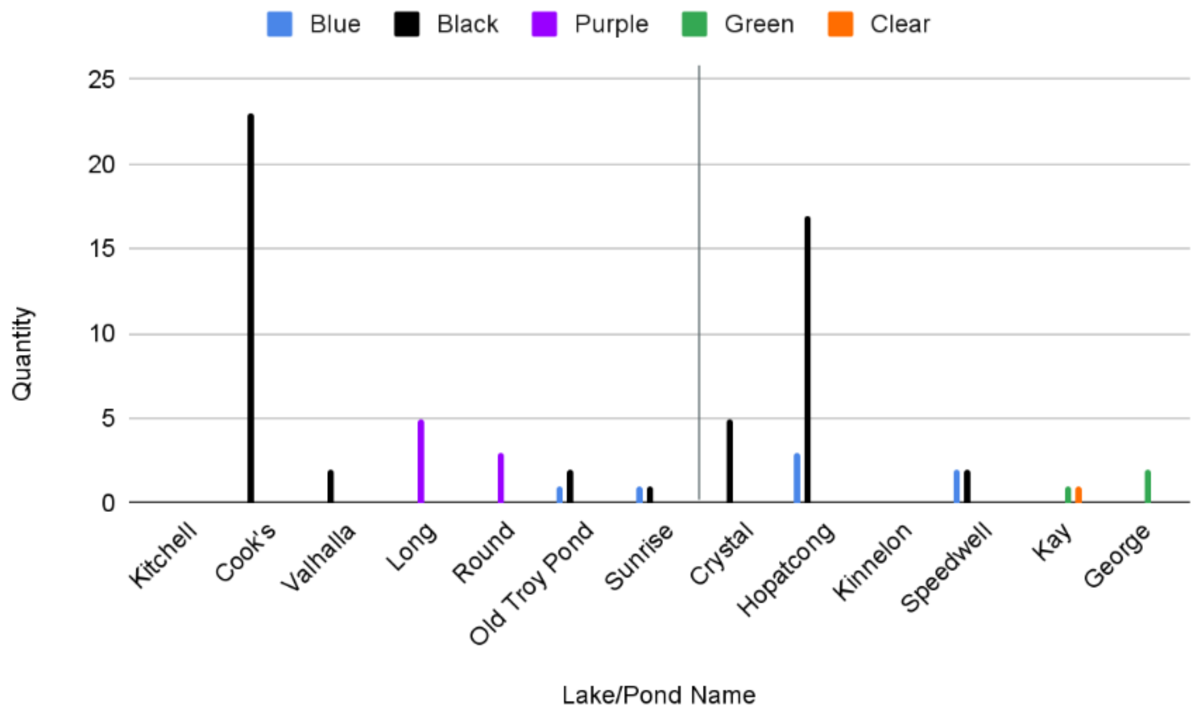


Figure 6: The color and quantity of microplastic in each respective lake and pond. Any body of water right of Sunrise is considered impacted by agriculture.

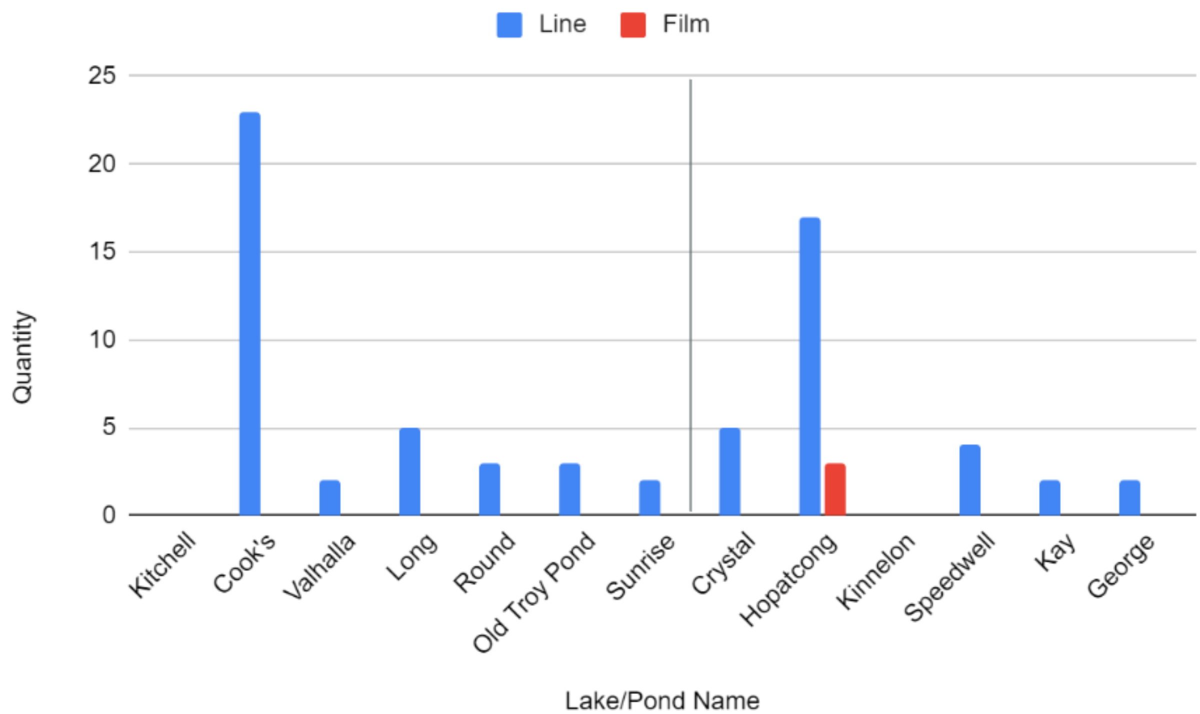


Figure 7: The shape of microplastics found in each respective lake and pond. Any body of water right of Sunrise is considered impacted by agriculture.

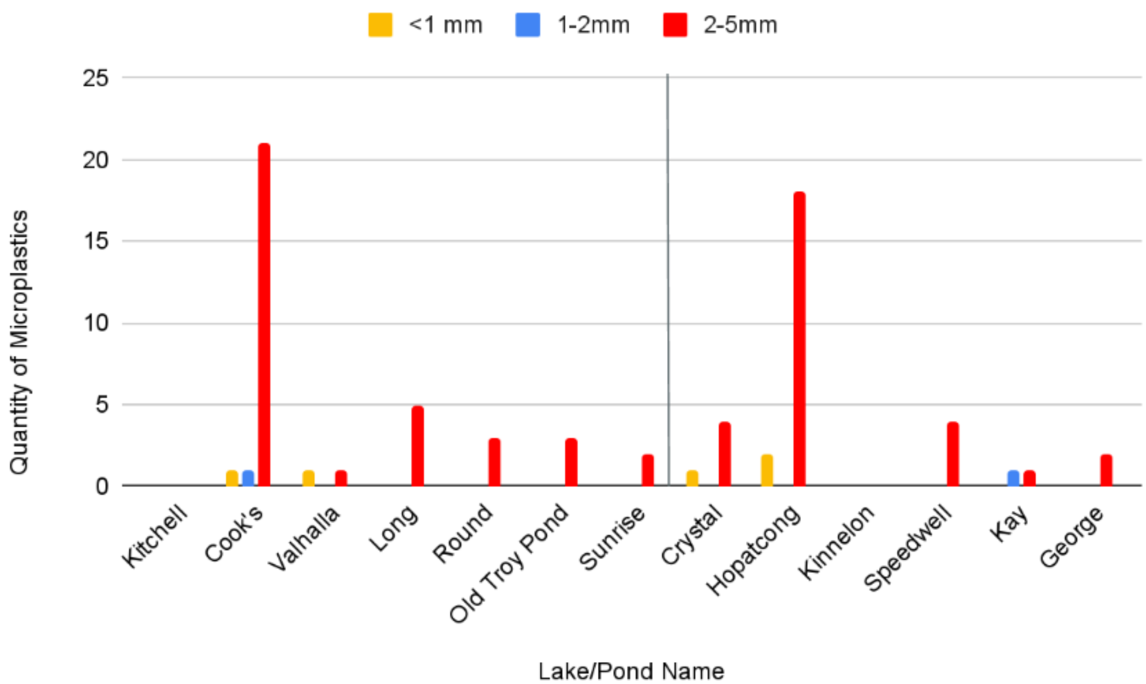


Figure 8: The size of microplastics found in each respective lake and pond. Any body of water right of Sunrise is considered impacted by agriculture.

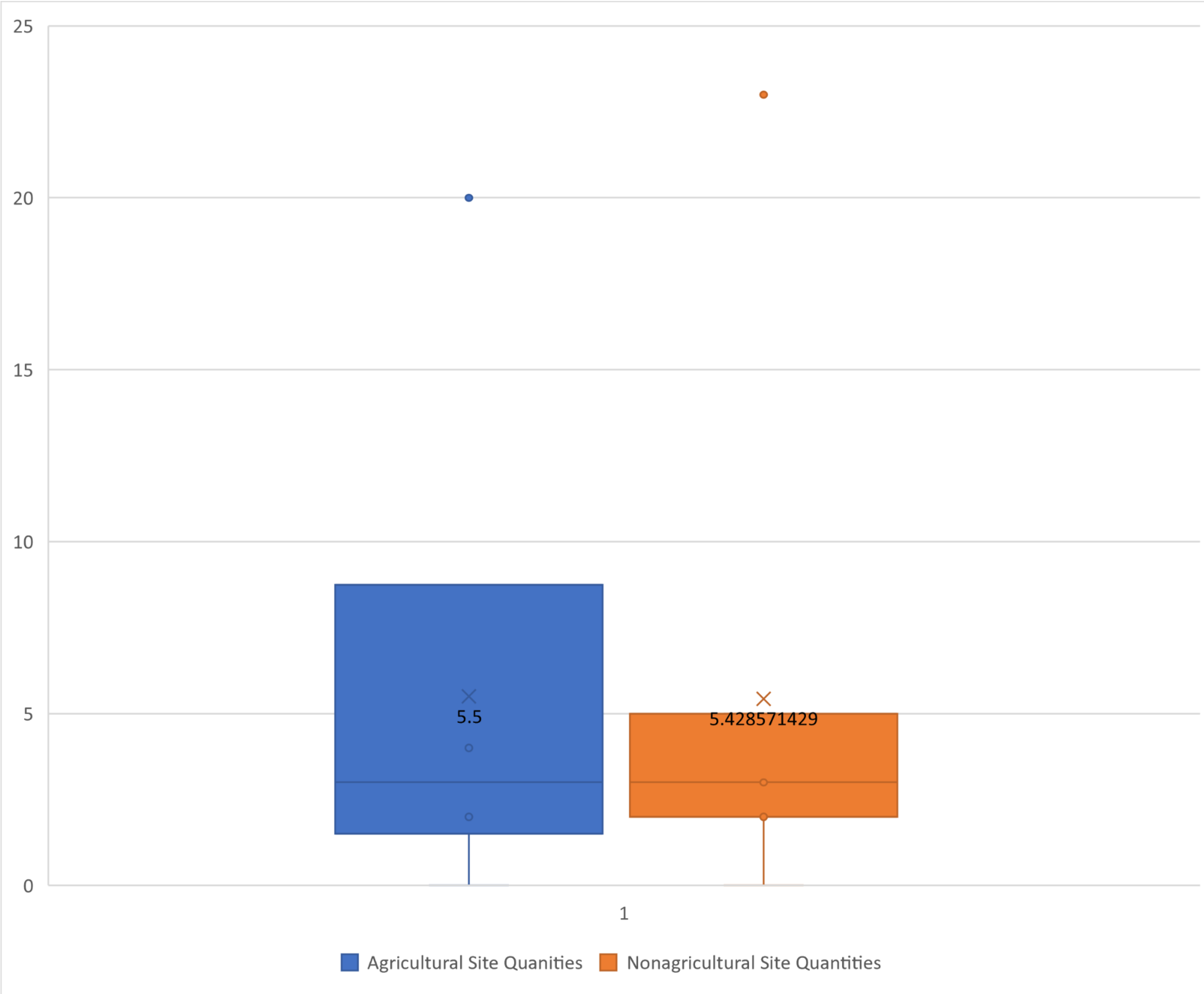


Figure 9: Box Plot depicting the distribution in quantity of microplastic quantities in sites impacted by agriculture and not impacted by agriculture with respective means

In each lake, black colored microplastics tended to be the most common, with fewer instances of colored microplastics and clear microplastics appearing (Fig. 5). Agricultural samples contained fewer purple microplastics than non-agricultural samples, and agricultural samples contained green and clear microplastics whereas non-agricultural samples contained none, however due to the low quantity of each no statistically significant conclusions can be drawn (Fig. 6). Overall, black microplastics, when accounted for all sites together, happened to bear the largest quantity overall, with clear microplastics appearing least frequently (Fig. 6). Microplastics in regards to the five colors observed respectively were not evenly distributed, with there being a significantly higher quantity of black microplastics (Chi-square test: $X^2_4 = 128.08$, $P < 0.001$). There was a statistically significant difference in color between microplastics found in agricultural samples and non agricultural samples – specifically, there was a significant difference in the quantity of blue microplastics between agricultural samples and non agricultural samples, with agricultural samples containing more blue microplastics (Chi-square test: $X^2_4 = 13.08$, $P < 0.05$).

Line shaped microplastics were the most common comprising 95.8% of microplastics collected, with the only other shape appearing being film (Fig. 7). Only one instance of a film shaped microplastic appeared, and it appeared in a lake marked as being impacted by agriculture.

Additionally, most microplastics found were around 2-5 mm in length comprising 90.1% of microplastics collected, with there being substantially fewer microplastics ranging from 1-2 mm to <1 mm (Fig. 8). Accordingly, no statistically significant relationship from this could be drawn from this data. Both treatments in total, between agricultural and nonagricultural sites appeared to have rather large maximum outliers, with both having at least one site that numbered 20 or greater microplastics collected total (Fig. 9). The quantities of microplastics however, outside of these outliers, remained fairly consistent between sites overall in terms of mean, median, upper and lower quartile ranges respectively (Fig. 9).

## DISCUSSION

### *Overview of Data*

My research found that microplastics were ubiquitous – found in 84.6% of the ponds and lakes studied. Many of the conclusions that will be drawn from the results will stem primarily from the color of the microplastics, as these ultimately provided the most variation in data and accordingly allow for more to discuss in terms of statistically significant results. There are many factors that will be discussed in this regard, especially as to why there was such a high presence of black microplastics and as to why there was a higher presence of blue microplastics in agricultural areas compared to non-agricultural areas. The shape of the microplastics collected, while appearing homogenous among one type of shape, that being line, still allows for some insightful discussion as to why most of the microplastics could have been line in the first place. The size of the microplastics, while also generally being favored toward one specific size, still allows for a bigger discussion as to why these microplastics appeared in the size they did and even allows for a potential discussion on the implications of microplastics versus nanoplastics.

### *Black Microplastics*

The gathered data revealed that among all of microplastics collected, when accounting for color, the most common color tended to be black, with 73.2% of collected microplastics being black. Upon my initial examination of the data, I was rather surprised at how common black microplastics were, as they appeared in many of my samples. Incidentally, I thought at first that there must have been some error, that these black microplastics were actually just fragments of twigs or other pond debris, but when I examined these particles under the microscope higher and higher powers, they held no organic properties, so I knew these had to be microplastics and required further study. The prevalence of black microplastics can be for a wide variety of reasons: however, since every black fiber also happened to be lined-shape, this can help to narrow down where these microplastics can potentially be sourced from. Of course however, without the use of mass spectrometry, it isn't entirely possible to say with absolute certainty what these could be. Mass spectrometry is the most accurate method to identify the origins of specific microplastics, and black microplastics are no exception – a paper published in 2021 proposes refined and tested methods in spectrometry in finding the origins of black microplastics specifically (Nogo et. al 2021). This paper also proposes that chemical imaging might be a viable alternative for spectrometry, however despite requiring more research still, spectrometry proves to be the most viable and up to date method of accurate microplastic identification (Nogo et al. 2021). Because of this, I felt it was necessary to address the current methodology of identifying microplastics in larger scale research, though as mentioned previously however, due to technical limitations and the smaller scale of my study, spectrometry wasn't performed. Instead, in order to attempt to identify what these black microplastics may be, where they're potentially sourced from, and why there was such a high presence of them compared to other microplastics, other scientific literature that yielded results and identification of similar microplastics will be referenced. Additionally, a majority of the literature considered will involve freshwater studies, as they will likely yield the most similar possibilities for the origins of these microplastics.

One study conducted in a river in Italy yielded results that found black fibers, and despite the study site being a river, it still provides useful insight into the possible origins of these microplastics (Campanale et al. 2019). They found that black microplastics accounted for 35% of all microplastics recorded, and this article also makes clear that it's possible for microplastics to change color due to degradation over time (Campanale et al. 2019). Additionally, it is mentioned how many lines and fibers can stem from the presence of fishing lines (Campanale et al. 2019).

All of the bodies of water are open to fishing, so this possibility cannot be entirely ruled out. While these are all possible, when considering strictly the implications of agriculture, it is entirely possible that these black microplastics may stem from the plastic mulch, or plastic covers, that are commonly used in farming vegetables (Lwanga et al. 2022). It is difficult to exactly pinpoint the origins of these black microplastics as such, though in this context, it could likely be from these plastic mulches, however I wouldn't completely disregard the possibility of microplastic fragmentation from fishing supplies as well upon reflection of the previous scientific literature.

*Blue Microplastics*

There are several factors as to why there may have been a larger presence of blue microplastics in bodies of water impacted by agriculture compared to bodies of water not impacted by agriculture. The quantity of blue microplastics is potentially the most telling element of the statistical analysis that may suggest there is a significant impact in the presence of microplastics between sites impacted by agriculture and in those not impacted by agriculture. As mentioned before, when considering strictly agriculture, plastic covers and plastic mulches could likely be the reasons for the introduction of these colored microplastics (Lwanga et al. 2022). Despite this however, from what I've observed most plastic mulches are black in color, so the possibility that they aren't strictly from plastic mulches is also possible. I'd be more inclined to come to this conclusion as the statistics would favor this conclusion, though as was the case with black microplastics, I wouldn't completely discount the possibility that they stem from microplastic contamination from fishing equipment. The reason for a difference in color, again, could be due to a degradation in color that occurs naturally over time due to a variety of factors.

(Campanale et al. 2019). This is just a possibility though and might not explain the full picture, the frequency that degradation may occur to this end might not be the most reliable conclusion to explain a potential origin of these microplastics (Campanale et al. 2019). Still, the occurrence of both fibrous black and blue microplastics specifically among other shapes and colors has been seen in other scientific literature (Sarkar et al. 2023).

*Other Colors*

While other color microplastics collected, such as green, purple, and clear, may not have yielded a statistically significant result in terms of difference in their presence in agriculturally affected sites and sites not affected by agriculture, it is still important to discuss what the presence of microplastics of other colors, or lack thereof, could potentially indicate. It’s possible that these plastics may not even stem agriculturally, and that the microplastics may be sourced from dumping of single use plastic products – this would be sensible as all of the sites sampled experience recreational activities to some extent (Mercy 2023). Additionally, as previously mentioned, fishing supplies may be the source of some of these plastics (Campanale et al. 2019). However, due to there being no statistically significant correlations in the data when considering agriculture, it’s difficult to say exactly where these microplastics could have stemmed from strictly agriculturally, though some possibilities could be sources such as plastic pipes, twines, or possibly bale nets to name a few (Lwanga et al. 2022).

*Microplastic Shape*

The most common shape for microplastics was substantially line, with only one instance of film shaped microplastics. It was surprising to me with all the data considered from shape, with almost all of the microplastics being line shaped with the exception from one site having films, it can be inferred that this is because of how these microplastics can stem from fishing products, especially fishing line (Campanale et al. 2019). Due to this, when I initially noticed the abundance of line shaped microplastics in my data, I began to consider that these specific microplastics might not even be traced back to agriculture and can be strictly related to fishing – after all, many of the ponds and lakes I chose to study are also recreational bodies of water (Lwanga et al. 2022). This of course is a large consideration for the potential origins of fibers specifically, however it is also important to consider how these microplastics also may have originated from agriculture as well. While it would be easy to say that this could be a result of microplastic pollution from agricultural twine, there haven't been many studies regarding microplastic pollution specifically with twine, though I cannot completely disregard twine as a potential microplastic contaminant here such as suggested in a study by Lwanga et al. (2022). Without statistical variation, it's difficult to conclude what the source of the microplastics here could be solely based on shape, and color seemed to be a stronger indicator when the data and statistical analysis were considered strictly based on my findings. Still, fishing line being the source of microplastics as well can't be completely disregarded as these sites see recreational activities like fishing (Campanale et al. 2019).

*Microplastic Size*

The most common size of microplastics that was collected ranged from 2-5 mm total accounting for 90% of all collected microplastics, with any amount under 2 mm generally appearing less frequently with 1-2 mm microplastics comprising 7% of microplastics and <1 mm microplastics comprising around 3%. There are a variety of reasons as to why this could be.

Firstly, it's important to reiterate where microplastics can be sourced from; particularly with primary and secondary microplastics, with primary microplastics being introduced into the environment as they are in their current size, being less than 5 mm, and secondary microplastics that enter the environment through fragmentation of a macroplastic (Frias & Nash 2019). With this considered however, there wasn't enough of a statistical connection between size and variation between agricultural and nonagricultural sites, and therefore a strong conclusion based on size cannot be made based on this. Generally, the size of microplastics seems to be used more for categorization rather than specific identification (de Carvalho 2021). Regardless, I still felt it was important to take note of the lengths, as if something statistically significant did occur, it would have been important in finding a potential source of these microplastics, however solely based on this it wouldn't be feasible to do so.

Given the specific trends presented in the collected data, it's reasonable to conclude that in these bodies of water, agriculture as a whole might be playing a role in introducing microplastics into them on a surface level. Given what is written in other studies investigating the impact of agriculture in introducing microplastics into the environment, the data collected here don't align with other studies that would display such a trend such as in Campanale et al. (2019), though studies like what is seen in Mercy et al. (2023) would also suggest similar results as to what I found. Microplastics from other agricultural sources such as nets, twine, pipes, and other indirect sources such as compost don't appear in the data either to any degree to make an effective conclusion on whether microplastics from these sources have been introduced to the water bodies I studied, though I can't completely discount their influence in microplastic introduction as they do appear in agriculture (Lwanga et al. 2022). It would appear more likely however that plastic mulches play a larger role in introducing the black lined microplastics I found (Lwanga et al. 2022).

Of course, these findings in terms of ubiquity are certainly concerning based on factors such as bioaccumulation, potential to spread bacteria, and implications on human health (Rodrigues et al. 2019, Jaafar 2021, Al Mamun 2023)

*Future Work and Suggested Changes*

I feel the impact of agriculture and its introduction of microplastics into the environment overall is a relatively well researched field, and when considering the impact on freshwater bodies, research also tends to be plentiful. However, as I mentioned before, there haven't been any long term studies on any freshwater bodies in New Jersey specifically. There are definitely surveys to assess water quality, for example such as those performed by the Great Swamp Watershed Association, though no attempt to differentiate to what degree agriculture contributes to the introduction of microplastics in freshwater bodies in New Jersey and to what extent the impact seems to have been made. Given how recent microplastics studies overall have been however, this is something that could potentially prove to be challenging, as there are no historical accounts of microplastic records over time within the past decades necessarily, especially with agriculture in mind. This isn't to say that surveys aren't being conducted and that they aren't useful to uncover the presence of microplastics in New Jersey's freshwater bodies overall – the Great Swamp Watershed Association recently employed methods to survey microplastics in rivers and streams for example (https://www.greatswamp.org/) – but a larger scale study would be required in order to truly see the extent of agriculture's impact on the introduction of microplastics overall. From my results, I was only able to conclude that the only statistically significant difference between ponds and lakes impacted and not impacted by agriculture was that there tended to be slightly more blue microplastics overall in ponds and lakes potentially impacted by agriculture. It is important to mention however that I ended up only being able to have access to a smaller sample size, and that the study was conducted in northern New Jersey which in general doesn't see a significant amount of agricultural activity as compared to other parts of the state. Additionally, it's entirely possible that the sites I chose weren't as impacted by agriculture as others and vice versa; I feel that having a broader study to determine a numerical and empirical relation of agricultural impact in ponds and lakes would be necessary in order to gather more accurate data. Afterall, the sites I chose were based on general geographic proximity to sites that potentially see agricultural activity, and it's entirely possible that during the course of my study these sites implemented more environmentally friendly practices or have already had them in the first place without me having knowledge of it. It would have also been better to use GPS technology in order to better identify proximities to agricultural sources – this would specifically be useful to identify the contributions of sheds and percent area of agricultural land contributing to ponds and lakes.

By extension, the methodology could have been improved, and there are several ways to potentially improve it in this study. As I mentioned before, performing certain methods proved to be either too logistically challenging or I simply didn't have access to certain ways of collecting data. The biggest element that would have helped me pinpoint exactly what data I had collected and what it could mean would be access to mass spectrometry. Mass spectrometry could have additionally been used in order to determine exactly what type of plastics were being observed, and on a larger scale study, this would likely be more feasible to do (Prata et al. 2019). Another way that the collection specifically could have yielded more data is if a manta trawl was used rather than filter paper. Manta trawls would have allowed for more water to flow through and therefore yielded a greater quantity of microplastics, however other factors would need to be included such as the amount of time a trawl is in the water, the distance covered, and how wide the head of the trawl is (Prata et al. 2019). For the scale of this specific study, however, I think simply using filter paper, especially given how small the pores are in the Whatman filter paper, proved to be sufficient in filtering out microplastics, though going through a first initial filter like a manta trawl could prove to yield better results and I felt it was important to mention. For a smaller scale project like this one however, I think the use of Whatman paper was sufficient.

Performing this study at a different time of year could help alleviate the logistical challenges that come with collecting data. While collecting from ponds and lakes wasn't as challenging and at most required the use of waders in order to get to the water source, collecting in rivers proved to be much more challenging in colder weather. Setting up a trawl, calculating the flow of a stream or river, and maintaining the trawl over an extended period of time in the cold weather in the late fall and early winter proved to be too challenging for myself alone to perform. Collection would be significantly less challenging during spring or summer. Unfortunately, the consideration to look for rivers as well was something not considered until after collection of the ponds and lakes data. More lakes and ponds could have been sampled to produce a greater sample size; however, as most lakes in Morris County are privately owned, obtaining access for most of them was not alway possible. Rivers and streams however are generally accessible to the public in most parts, and gaining access to them is far less challenging by extension, so the incorporation of rivers would be interesting.

It's possible more water could have also been sampled, though given the size of the filter paper being used, it would have proven challenging for this study and given the scale of microplastics as a whole, so sampling 100 mL of water per each site seemed to be a fine medium. Inherently, measuring more water would yield a greater quantity of microplastics and in theory would allow for larger sample sizes strictly due to more water being sampled and would therefore partially alleviate the need for sampling more sites; however, it's important to consider that it's possible for the Whatman paper to tear the more it is used and if too much pressure is applied to it over time. I think beyond strictly looking at water quality, it would also be interesting to observe how microplastics bioaccumulate in New Jersey's freshwater bodies and to see how agriculture may impact this. Studies like the one performed by Parvin et al. (2021) is an example of a study of microplastic bioaccumulation in fish in freshwater bodies in Bangladesh that includes a categorization of microplastics based on shape and color. Of course, this study took place in Bangladesh, and the results in New Jersey may be different than expected based on that study. I mentioned that I initially proposed to perform gut tract samples in order to see the presence of microplastics in fish populations, though it would have proven far too logistically challenging to do for the purposes of the scale of this study. I think on a larger scale it would be interesting to see the presence of microplastics in different fish species at different trophic levels in New Jersey, and it would also be interesting to see the presence of microplastics on aquatic flora as well. Bioaccumulation of microplastics I feel is something that is relatively well researched and there is plenty of scholarly literature regarding it, and I feel the addition to the study of New Jersey's aquatic wildlife would help to further microplastic research overall.

*Future of Microplastics*

The discussion of microplastics in the environment in general raises a question; what are the long term solutions to removing them? While an absolute universal and long term solution hasn't been agreed upon, there have been many strides made in an attempt to find methods of removing microplastics from the environment. In recent years, there have been many scholarly publications on such potential ways. One interesting study tested the use of sand filtration in order to filter out microplastics from drinking water (Na et al. 2021). This particular study was actually successful in removing microplastics over 45 micrometers from drinking water, however microplastics that were under 20 micrometers were not only unable to be filtered, but also caused contamination to the experimented water through UV oxidation (Na et al. 2021). In this regard, a lot of microplastic treatments are still experimental. Other methods however have proven to yield a bit more success. Methods such as absorption of microplastics by green algae, chemical removal, and bacterial removal for example have all yielded some form of success to this end, however these methods aren't completely effective yet and some of them require more research in order to be implemented at a higher level (Padervand 2020). The use of enzymes seems to be the direction that eliminating microplastics in the environment is heading towards as of recent – a study done by Lu et al. (2022) found that AI could be used to engineers an enzyme that could effectively degrade Polyethylene terephthalate (PET). At the end of the day however, the best way to effectively reduce microplastic presence in the environment overall is to simply reduce the amount of plastic waste that is produced, though with the increase of plastic production over the years, it's not entirely likely that this will be the case (Padervand 2020).

Though the topic of microplastics as a whole may seem obscure, confusing, and bleak, I'm still overall hopeful that with the increase of microplastic related studies that we will be able to fully understand the extent of their harm and eventually find clear cut methods to remove them from the environment.

# CONCLUSION

To conclude, the major findings from this study is that a significant portion of the microplastics found collectively were black in color, and there was a statistically significant difference in blue colored microplastics between non agricultural and agricultural sites, with non agricultural sites containing more blue microplastics. The size and shape of microplastics did not seem to be statistically significant between sites, though it is important to note that a majority of the microplastics found were greater than 2 millimeters and less than 5 millimeters, and all except one microplastic found were line shaped. It is entirely likely that many of these microplastics, strictly speaking in terms of agricultural impact, stem from the use of plastic mulches. What stood out the most however were how the presence of microplastics was almost ubiquitous throughout each site. These findings, in terms of color, have appeared to align with some other scientific literature, though further research would definitely provide more insight as to why the data collected could be. While the full impacts of microplastics are still not yet fully understood, it is important that more research is done in this area in order to fully understand the extent to their impact as a whole.

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