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Gray Seal (Halichoerus Grypus) Diet and Microplastic Ingestion on Great Point, Nantucket

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GRAY SEAL (*HALICHOERUS GRYPUS*) DIET AND MICROPLASTIC INGESTION ON GREAT POINT, NANTUCKET

A Thesis Presented

by

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ABSTRACT

GRAY SEAL (*HALICHOERUS GRYPUS*) DIET AND MICROPLASTIC INGESTION ON GREAT POINT, NANTUCKET

August 2024

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Due to their semi-aquatic lifestyle, pinnipeds are an ideal sentinel group used to study anthropogenic threats to the marine environment. Microplastics, primarily transported to the ocean through river discharge or weathering of larger plastics, are a threat to both pinnipeds and humans. Bioaccumulation of microplastics within the marine food web has been observed, with pinnipeds indirectly ingesting microplastics through their prey. As generalists, gray seals (*Halichoerus grypus*) are a pinniped species that can provide information on microplastic exposure to many lower trophic level organisms. This thesis explores the relationship between the diet and microplastic ingestion of gray seals on Great Point, Nantucket. Firstly, gray seal diet was assessed using two methods, prey hard parts and DNA metabarcoding, from 112 scat samples. Our results support previous findings that DNA metabarcoding reduces the biases of prey hard parts, identifying more prey types in more samples. We then compared the DNA metabarcoding diet results to microplastic concentration, type, fiber color, and polymer type. Anthropogenic microparticles were found

in 111 out of 112 gray seal scat samples. Our findings suggested weak relationships between the microplastic variables and diet, however our methods were not able to determine the abundance of each prey type, making it difficult to draw any real conclusions. More research using quantitative methods is needed to determine whether gray seals' diet is influencing their microplastic consumption. Given their role as a sentinel species, gray seals, along with other pinnipeds, offer valuable insights into the distribution and impact of microplastics throughout their range. Future research should continue to utilize pinnipeds as indicators to further investigate microplastic pollution in marine ecosystems.

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CHAPTER 1

PINNIPEDS AS A LINK BETWEEN THE LAND AND THE OCEAN

1.1 Abstract

In the present day, where human activities are threatening coastal regions, the necessity of studying their impacts on the marine environment is pertinent. Pinnipeds are an ideal sentinel group for investigating this relationship due to their semi-aquatic lifestyle. Being high trophic level organisms, they are also particularly vulnerable to anthropogenic pollution. Their exposure to chemical and physical pollution highlights the pathway of the pollutants from the terrestrial to marine ecosystems. Climate change causes additional challenges, impacting pinniped habitats, prey availability, and disease dynamics. As sea surface temperatures rise, the distribution of pinniped prey is shifting, affecting food availability and quality. Concurrently, the frequency and intensity of severe weather events erodes coastlines, reducing haul-out spaces for pinnipeds and increasing human-wildlife interactions. Increases in sea surface temperature and decreases in pinniped haul-out space are positively linked to the spread of infectious diseases. Pinnipeds and humans are also experiencing competition for resources such as land and economically important prey species. This chapter adopts a "one health" perspective, recognizing the interconnectedness between human, animal, and ecosystem health. Through pinnipeds, changes in both

terrestrial and marine ecosystems, and threats to overall ecosystem health, can be monitored. Understanding these interactions is crucial for effective conservation and management strategies in the face of ongoing environmental changes.

1.2 Introduction

Pinnipeds (*e.g.* seals, sea lions, and walruses) live both on land and in the ocean (Berta et al., 2015). Some species, like harbor seals (*Phoca vitulina*), will often haul-out on land just to rest (NOAA Fisheries, 2022a). Others, like elephant seals (*Mirounga sp.*), spend much of their time in the water and primarily haul-out during specific life history events (Berta et al., 2015). All pinnipeds use land or ice to rest, breed, pup, and molt, and they forage in the ocean (Berta et al., 2015). This semi-aquatic lifestyle makes pinnipeds an ideal sentinel for monitoring ecosystem and human well-being and resiliency.

Forty percent of the global human population lives within 100 km of the coast (Division for Sustainable Development, 2007). Highly populated coastal regions provide significant economic value due to tourism and their accessibility for transportation and trade (Borja et al., 2020). These areas are also threatened by climate change, storms, and other natural disasters (Borja et al., 2020). They are dynamic regions, impacted by natural and human-derived processes, and activities originating both from the ocean and the land. Thus, species that move between these two realms play an increasingly important role in linking stressors and providing warning for threats between terrestrial and marine systems. In this age of climate change and global human activity, these regions are at increased risk to sea-level rise, habitat loss, pollution, and disease outbreaks. (Berta et al., 2015; Borja et al., 2020; Calvin et al., 2023; Nehasil, 2010).

Along with the exponential growth of the human population comes increases in pollution. Pinnipeds are often exposed to anthropogenic chemical and physical pollution in the marine environment that originates on land. Trace elements, persistent organic pollutants (POPs) and plastic can all enter the ocean through river and wastewater discharge (Walker et al., 2012). As high trophic level organisms, pinnipeds are at risk of significant exposure to these contaminants through bioaccumulation and biomagnification within the marine food web (Berta et al., 2015). They can also become entangled in larger marine debris, like fishing ropes, nets, and line (Henderson, 2001; Raum-Suryan et al., 2009).

Human activity has also increased the amount of greenhouse gasses in the atmosphere, resulting in a 1.07°C increase in global surface temperature from 1850-1900 to 2010-2019 (Calvin et al., 2023). As the mean sea surface temperature (SST) rises, the distribution and abundance of pinniped prey is predicted to shift, causing a decrease in food availability (Baker et al., 2020; McHuron et al., 2023). Furthermore, this increase in SST is positively correlated with the number of infectious disease outbreaks (Calvin et al., 2023; Sanderson & Alexander, 2020). Another effect of climate change is the increasing frequency and intensity of severe weather events, which can erode beaches and coastlines, shrinking pinniped habitat (Nehasil, 2010; Theuerkauf et al., 2014).

As climate change and pollution continue to threaten the earth, pinnipeds and humans will experience similar challenges and may have to compete for resources. Pinnipeds often consume prey from commercially valuable fisheries, such as Atlantic cod and salmon (Ampela, 2009; Weise & Harvey, 2008). Many chemical and physical pollutants are transported up the food web resulting in negative health effects to higher trophic level organisms, like pinnipeds and humans (Andrady, 2011; Berta et al., 2015; Frouin et al., 2010;

Kakuschke et al., 2005, 2011; Mos et al., 2006; Nelms, Barnett, et al., 2019; Rotjan et al., 2019; Soulen et al., 2022; Walker et al., 2012). Both groups may also experience a decrease in food quality and availability as prey abundance and distribution changes. Fisheries are economically and culturally important, with 37.9 million people employed in the global fisheries primary sector (FAO, 2022). Range shifts, primary productivity changes, and divergence in the timing of important biological events due to climate change are expected to decrease global fishing revenue by 35% by the 2050s under high CO_2 emission scenarios (Lam et al., 2016). These changes in fish distribution and biology will also negatively affect the feeding ecology of pinnipeds (Baker et al., 2020; Florko et al., 2021; Kauhala et al., 2017; McHuron et al., 2023). Competition between humans and pinnipeds may arise as fisheries significant to both groups experience these shifts.

Another resource that is important to both pinnipeds and humans is land. Coastal erosion and sea level rise will result in less space for pinnipeds to haul-out, and governments will have to decide whether to designate these haul-out areas for pinniped or human use (Nehasil, 2010). Smaller coasts could also increase human interaction with these animals. Pinnipeds carry zoonotic diseases (e.g. avian influenza), which can be transmitted to humans (Hunt et al., 2008). As the earth continues to warm, not only will infectious disease outbreaks occur more frequently, but transmission of zoonotic diseases may increase due to more frequent contact (Hendrik et al., 2023).

This review will focus on the interactions between terrestrial and marine ecosystems through the study of pinnipeds. Since the first industrial revolution, the relationship between the land and the ocean has become more stressed (NOAA Education, 2020). Additionally, the "one health" perspective intertwines the well-being of humans, animals, and the ecosystem

(Wilcox & Aguirre, 2004). Pinnipeds are an exemplary group for studying this relationship since they serve as link between the land and the ocean. By studying pinnipeds, we can detect changes in both ecosystems, observe resiliency, and monitor threats to the health of humans, animals, and the ecosystem.

1.3 Infectious disease

Human health and the oceans have been linked for centuries and will continue to be in the future (Borja et al., 2020; Wheeler et al., 2014). Humans have visited and used the ocean as a form of medicine for centuries (Wheeler et al., 2014). “Sea bathing” was popularized in Europe in the 1800s, with the belief that sea water has healing properties (Wheeler et al., 2014). In the present day, increased dumping of chemical and physical waste is negatively impacting the health of both humans and the ocean (Borja et al., 2020). Additionally, increased anthropogenic greenhouse gas emissions are causing the ocean to warm, which has been linked to the spread of infectious diseases (Calvin et al., 2023; Sanderson & Alexander, 2020). Marine mammals are known for being sentinels for ocean and human health because they are susceptible to many of the same threats that humans face, including infectious disease (Simeone et al., 2015).

Infectious diseases are illnesses caused by bacteria, viruses, and fungi (NCEZID, 2023). Two infectious diseases, avian influenza and phocine distemper virus (PDV), have caused unusual mortality events (UMEs) in pinnipeds in the northwest and northeast Atlantic (Table 1.1) (Anthony et al., 2012; Bodewes et al., 2013; Puryear et al., 2021, 2023; Puryear et al., 2016; van den Brand et al., 2016). PDV has also spread to Pacific pinniped populations, however it has not caused any UMEs in the area as of yet (Duignan et al., 1997).

A UME is “a stranding event that is unexpected, involves a significant die-off of any marine mammal population, and demands immediate response” as defined by the Marine Mammal Protection Act (MMPA) (Marine Mammal Protection Act of 1972, 2022). While the term UME is only used for marine mammals strandings in the United States, there have been significant avian influenza and PDV outbreaks in Europe as well (Bodewes et al., 2013; Bodewes, Rubio García, et al., 2015).

1.3.1 Influenza A

Avian influenza is a zoonotic disease, meaning that it can spread between animals and humans (NCEZID, 2021). The transmission of a disease from one species to another is called a zoonotic spillover (Ellwanger & Chies, 2021). Multiple avian influenza A virus strains have spilled over into harbor and gray seal populations from wild birds. In the northwest Atlantic, H3N8 and H5N1 caused UMEs in harbor seals in 2011 (n = 162) and 2022 (n = 164), respectively (Anthony et al., 2012; Puryear et al., 2023). Both strains were found to have amino acids changes that allowed them to infect mammals (Anthony et al., 2012; Puryear et al., 2023). A 2014 outbreak of H10N7 also fatally infected thousands of harbor seals in Sweden (n = 425), Denmark (n = 152), and Germany (n = 1400) (Bodewes, Bestebroer, et al., 2015; Krog et al., 2015; van den Brand et al., 2016; Zohari et al., 2014).

While harbor seals have experienced the more significant impact of these spillovers, gray seals have been found with antibodies for many influenza A strains. In the Netherlands, 26% of adult gray seals had antibodies from the H10N7 outbreak (Bodewes, Rubio García, et al., 2015). Fifty percent of (sub)adult and 19.3% of weanling gray seals sampled from 2013 to 2015 in Cape Cod, MA and Nova Scotia, Canada had antibodies from a combination of strains (Puryear et al., 2016). Gray seals are thought to be a reservoir for influenza A since

they can carry the virus but may not display symptoms during infection (Puryear et al., 2016).

Van de Brand et al. (2016) were able to experimentally infect ferrets with H10N7. These ferrets experienced respiratory tract inflammation, secondary bacterial infection, and fatal pneumonia, similar to harbor seals (van den Brand et al., 2016). The ability of this virus to infect terrestrial mammals is a warning sign for its zoonotic potential (van den Brand et al., 2016). Additionally, there have been over 800 cases of the H5N1 strain of avian influenza in humans since 2003 (World Health Organization, 2024). Continued monitoring of influenza A in seals is important for preparing for prospective future pandemics.

1.3.2 Phocine Distemper Virus (PDV)

There are several different types of Morbilliviruses, for example PDV, measles virus, canine distemper virus (CDV), and cetacean morbillivirus (CeMV) (Jo et al., 2018). CDV has previously infected both dogs and pinnipeds, but it is unknown whether PDV can be transmitted to dogs (Northeast Fisheries Science Center, 2022). While PDV is not zoonotic, it is an infectious disease of concern and outbreaks in pinnipeds can provide information on the overall health of the ocean.

There have been multiple PDV epidemics in both the northwest and northeast Atlantic harbor seal populations. Outbreaks in Europe occurred in 1988 and 2002, and UMEs were declared in the United States in 2006 and 2018 (Bodewes et al., 2013; Puryear et al., 2021). Harp seals (*Pagophilus groenlandicus*) were a suspected reservoir species for PDV prior to the 1988 outbreak (Duignan et al., 1997). Eighty-three percent of harp seals tested in the Gulf of St. Lawrence, Canada between 1988 and 1993, and 30% tested in eastern Greenland from 1985-1986, were seropositive, indicating that this species has been exposed

to PDV since at least 1985 (Dietz et al., 1989; Duignan et al., 1997). With their large population and high-density aggregations, disease transmission can occur easily in this group (Duignan et al., 1997; NOAA Fisheries, 2022b). A harp seal mass migration from the Barent's Sea to the North Sea was suspected to have led to the 1988 harbor seal outbreak (Dietz et al., 1989; Duignan et al., 1997). Since harp seals disperse as juveniles and can transmit diseases to other Arctic pinnipeds, it is plausible that harp seals in the Barent's Sea also carried PDV and were the source of this outbreak (Duignan et al., 1997).

The distribution of harp seals ranges from the northwest Atlantic Ocean to the Barent's Sea (Figure 1.1) (NOAA Fisheries, 2022b). Since their distribution spans the entire width of the Atlantic Ocean, it is likely that they also introduced PDV to the northwest Atlantic harbor seal population before the 2006 UME (Puryear et al., 2021). Other arctic seals, including ringed (*Pusa hispida*), hooded (*Cystophora cristata*), bearded (*Erignathus barbatus*), and spotted seals (*Phoca largha*) have been infected with PDV (Duignan et al., 1997; VanWormer et al., 2019). A 2002 Arctic Sea ice minimum connecting the eastern Atlantic Ocean with the Pacific Ocean was positively correlated with PDV exposure in Pacific pinnipeds (Figure 1.1) (VanWormer et al., 2019). Steller sea lions (*Eumetopias jubatus*) and northern fur seals (*Callorhinus ursinus*) in the Pacific reached a peak in PDV exposure in 2009 (VanWormer et al., 2019).

In addition to influenza A, gray seals may be a reservoir species for PDV. Reverse transcription-polymerase chain reaction (RT-PCR) is a common test for detecting a virus in an individual. In the 2018 PDV outbreak, 73.5% of harbor seals and 35.7% of gray seals tested were RT-PCR positive for PDV (Puryear et al., 2021). Between 2016 and 2020, 9.78% to 21.88% of gray seal pups sampled in the Gulf of Maine were RT-PCR positive but did not

show any clinical symptoms of PDV (Puryear et al., 2021). While harbor seals have been found with PDV antibodies up to a decade after an outbreak, the proportion of immune individuals decreases over time, leading to increased susceptibility (Bodewes et al., 2013). PDV can also circulate within pinniped populations at low numbers after outbreaks (Puryear et al., 2021). Increased temperatures are expected to increase the frequency of infectious disease outbreaks (Calvin et al., 2023). Coupled with habitat loss and increased interspecies interactions due to climate change, the spread of viruses like PDV and influenza A is likely to grow in the future.

1.4 Climate Change

1.4.1 Coastal erosion

As climate change continues, pinnipeds are losing their terrestrial habitats due to sea level rise, erosion, and ice melt. Sea level anomalies are a major consequence of climate change, and it contributes to coastal erosion just as much or even more than severe storms (e.g. hurricanes) (Theuerkauf et al., 2014). Under the IPCC A2 scenario, the sea level in California is projected to rise 1.4 meters by 2100 (DR et al., 2009). Nehasil (2010) found that 99% of California sea lion (*Zalophus californianus*), northern elephant seal (*Mirounga angustirostris*), and Pacific harbor seal haul-outs in central and southern California will be affected by this 1.4 m increase. Pinnipeds often choose haul-out locations that are close to high quality foraging areas and that are suitable for breeding. If they are displaced due to eroding coastlines, their health and survival will likely decline.

The Northwestern Hawaiian Islands (NWHI) includes French Frigate Shoals (FFS), where Hawaiian monk seals (*Monachus schauinslandi*) pup. Three FFS islands (Whaleskate,

Trig, and East Islands) that this population uses for pupping have been eroding and are now almost fully submerged due to sea level rise and storms (Baker et al., 2020). This has severely decreased pup survival and the Hawaiian monk seal birth rate overall (Baker et al., 2020). The erosion of these islands is providing Galapagos sharks (*Carcharhinus galapagensis*) with easy access to pups, resulting in more predation events (Baker et al., 2020). Out of the 8 NWHI, FFS has the third lowest amount of land area, but has the greatest abundance of Hawaiian monk seals (Baker et al., 2020). While the amount of land available is decreasing in FFS, these seals are continuing to return because it allows them to access 31% of all NWHI foraging area (Baker et al., 2020). As the FFS islands disappear, access to this foraging area will decrease. Food availability is important for pinniped health, and changes in foraging may result in more strandings (SWFSC, 2016).

Strandings of harbor, gray, and harp seals in Maine frequently involved human interaction in high population and tourist areas (Haverkamp et al., 2023; Newcomb et al., 2021). This was especially true for harbor seals who pup during tourist season (May and June) (Newcomb et al., 2021). The most common type of human interaction that pinnipeds in Maine experience is harassment, including human approach, displacement, and unauthorized collection (Newcomb et al., 2021). Harassment from humans can alter pinniped behavior, leading to maternal pup abandonment and increased stress levels in these animals (Newcomb et al., 2021).

Increased interactions with pinnipeds can be negative for humans too, as they can contract zoonotic diseases from pinnipeds. Marine mammal workers have attributed bacterial infections and other illnesses to direct marine mammal contact (Hunt et al., 2008). As haul-out space becomes limited, pinnipeds may haul-out in denser groups, resulting in increased

disease transmission. Zoonotic spillover events are more likely to occur when species are living in high density groups near each other (Ellwanger & Chies, 2021). Gray seals are typically a reservoir species for infectious diseases, and they have been seen to haul-out in denser groups than harbor seals who are more susceptible to diseases (Hoekendijk et al., 2023).

Additionally, humans will have to compete with pinnipeds for space and other resources. This has already been seen at Children's Pool in La Jolla, California and Great Point, Nantucket (Nehasil, 2010). Use of beach space by pinnipeds at Children's Pool demonstrates the need for policy makers to adapt to changing coastlines to determine whether an area should be designated for pinniped or human use. Competition between recreational fishermen and gray seals for fish near Great Point has also caused tension, resulting in the formation of the Seal Abatement Coalition (SAC). This group created a petition with the goal of amending the MMPA to allow for the disturbance of these seals (Seal Abatement Coalition (SAC), 2012). The petition has since been closed and no changes were made to the MMPA, however it is not unlikely that competition for resources will cause more groups like the SAC to form in the future.

1.4.2 Sea ice

Arctic pinnipeds rely on sea ice for pupping, nursing, and resting. As climate change worsens and ice availability decreases, bearded and Pacific harbor seal movements have changed (Boye et al., 2020; Cameron et al., 2018; Womble et al., 2021). Young bearded seals in the Bering Sea prefer habitat that is close to the ice edge (Cameron et al., 2018). As the ice retreats, seasonally and due to climate change, these seals move with the ice edge (Boye et al., 2020; Cameron et al., 2018). Pacific harbor seals in Glacier Bay National Park, Alaska

rely on icebergs calved by tidewater glaciers for pupping and molting (Womble et al., 2021). During the pupping season in June when there is greater ice cover, the seals are more spread out than during the molting season in August (Womble et al., 2021). Additionally, in years with greater ice cover there was a larger abundance of harbor seals in the area (Womble et al., 2021). With decreasing ice cover, Arctic pinnipeds will have to compete with each other for this space, or they may opt to haul-out on land (Stenson & Hammill, 2014).

Decreasing sea ice has also been linked to an increase in shipping activity in the Arctic (Druckenmiller et al., 2022). The impacts of increased icebreaker activity in the Caspian sea include mother-pup separation, breeding site collapse, and ship strikes on Caspian seals (*Pusa caspica*) (Wilson et al., 2017). In 2006, 9.6% and 1.4 to 1.9% of Caspian and harp seal pups, respectively, had potential exposure to collision risk (Wilson et al., 2020). Shipping activity along the Northern Sea Route (NSR) has shifted in recent years. NSR activity was previously highest from July to November, however an increase in usage from December to May has been observed, now overlapping with Arctic pinniped pupping season (Humpert, 2019; Wilson et al., 2020). Walruses (*Odobenus rosmarus*) and harp, ringed, gray, ribbon (*Histriophoca fasciata*), spotted, and bearded seals breed in areas with moderate to frequent shipping activity, putting them at risk of collisions and other negative effects from ships (Wilson et al., 2020).

As less ice and more land becomes available to ice-obligate pinnipeds, their feeding and reproductive success has declined (Jay et al., 2017; Jüssi et al., 2008; Stenson & Hammill, 2014). The Pacific walrus commonly hauls out on ice to rest between foraging trips (Jay et al., 2017). Models showed that walruses spend more time foraging when sea ice is available, in contrast to when only land is available (Jay et al., 2017). Gray seals in the Baltic

Sea and harp seals in the Gulf of St Lawrence also have lower reproductive fitness when less ice is available (Jüssi et al., 2008; Stenson & Hammill, 2014). Gray seal pups that are born on land have higher mortality and poorer body conditions than those born on ice (Jüssi et al., 2008). Harp seals have not been found to pup on land, however they will give birth on extremely thin ice, resulting in high pup mortality (Stenson & Hammill, 2014). Antarctic seals utilize underwater vocalizations to initiate breeding (Roca et al., 2023). Negative sea ice anomalies are correlated with a decrease in the vocalizations, indicating that a decrease in ice cover may negatively impact reproductive success (Roca et al., 2023). Sea ice is essential for ice-obligate pinniped health, and its decline will likely result in higher mortality, lower reproductive success, and behavioral changes.

1.4.3 Prey shifts

Along with rising temperatures, many marine organisms are expected to shift their geographic distributions in order to stay within suitable temperature ranges. As generalist feeders, information on the status of many lower trophic level fish can be obtained from studying pinniped diet. Many pinnipeds also feed on commercially important species. This is especially true for California sea lions who typically consume sardine (*Sardina sp.*), anchovy (*Engraulis sp.*), rockfishes (*Sebastes sp.*), squid (Decapodiformes), and hakes (Phycidae) (Robinson et al., 2018; Weise & Harvey, 2008). California sea lion diet composition is positively related to what prey is most abundant in the environment at the time (Robinson et al., 2018; Weise & Harvey, 2008). In addition to providing information to fisheries regarding stock status, California sea lion (and other pinniped species) diet composition can be used as a preliminary assessment for how climate change and other anthropogenic factors are impacting lower trophic level distribution and abundance.

Northern fur seals (NFS) in California are income breeders, meaning they nurse their pups for several months, occasionally leaving for short foraging trips (Berta et al., 2015). NFSs typically forage in prey patches that are close to their breeding sites, however those prey patches are predicted to move as climate change worsens (McHuron et al., 2023). Models showed that NFSs will feed in their usual high quality prey patches until they are 400 km away from their breeding site (McHuron et al., 2023). After that, they will feed in lower quality prey patches that are closer to "home" (McHuron et al., 2023). Observations of NFS feeding behavior will provide information on prey habitat and quality.

Changes in prey quality can also be seen in ringed and gray seal diets. A model of ringed seal diet under different greenhouse gas emissions scenarios showed that under high emissions, all prey will have a lower mean body size, but total prey biomass will increase (Florko et al., 2021). Baltic gray seal blubber thickness was found to increase with individual herring weight but decrease with the quantity of herring consumed (Kauhala et al., 2017). Pinniped health, such as blubber thickness, will play in important role in investigating changes in prey quality over time. Additionally, the quality of pinniped prey will be determined by primary productivity and changes in lower trophic levels.

Long-term changes in pinniped diet can be measured from stable isotope ratios in their teeth and bone collagen. Stable isotope levels in marine mammals can show changes in lower trophic levels. If phytoplankton experience changes in Carbon and Nitrogen sources, this will be reflected in higher trophic level organism, like pinnipeds (Hirons et al., 2001). Carbon ($\delta^{13}C$) and Nitrogen ($\delta^{15}N$) isotope ratios were measured in Antarctic fur seal (*Arctocephalus gazella*) (AFS) teeth from 1983-2015 (de Lima et al., 2022). de Lima et al. (2022) found a decrease in $\delta^{13}C$ and an increase in $\delta^{15}N$, which may indicate changes in krill

(*Euphuasia sp.*) diet or a shift in AFS diet towards higher trophic level prey. $\delta^{13}C$ and $\delta^{15}N$ were also measured in the bone collagen of steller sea lions, northern fur seals, and harbor seals in the Bering Sea and Gulf of Alaska from 1951-1997 (Hirons et al., 2001). Hirons et al. (2001) saw no change in $\delta^{15}N$ and a decrease in $\delta^{13}C$. The decrease in $\delta^{13}C$ may be the result of declines in phytoplankton growth rates and primary productivity (Hirons et al., 2001). Changes in prey distribution, quality, and quantity will not only impact pinniped health but will effect commercial fisheries as well. Fishermen may be required to shift where they typically catch seafood. Additionally, if the quality of their catch decreases, it may be seen as less valuable (Shalders et al., 2022).

1.5 Toxicology

As a result of industrialization, chemical pollutants are frequently released into the environment. Two major classes that affect pinnipeds are trace elements and persistent organic pollutants (POPs). Trace elements (e.g. iron, zinc, copper, aluminum, and mercury) are minerals that are present in the natural environment (National Research Council (US) Committee on Diet and Health, 1989). Essential trace elements (e.g. selenium (Se), copper (Cu), and zinc (Zn)) are biologically required by pinnipeds, however excess exposure can cause adverse effects, while non-essential trace elements (e.g. lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg)) are toxic at any level (Ashley et al., 2020; Puchades et al., 2022). POPs are also harmful to pinnipeds, as they are manmade chemicals that have low solubility in water, allowing them to persist in aquatic environments (Walker et al., 2012).

Anthropogenic sources of both trace elements and POPs in the ocean include sewage outfalls, commercial activity, runoff from land, precipitation, pesticide spraying, and

shipwrecks (Walker et al., 2012). Pinnipeds' primary route of exposure to these toxins is through their prey (Berta et al., 2015). Both trace elements and POPs have been found to bioaccumulate within the marine food web (Walker et al., 2012). The transfer of these toxins from prey to predator is known as bioaccumulation (Walker et al., 2012). Only POPs biomagnify, meaning their concentrations multiply as they are transferred to higher trophic levels (Walker et al., 2012). As predators, pinnipeds are often exposed to high levels of these contaminants.

1.5.1 Trace Elements

The level of trace element exposure measured in pinnipeds can provide insight into the concentrations of trace elements in different geographic locations. Two subpopulations of harbor seals in the Wadden Sea forage in different habitats (Griesel et al., 2008). There were differences in Al (aluminum), Mn (manganese), Cu, and Pt (platinum) concentrations between these subpopulations, indicating a difference in trace element concentration in their prey (Griesel et al., 2008). Differences in Cd, Cu, Zn, As, MeHg (methylmercury), Se, and Ag (silver) have also been documented in harbor seal subpopulations in San Juan County and South Puget Sound, Washington (Akmajian et al., 2014; Ashley et al., 2020). Juan Fernandez fur seals (*Arctocephalus philippii*) reside on an isolated archipelago off the coast of Chile (Toro-Valdivieso et al., 2023). Despite their remote location, far from any urban cities, high levels of Cd and Hg were found in their feces (Toro-Valdivieso et al., 2023). This indicates that trace elements are able to be transported throughout the ocean.

Changes in trace element concentrations over time have been observed in pinnipeds. The South American fur seal (*Arctocephalus australis*) (SAFS) and South American sea lion (*Otaria flavescens*) (SASL) had higher Cr (chromium) levels from 1970 to 89 compared to

1944 to 69 and 1990 to 2013, likely due to industrial activity (De María et al., 2021). Caspian seals also experienced a decrease in Zn and Se, and an increase in Pb from the late 90s and early 2000s to 2013 to 2016 (Hoseini et al., 2022). While the release of trace elements into the environment is regulated, there are still unregulated instances of their transport into the marine environment, leading to fluctuations in their concentrations (Piwowarska et al., 2024).

Diet has been found to affect the concentrations of certain trace metals in pinnipeds as well. The SAFS, who feed on squid offshore, had higher Cd levels than the SASL, who have a coastal, benthic diet (De María et al., 2021). However, SASLs had higher Pb and Cu concentrations than SAFSs (De María et al., 2021). Caspian seals, which feed primarily on fish, were found with lower Cd levels than other pinnipeds with an invertebrate diet (Hoseini et al., 2022). It appears that which trace elements pinnipeds are exposed to depends on which trophic position they feed at.

Trace elements can be transferred from the mother to the fetus/pup through the placenta and lactation, as observed in a spotted seal mother-fetus pair in the Sea of Japan and gray seal mother-pup pairs in Scotland (Habran et al., 2013; Simokon & Trukhin, 2021). All trace elements found in the spotted seal mother were detected in the fetus, with Be (beryllium), Sb (antimony), Th (thorium), and U (uranium) at higher concentrations in the fetus (Simokon & Trukhin, 2021). Similarly, all trace elements measured, except Cd, were transferred through lactation in the gray seal pairs (Habran et al., 2013). Pups may excrete these metals by the time they're weaned by shedding their lanugo (Trukhanova et al., 2022). Lagoda ringed seal pups had higher concentrations of Hg, Cr, and Zn in their lanugo before they molted (Trukhanova et al., 2022). Additionally, Cd concentrations were much higher in pup lanugo than hair from adult ringed seals (Trukhanova et al., 2022). Still-born ringed seal

pups also had higher Ni (nickel) concentrations in their hair than live-born pups and adults (Hyvärinen & Sipilä, 1984).

There is limited information on the toxic effects of trace elements when above their "normal" thresholds in pinnipeds. Some harbor seals have shown a hypersensitivity response when exposed to heavy metals, meaning they experience an extreme immune response (Kakuschke et al., 2005; Momtazmanesh & Rezaei, 2022). These hypersensitive seals displayed elevated liver function, possibly due to a detoxing response to the elevated metal concentrations in their blood (Kakuschke et al., 2011). The effects of trace metals were also tested on the harbor seal lymphoma B cell line, which can be used as an immune model (Frouin et al., 2010). High levels of As, Se, V (vanadium), Ag, Zn, and Fe reduced the functional activities of the immune cells, which may weaken the immune system and alter harbor seal disease resistance (Frouin et al., 2010).

1.5.2 Persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) are endocrine disrupting chemicals, meaning that they can negatively impact hormones involved in growth and reproduction (NIEHS, 2023; Tanabe, 2002). In northern fur seals in Alaska, POP concentrations were correlated with changes in gene expression related to blubber metabolism pathways (Soulen et al., 2022). Additionally, POPs have been found to negatively impact lymphocyte and immune function in Pacific harbor seals (Mos et al., 2006). Reductions in blubber metabolism and immune response both have the ability to increase pinniped disease susceptibility.

Measuring POP concentrations in pinnipeds can provide information on their levels in the environment over time. The Stockholm Convention, put into effect in 2004, is a global treaty that aims to "protect human health and the environment from persistent organic

pollutants" (Lallas, 2001). Since 2004, POP levels in pinnipeds around the world have decreased. Scotland gray seals sampled from 2015 to 2017 had 75% of the polychlorinated biphenyl (PCB) concentrations that were measured in 2002 (Robinson et al., 2019). Similarly, PCB, Dichlorodiphenyltrichloroethane (DDT), chlordane (CHL), and hexachlorocyclohexane (HCH) levels in Canadian Arctic ringed seals decreased from 1972 to 2016 (Houde et al., 2019). To measure current usage of DDT, the ratio between DDT and its metabolites, dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD), is determined. In Antarctic Weddell seals (*Leptonychotes weddellii*), the ratio of DDE:DDT has increased, indicating a decrease in the use of DDT (Trumble et al., 2012). However, in the Baikal seal (*Pusa sibirica*) from Russia and the Caspian seal from the Caspian Sea, ratios of DDT and its metabolites show recent use of DDT (Hoseini et al., 2022; Mamontov et al., 2019). Baseline measurements of POP concentrations in pinnipeds can help to determine changes in POP usage and exposure in the future.

Pinnipeds can also provide information on geographic differences in POPs. Pacific harbor seals in Puget Sound, Washington, had PCB concentrations 7 times greater than harbor seals in the Strait of Georgia, Canada (Cullon et al., 2005). Differences in hexachlorobenzene (HCB) and β-HCH concentrations were found between ringed seals in different areas of the Canadian Arctic (Houde et al., 2019). Additionally, ringed seals in Barrow, Alaska had higher concentrations of multiple POPs than those in Nome, Alaska (Kucklick et al., 2006). The Pechora Sea and Svalbard walrus populations exhibited differences in PCB, polybrominated diphenyl ether (PBDE), and oxychlordane concentrations (Boltunov et al., 2019). Antarctic pinnipeds also had high levels of HCHs,

HCB, Heptachlor, Aldrin, Endrin, DDTs, and Methoxychlor, showing that POPs can be transported to remote areas (Vergara et al., 2019). Cullon et al (2019) investigated the difference in PCB levels between Puget Sound and Strait of Georgia harbor seals using food baskets of the two subpopulations' prey. They found that the greater PCB concentrations in Puget Sound seals were due to local environmental contamination, not a difference in diet (Cullon et al., 2005). However, caution should be used when observing differences in environmental POPs between pinniped subpopulations, as their diet may also be impacting their exposure. Walrus populations that consume seals may have higher POP concentrations than those that feed primarily on benthic invertebrates due to biomagnification (Boltunov et al., 2019; Kucklick et al., 2006; Tsygankov et al., 2015).

Maternal transfer of POPs has been observed in multiple pinniped species. Per- and polyfluorinated substances (PFAS) were measured in Australian sea lion (*Neophoca cinerea*), Australian fur seal (*Arctocephalus pusillus*), and New Zealand fur seal (*Arctocephalus forsteri*) pups as a result of maternal transfer (Taylor et al., 2021). A model produced by Hickie et al. (2005) showed that nursing ringed seal mothers can transfer 5 to 40% of their POP levels to their milk, which is then transferred to their pups. Selective transfer of these contaminants has been observed in harp and gray seal mother-pup pairs in Canada (Frouin et al., 2012; SORMO et al., 2003). Pup blubber and milk from the mothers had similar POP makeup and concentrations in both species (Frouin et al., 2012; SORMO et al., 2003). Hydrophilic compounds like HCH are also more easily transferred from the mother's blubber to milk (Frouin et al., 2012; Sormo et al., 2003). Knowledge of POP maternal transfer is important when investigating the toxic effects of POPs and their changes throughout time and regions.

1.6 Plastic

1.6.1 Macroplastic

Physical pollution is overwhelming the oceans. A major threat to pinnipeds has become entanglement in microplastic, which is plastic that is larger than 5 mm. Most entanglements are caused by fishing gear including lines, nets, ropes, and packing bands (Jepsen & de Bruyn, 2019). As a result, foraging, swimming, and reproductive behaviors may become negatively impacted (Butterworth, 2016). Many entanglements occur in the northern Pacific ocean, impacting Hawaiian monk seals, steller sea lions, California sea lions, Northern elephant seals, and harbor seals (Dau et al., 2009; Henderson, 2001; Raum-Suryan et al., 2009). Perez-Venegas et al (2023) modeled pinniped entanglement across the globe, and found that the Hawaiian Islands, USA and Kaikoura Island, New Zealand were two major hotspots. They attributed this to the transport of debris via ocean currents and these locations' close proximity to ocean gyres (Perez-Venegas et al., 2023). Similarly, entanglements have been observed in Antarctica, which is uninhabited by humans (Hofmeyr et al., 2006). Antarctic fur seals have been seen entangled in fishing gear, which either occurred far offshore or the debris drifted to Antarctica (Hofmeyr et al., 2006).

While global rates of entanglement appear to have remained constant, the number of successful disentanglements has increased (Jepsen & de Bruyn, 2019). However, a review by Jepsen and de Bruyn (2019) found that pinniped entanglements may be underreported due to unpublished monitoring data and lower representation from developing nations, possibly skewing this data. Observations of entanglements in remote locations can provide information on unreported abandoned or lost fishing gear (Perez-Venegas et al., 2023).

Overall, at least 1 individual from 67% of pinniped species have been reported entangled , making them an excellent group to study when monitoring marine debris (Jepsen & de Bruyn, 2019).

1.6.2 Microplastic

Microplastics (1-5000 μm) are the result of either physical and chemical weathering of macroplastic, or they are created that size, for example plastic pellets and microbeads (Andrady, 2011). Ingestion of microplastics by pinnipeds is common and is mainly due to indirect ingestion through their prey (Nelms et al., 2018). The amount and types of microplastics consumed in pinnipeds can be used to gather estimates of microplastic in the environment (Ortega-Borchardt et al., 2023a). Similar to findings of microplastics in the environment, pinnipeds typically consume more fibers than fragments (Bosker et al., 2018; Covernton et al., 2019; Hogan, 2022; Nel & Froneman, 2015; Nelms, Barnett, et al., 2019; Ortega-Borchardt et al., 2023b; Yu et al., 2018).

The most common polymers of microplastics found in pinnipeds have been rayon, polyethylene terephthalate or polyester (PET), polypropylene (PP), polyethylene (PE), and nylon (Eriksson & Burton, 2003; Nelms, Barnett, et al., 2019; Ortega-Borchardt et al., 2023b). Fishing lines, ropes, and nets are typically made of PET, PP, or nylon (NOAA Marine Debris Program, n.d.). Additionally, most studies have found common colors to be blue, black, gray, red, clear, and white (Eriksson & Burton, 2003; Hogan, 2022; Nelms, Barnett, et al., 2019; Ortega-Borchardt et al., 2023b; Perez-Venegas et al., 2020).

During the plastic making process, chemicals called additives are incorporated into the plastic (Andrady, 2011). When the plastic starts to fragment and microplastics are formed, these additives can leach into the surrounding environment (Andrady, 2011). In

Mediterranean monk seals (*Monachus monachus*), concentrations of microplastics were positively correlated with the number of phthalates in their scat (Hernandez-Milian et al., 2023). Phthalates are a common plastic additive, which are also endocrine disruptors (Hernandez-Milian et al., 2023). While the effects of phthalates on pinniped health have not been studied, this research shows that they are exposed to this chemical through microplastics. In marine mammals stranded along the British coast, including pinnipeds and cetaceans, those that died due to infectious disease had higher microplastic abundances than those with trauma or "other" as their cause of death (Nelms et al., 2019). There is no clear evidence of microplastics causing infectious disease in pinnipeds, however their function as a vector for harmful chemicals and bacteria should be studied further (Andrady, 2011; Nelms, Barnett, et al., 2019; Rotjan et al., 2019).

1.7 Summary

The anthropogenic threats that pinnipeds currently face are interconnected. The severity and spread of infectious disease between pinnipeds is likely to increase as climate change and pollution intensify. Some infectious diseases thrive in warmer temperatures (Calvin et al., 2023). Additionally, habitat loss due to climate change will cause pinnipeds to haul-out in higher density groups, which may increase the spread of disease between individuals (Hoekendijk et al., 2023; Nehasil, 2010). Increasing exposure to toxic chemicals and microplastic may weaken pinniped species' immune systems, making them more susceptible to infectious diseases (Frouin et al., 2010; Kakuschke et al., 2011; Nelms, Barnett, et al., 2019). Chemicals can adsorb onto and leach out of microplastics, contaminating the surrounding environment and any organisms that consume them (Andrady,

2011; Nelms et al., 2018). Pinniped prey quality may not only be worsened by climate change and shifting habitat ranges, but also by exposure to toxins and microplastics.

Since pinnipeds mainly consume microplastics via their prey, it is important to understand the relationship between pinniped diet and microplastic ingestion. Hogan (2022) and Hudak & Sette (2019) performed preliminary analyses of gray seal microplastic exposure in Massachusetts. Both studies found microplastics in scat samples. Hogan (2022) extracted microplastics from 19 scat samples from Great Point, Nantucket. All of their samples included fibers, and 18 had fragments (Hogan, 2022). The main purpose of Hudak & Sette (2019)'s study was to analyze the diet of gray seals on Cape Cod, however they also found microplastic fragments in 2 out of 129 samples.

Gray seals are an excellent sentinel species for ecosystem health because they are generalist predators, giving them the ability to provide information on microplastic consumption for many lower trophic level and commercially important fish and invertebrate species. In the present study, I have isolated microplastics and prey hard parts, and extracted DNA from 112 gray seal scat samples from Great Point, Nantucket, MA. The main objective is to investigate the relationship between gray seal diet and their ingestion of microplastics. The findings from this study will give insight into microplastic presence around Great Point and will be useful in guiding future research surrounding the trophic transfer of microplastics.

Tables

Table 1.1 Pinniped exposure to Phocine Distemper Virus (PDV) and Avian Influenza. Species exposed: *Pagophilus groenlandicus* (Pg), *Phoca vitulina* (Pv), *Halichoerus grypus* (Hg), *Eumetopias jubatus* (Ej), and *Callorhinus ursinus* (Cu). Exposure type: Antibodies, positive, positive with no symptoms, and Mass Mortality Event (MME).

Infectious Disease	Species	Location	Year	Exposure	Source
PDV	Pg	Greenland	1985-86	Antibodies	Dietz et al., 1989
	Pg	Canada	1988-93	Antibodies	Duignan et al., 1997
	Pv	Europe	1988	MME	Bodewes et al., 2013
	Pv	Europe	2002	MME	Bodewes et al., 2013
	Pv	New England, USA	2006	MME	Puryear et al., 2021
	Ej	US Pacific	2009	Positive	VanWormer et al., 2019
	Cu	US Pacific	2009	Positive	VanWormer et al., 2019
	Pv	New England, USA	2018	MME	Puryear et al., 2021
	Hg	New England, USA	2018	Positive – no symptoms	Puryear et al., 2021
Influenza A	Pv	New England, USA	2011	MME	Anthony et al., 2012
	Hg	NW Atlantic	2013-15	Antibodies	Puryear et al., 2016
	Pv	Denmark	2014	MME	Krog et al., 2015
	Pv	Germany	2014	MME	Bodewes et al., 2015
	Pv	Sweden	2014	MME	Zohari et al., 2014
	Hg	Netherlands	2015	Antibodies	Bodewes et al., 2015
	Pv	New England, USA	2022	MME	Puryear et al., 2023

Figures

Figure 1.1 Distribution of harp, harbor, and gray seals in the Arctic Ocean (NOAA Fisheries, 2022a, 2022b, 2022c). The direction of the 2002 sea ice minimum arrow indicates the spread of PDV to the Pacific Ocean (VanWormer et al., 2019). Map from Price (2023). (Price, 2023)

CHAPTER 2

UTILIZING PREY HARD PARTS AND DNA METABARCODING TECHNIQUES TO ASSESS GRAY SEAL (*HALICHOERUS GRYPUS*) DIET

2.1 Abstract

As the gray seal (*Halichoerus grypus*) population in the Northwest Atlantic has recovered from near-extirpation, interest in their diet has grown. Traditional methods of studying gray seal diet include isolating and identifying prey hard parts (e.g. otoliths, dermal denticles, squid beaks, etc.) from scat and stomach samples. Otoliths, specifically, are used to identify fish species, however these structures may be partially or fully digested, biasing hard parts analyses toward certain prey types. Additionally, gray seals have been observed eating only the body of certain fish species, excluding the head and otoliths of these prey. New research has employed DNA metabarcoding to construct gray seal diet. This method has been able to detect prey types that previous hard parts studies have not. This chapter explores differences between prey hard parts and DNA metabarcoding, using the mtDNA 16S gene, in 112 gray seal scat samples collected from Great Point, Nantucket. Our results support previous findings that DNA metabarcoding reduces the biases found with prey hard parts. DNA metabarcoding recovered 44 prey taxa from 101 out of 112 samples, whereas prey hard parts identified 21 taxa from 71/112 samples. However, there are still limitations to using

DNA metabarcoding, as it cannot provide information on the abundance or biomass of prey types in a sample. Our findings suggest that DNA metabarcoding offers a more comprehensive assessment of gray seal diet than prey hard parts, although future work is needed to quantitatively assess diet using metabarcoding.

2.2 Introduction

Once nearing extirpation due to hunting, gray seals (*Halichoerus grypus*) have recolonized much of their historic range in the Northwest (NW) Atlantic since becoming a protected species (den Heyer et al., 2021; Wood et al., 2020). As the population has recovered, new breeding and haul-out sites have been established in the northeast U.S. including Great Point, Nantucket, where pupping was documented in 2018 (Wood et al., 2020). Along with the resurgence of their population, interest in gray seal diet has grown. This is largely due to perceived competition between gray seals and fishermen for commercially important fish species (Baraff & Loughlin, 2000). Gray seals are known to feed on Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), pollock (*Pollachius pollachius*), flatfish (Pleuronectiformes), and skates (Rajiformes) (Ampela, 2009; Bowen & Harrison, 1994). Being generalist feeders, studying their diet can help determine the abundance and distribution of the numerous lower trophic level species they consume (Berta et al., 2015; Moore, 2008).

The most common method of studying pinniped diet is hard parts identification from scat samples. Since pinnipeds haul-out on shore, collecting scat is easy and non-invasive. Fish otoliths, skate dermal denticles, squid beaks, and other bony structures are commonly found in scat. Otoliths are used to identify fish species; however these structures may be

partially or fully digested, biasing hard parts analyses toward certain prey types (Bowen, 2000). Additionally, gray seals may exclude the heads of fish when eating them, leaving out any otoliths (Ampela, 2009). It is possible that vertebrae from these fish will be found in seal scat, but without otoliths, the species of fish are unidentifiable. Gray seals also consume invertebrates, whose remains are often not found in scat (Lerner et al., 2018; McCosker et al., 2020). Due to the unreliability of hard parts, other methods of diet analysis have been developed in more recent years.

More recently, stable isotopes, fatty acids, and prey DNA have been utilized in gray seal diet studies (Flanders et al., 2020; Ono et al., 2019; Tucker et al., 2008). Analysis of carbon (^{13}C) and nitrogen (^{15}N) stable isotopes can identify prey trophic levels from skin, vibrissae, and lanugo samples (Lerner et al., 2018; Tucker et al., 2007). Similar to stable isotopes, fatty acid signatures found in blubber are useful in long-term diet analyses, for example determining the diet of an individual over weeks or months (Ampela, 2009; Beck et al., 2007). Prey DNA extracted from scat samples can provide information on short-term diet (days). All of these methods are able to detect invertebrates and cartilaginous fish, whose presence may be underestimated in hard parts analyses.

Stable isotope and fatty acid analyses both involve physical contact with the seals to collect samples (Lerner et al., 2018; Tucker et al., 2007). Prey hard parts and DNA can both be extracted from scat samples, which causes less disturbance to seals compared to sampling blubber and skin tissue. One drawback to the non-invasive collection of seal scat is that it is not known which individual the sample came from. Age, weight, health status, and other characteristics are unknown, however sex can be determined using a primer that targets the ZFY gene on the Y chromosome of male gray seals (Dufault et al., 2021; Flanders et al.,

2020; McCosker et al., 2020). An advantage of prey DNA is that is uses the same sample type as hard parts analyses. By identifying diet using hard parts and DNA metabarcoding from the same scat samples, we can easily compare the results between the two methods.

Species-specific primers can be used to target species of interest within a predator's diet (Casper et al., 2007; Dufault et al., 2021; Marshall et al., 2010; Ono et al., 2019). This technique is useful when monitoring the consumption of certain species to answer ecological questions. For example, investigating the presence of Atlantic cod in gray seal diet as the gray seal population grows. However, a more efficient way to study predator diet is through DNA metabarcoding using group-specific primers (Deagle et al., 2009; Jarman et al., 2004). These primers can be used to evaluate the diversity of a species' diet. While group-specific primers do not offer as much taxonomic resolution as species-specific assays, they detect a broader range of prey types (Deagle et al., 2009).

Although DNA metabarcoding reduces the biases of hard parts analyses, certain prey types may still be excluded (McCosker et al., 2023). This depends on which primer is chosen and the degree to which DNA is degraded in the scat. Metabarcoding with group-specific primers will omit other prey groups. Furthermore, DNA from scat is often degraded, limiting the quality of DNA in the analysis (Waits & Paetkau, 2005).

As generalist predators, gray seals have a diverse diet that can provide information on lower trophic levels (Berta et al., 2015; Moore, 2008). Great Point gray seal diet has not yet been studied, and understanding their feeding habits can give insight into the local food web structure. Ampela (2009) discovered differences in gray seal diets between Muskeget and Monomoy Islands, which are approximately 30 km apart. It's possible that gray seals on Great Point, situated about 20 km from both sites, may have a unique diet as well (Ampela,

2009). This study aims to evaluate the diet composition of gray seals hauled-out on Great Point, Nantucket, comparing the effectiveness of prey hard parts identification and DNA metabarcoding techniques. Both invertebrates and vertebrates can be identified from hard parts, and Primer set B from Deagle et al. (2009) will detect chordate prey. The results of this study will not only provide information on the diet composition of Great Point gray seals but will also allow for comparison of temporal trends in diet between prey hard parts identification and DNA metabarcoding.

2.3 Methods

2.3.1 Scat Collection

A total of 112 gray seal scat samples were collected from Great Point, Nantucket on 7 dates between November 2017 and June 2022 (Table 2.1). The samples were collected at low tide using a slotted metal scoop to minimize contamination from the sand. They were then individually wrapped in aluminum foil and transported in a cooler to a -20°C freezer at the University of Massachusetts, Boston (UMB). A subsample of ~200 mg of each scat was stored in 1 mL of DNA/RNA shield (Zymo Research) and kept in a -20°C freezer until DNA extraction.

2.3.2 Hard Parts Identification

The weight, length, and width of each sample was recorded. The scat was then defrosted in a glass mason jar for 24 hours. The next day, 100 – 500 mL of hot deionized (DI) water was added to the scat and stirred until the mixture was homogenized. The volume of DI water added was dependent on the size of the scat sample. The scat mixture was poured through a stack of 4mm, 2mm, 1mm, and 500µm sieves. DI water was used to rinse the scat

through the sieves and to clean any hard parts that were found. Fish otoliths, vertebrae, lenses, skate dermal denticles, cephalopod beaks, and crustacean remains were picked out and stored in glass vials for further identification. Guides were used to identify fish taxa from otoliths (Brodeur, 1979; Campana, 2004). Samples with only vertebrae and lenses, and no otoliths, were classified as containing unidentified fish. Samples with dermal denticles were identified as the Rajidae family, cephalopod beaks were identified as the Cephalopoda family, and crustacean claws and shells were identified as the Crustacea sub-phylum.

2.3.3 DNA Metabarcoding

DNA was extracted using the *Quick*-DNA/RNA™ Magbead kit (Zymo Research). Samples were incubated in 25µl of Proteinase K at room temperature for 24 hours. Sample placement was randomized across the 96-well plates, and 1 extraction blank per plate (2 total) were used to test for contamination.

PCR was performed in duplicate to amplify the mtDNA 16S gene. Primer set B from Deagle et al (2009) (Chord_16S_F_TagA 5'- ATG CGA GAA GAC CCT RTG GAG CT -3', Chord_16S_R_Short 5'- CCT NGG TCG CCC CAA C -3') and a gray seal blocking primer designed by Flanders et al (2020) (Grayseal-block 5'- ATG GAG CTT TAA TTA ACT AAC TCA ACA GAA CAA /3SpC3/ -3') were used to amplify chordate DNA while reducing the amount of gray seal sequences. Forward and reverse primers were tagmented with Nextera adapter sequences (forward 5'- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG, reverse 5'- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G). PCR was done following the methods from Flanders et al (2020). Samples and blanks were amplified using Molecular Grade Water (12.8µl), 5x MyTaq Reaction Buffer (5µl), MyTaq DNA Polymerase (0.2µl), 10µM forward primer (2µl), 10µM reverse primer (2µl),

100µM gray seal blocking primer (2µl), and template DNA (1µl) for a total of 25µl per well. Cycling conditions are described in Table 2.2. After thermocycling, PCR reactions were tested for amplification using a 1.6% agarose gel.

A second PCR was done to attach a unique combination of Nextera XT indices to each sample. Duplicates were pooled and PCR was done using Index 1 (i7) adapters (5µl), Index 2 (i5) adapters, Azura 2x Taq Mix (15µl), and PCR product (25µl) for a total of 50µl per well. Cycling conditions are described in Table 2.3. PCR reactions were again tested for amplification using a 1.6% agarose gel.

PCR products were then cleaned and normalized using the SequalPrep Normalization Plate Kit. All samples were then pooled for sequencing. The library was sequenced on an Illumina MiSeq using a MiSeq Reagent Kit v2 (300-cycles) with 2 x 250 bp chemistry at UMass Boston.

2.3.4 Bioinformatics and Data Analysis

Bioinformatics were done using Qiime2(Bolyen et al., 2019). The sequences were provided in a demultiplexed format, so reads were entered into Qiime2 as SampleData[PairedEEndSequencesWithQuality] using a manifest file. Next, the data was visualized and inspected for quality. Reads were trimmed where the median quality score dropped below 30. In this case, all bases had a median quality score above 30, so the reads were not trimmed. The data was then denoised using dada2. Taxonomy was assigned via a command-line BLAST using the NCBI non-redundant database. All OTUs that were returned as bacteria or had 2 or less reads were removed. The remaining taxa were inspected and filtered for suspected prey items in the Gulf of Maine (McCosker et al., 2023).

Diet from both prey hard parts and DNA metabarcoding was analyzed by calculating the frequency of occurrence (FO) for each prey taxa. FO describes the presence of a prey type in a sample. FO was calculated as:

$$FO_i = \frac{n_i}{n} \text{ or } \%FO_i = \frac{n_i}{n} \times 100$$

Where: n = total number of samples
n_i = total number of samples with prey type i

FO was compared qualitatively between the two methods. Alpha diversity, or prey richness, was calculated as the number of unique prey types in a sample.

All statistical analyses were performed in R Studio Version 2023.06.1+524. Generalized Linear Models with the 'glm' function in the package 'car' were used to compare alpha diversity between methods. To assess the relationship between prey, sampling dates, seasons, and years, redundancy analyses (RDA) were performed using the 'rda' function from the 'vegan' package. Prey were classified to their lowest taxonomic level (Table 2.4). Diet was analyzed in the RDA using a prey presence/absence matrix. A permutational multivariate ANOVA (PERMANOVA) was done using the 'adonis2' function from the 'vegan' package to analyze the significance of the environmental variable's contribution to the response variable's diversity. The environmental variables tested were sampling date, season, and year group. Since samples were collected from fall to spring/summer, samples from 2017 to 2018 and 2021 to 2022 were grouped into "year groups" (Table 2.1). If the PERMANOVA was significant ($p < 0.05$), a chi-square test of independence was done for each prey type and the environmental variable to determine which prey were influencing the RDA.

2.4 Results

2.4.1 Prey Hard Parts

Prey hard parts identified 21 prey taxa (Table 2.4) from 71 out of 112 samples (Table 2.5). Prey were classified at the species, genus or order levels (Table 2.4). The 5 prey taxa with the highest % FO were skates (28.57%), sand lance (*Ammodytes sp.*) (21.45%), unidentified fish (19.64%), red/white/spotted hake (Phycidae) (9.82%), and windowpane flounder (*Scophthalmus aquosus*) (8.04%) (Table 2.6). Prey composition from hard parts was significantly different between seasons ($p = 0.001$, Figure 2.1), year groups ($p = 0.046$, Figure 2.2), and sampling dates ($p = 0.001$, Figure 2.3).

Phycid hake ($p = 0.028$), Gadiformes ($p = 0.018$), sand lance ($p = 0.013$), Perciformes ($p = 0.017$), and skates ($p = 3.37e\text{-}07$) showed significant differences in presence between seasons. Unidentified Gadiformes hard parts were found in more samples from 2021 to 2022 than 2017 to 2018 ($p = 0.031$). However, when all samples with identified and unidentified Gadiformes hard parts were grouped, there was no significant difference between the number of samples with Gadiformes between year groups ($p = 0.397$). Sand lance ($p = 0.035$), Gadiformes ($p = 0.023$), skates ($p = 8.099e\text{-}07$), and unidentified Pleuronectiformes ($p = 0.024$) showed significant differences in presence between sampling dates. There was no significant difference in the number of samples with all identified and unidentified Pleuronectiformes hard parts between sampling dates ($p = 0.373$).

2.4.2 DNA Metabarcoding

A total of 1,737,352 prey reads were included in this analysis. DNA metabarcoding recovered 44 (Table 2.4) taxa from 101 out of 112 samples (Table 2.5). All of the samples in which prey did not amplify were in adjacent wells on the extraction and PCR plates. The

absence of prey DNA in these samples is likely due to human error. Prey were classified at the species, genus, or family level. The 5 taxa with the highest % FO from were skates (87.5%), longhorn sculpin (*Myoxocephalus octodecemspinosus*) (82.14%), Atlantic cod (78.57%), sand lance (77.68%), and windowpane flounder (76.79%) (Table 2.6). Prey composition was not significantly different between seasons ($p = 0.223$, Figure 2.4), year groups ($p = 0.138$, Figure 2.5), or sampling dates ($p = 0.065$, Figure 2.6).

2.4.3 Comparing methods

In total, 108 scat samples contained either prey DNA, prey hard parts, or a combination of both (Table 2.5). Mean prey richness was significantly higher when using DNA metabarcoding (13.58 ± 6.96) than prey hard parts (1.31 ± 1.34) ($p < 0.0001$) (Figure 2.7). Both methods combined recovered 56 prey taxa. Prey hard parts identified 13 unique taxa, metabarcoding identified 36 unique taxa, and both methods shared 8 taxa (Table 2.4). Of the shared taxa, all had a higher % FO from metabarcoding except for phycid hakes (Table 2.6).

2.5 Discussion

2.5.1 Prey Hard Parts vs DNA Metabarcoding

Overall, DNA metabarcoding was able to identify more taxa in more samples than prey hard parts (Table 2.6). The limited identification of prey from hard parts is partly a result of differences in otolith strength between prey and feeding behaviors of gray seals. The presence of Clupeids (e.g. river herring, Atlantic herring, Atlantic menhaden, etc.) has previously been underestimated in gray seal diet studies due to their fragile otoliths, which easily break down while being digested (Bowen, 2000; Browne et al., 2002). Ampela (2009)

has also reported gray seals avoiding the heads and/or only eating the viscera of summer flounder (*Paralichthys dentatus*), sea bass (Serranidae), sea robin (Triglidae), Atlantic menhaden (*Brevoortia tyrannus*), scup (*Stenotomus chrysops*), alewife (*Alosa pseudoharengus*), and blueback herring (*Alosa aestivalis*). In the present study summer flounder, sea robin, Atlantic menhaden, and river herring (alewife/blueback herring) were only identified using metabarcoding. Black sea bass (*Centropristis striata*) and scup had a much greater % FO from metabarcoding (50.89%; 36.61%) than hard parts (0.89%; 0.89%).

Flatfish (Pleuronectiformes) have also been underestimated in gray seal diet when using prey hard parts. Dufault et al. (2021) found flatfish otoliths in 6.8% of samples while 35.6% of samples contained flatfish DNA. The results from the present study align with this as 16.96% of samples had flatfish otoliths and 83.94% of samples had flatfish DNA. Dufault et al (2021) created a species-specific assay to assess the presence of 4 flatfish species: American plaice (*Hippoglossoides platessoides*), Atlantic halibut (*Hippoglossus hippoglossus*), winter flounder (*Pseudopleuronectes americanus*), and yellowtail flounder (*Pleuronectes ferruginea*). The present study used a group-specific primer for metabarcoding and was able to identify 8 flatfish taxa. The use of a broader primer may be influencing the higher flatfish FO in this study compared to Dufault et al (2021).

Gadoids, including phycid hakes and cod, have stronger otoliths and are less susceptible to erosion (Bowen, 2000). The proportion and importance of Gadoids (e.g. Atlantic cod, phycid hakes, etc.) in gray seal diet has been overestimated in the past (Browne et al., 2002). The five most common prey types differed between methods. Skates, sand lance, and windowpane flounder were in the top five from both methods (Table 2.7). In the hard parts dataset, unidentified fish and phycid hakes were also in the top five; however,

from metabarcoding longhorn sculpin and Atlantic cod were in the top five (Table 2.7). In the present study, phycid hakes were determined to be a common prey type using hard parts (FO = 9.92%), however when using metabarcoding they are slightly less present (FO = 7.14%). The number of samples with phycid hakes did not differ between methods (n = 7), indicating that the strength of these otoliths may allow them to be accurately represented in hard parts. While Atlantic cod also have strong otoliths, Read (2008) has reported incidents of gray seals "belly biting" cod, resulting in exclusion of the head and otoliths. The present study supports this observation, with cod having little presence in hard parts (FO = 2.68%) but being the third most common prey type from metabarcoding (FO = 78.57%). All prey were able to be identified to at least the family level from metabarcoding, whereas unidentified fish were very common in the hard parts data. This further supports the idea that metabarcoding is more reliable and better at identifying prey than hard parts.

2.5.2 Comparisons to past gray seal diet studies

Sand lance has been an important prey type in all gray seal diet studies (Table 2.7). Skates have been important in more recent studies, which have also taken place in Southern New England (SNE) (Table 2.7). The first gray seal diet studies took place on Sable Island, Canada and did not include skates as important prey (Table 2.7). As many Rajidae species', including little skate (*Pleuronectes ferruginea*), distributions expand into Canadian waters, this indicates that skates are either only an important prey type for gray seals in SNE or that they have become more important in their diet over time (Sulak et al., 2009).

The present study's hard parts results are similar to Ampela (2009)'s hard parts results from Muskeget and Monomoy Islands in Massachusetts. Both the present study and Ampela (2009) found skates, sand lance, phycid hake, and windowpane flounder to be in the

five most common prey types (Table 2.7). Ampela (2009) also identified winter flounder hard parts whereas neither winter flounder hard parts nor DNA were found in the present study. McCosker et al (2023) also had skates, phycid hake, and sand lance in their top five prey, however invertebrates (crustaceans and echinoderms) were more common than in the current study (Table 2.7).

The results of the present study and McCosker et al (2023) show that DNA metabarcoding is better at detecting and identifying prey in seal scat than hard parts. Flanders et al (2020), McCosker et al (2023), which collected samples from Monomoy, and the present study used primer set B from Deagle et al (2009) to identify prey in gray seal scat. All 3 studies had sand lance in their 5 most common prey types (Table 2.7). The Monomoy studies also had Atlantic menhaden and Pleuronectidae in their top 5 prey types (Table 2.7). McCosker et al (2023) and the present study included skates and windowpane flounder in their top five prey as well. Prey types that were in the top five in only the present study were longhorn sculpin and Atlantic cod. Atlantic mackerel (*Scomber scombrus*) and northern sea robin (*Prionotus carolinus*) were only in the top five in Flanders et al (2020). McCosker et al (2023) did not have any unique prey types in their top five prey.

McCosker et al (2023) and Flanders et al (2021)s' scat samples had an average prey richness of 3.11 and 2.66 respectively. This is much lower than the present study's average prey richness of 13.6. Flanders et al (2021) did not identify flatfish lower than the family level (Pleuronectidae and paralichthyidae). The only flatfish that McCosker et al (2023) identified to the species level was windowpane flounder. Our identification of 6 flatfish to at least the genus level could be increasing prey richness in the present student. Additionally, the previous studies collected scat from Monomoy Island, which is a more established

breeding site with breeding adults and pups (Wood et al., 2020). The gray seals observed at Great Point were majority juveniles or non-breeding adults, which have been observed having a more diverse diet than adults (Ampela, 2009; Beck et al., 2007; Wood et al., 2020). However, it is impossible to determine the age of the seal that a scat sample came from.

2.5.3 Trends in diet

Hard parts data showed significant differences in prey composition between seasons, sampling dates, and years while metabarcoding data did not. The hard parts data also had a much lower % FO for all prey than the metabarcoding data. Since the % FO was lower, it may be showing false trends that do not appear when using DNA metabarcoding. Since metabarcoding was able to identify prey in more samples than hard parts, this created a more uniform composition of prey between samples, resulting in no temporal trends from DNA metabarcoding.

Otolith size is positively correlated with the length of the fish it came from. Sand lance reach their maximum size in the summer each year, meaning their otoliths are largest in the summer (Suca et al., 2021). The results of this study show no sand lance otoliths found in scat samples collected in the summer, indicating that otolith size is not related to its presence in seal scat. Little skates mature around 7 years of age, and their growth or dermal denticle size does not appear to have a relationship with season (Frisk & Miller, 2006). Additional information is required to determine whether temporal trends in diet reflect biological factors for specific prey types or are influenced by biases in hard parts analysis.

In gray seal diet studies using fatty acids and stables isotopes, differences in prey diversity between sexes were observed (Ampela, 2009; Beck et al., 2007; Tucker et al., 2007). Flanders et al (2021) was able to determine genetically determine sex from gray seal

scat, however their results did not display any differences in diet diversity between sexes. Similar to this study, Flanders et al (2021) used the presence and absence of a prey type in a sample to assess differences between sexes. The studies analyzing fatty acids and stable isotopes used the proportions of prey types to investigate trends (Ampela, 2009; Beck et al., 2007; Tucker et al., 2007, 2008). Currently, DNA metabarcoding is only able to determine the presence or absence of taxa.

Seasonal trends in stock distribution and abundance have been documented in a few gray seal prey types. Windowpane flounder is more abundant in Georges Bank in the spring, while American Fourspot flounder (*Hippoglossina oblonga*) is more abundant in the fall (Methratta & Link, 2007; Stokesbury et al., 2019). In the summer, Atlantic cod tends to move away from the coast into deeper, colder water (Langan et al., 2020). Atlantic menhaden migrates into the Gulf of Maine in early spring and returns to the southern US east coast to spawn in the fall (Buchheister et al., 2016). Any trends in the abundance of these fish in gray seal diet would be absent due the qualitative nature of DNA metabarcoding.

2.5.4 Limitations of DNA metabarcoding

While the results of this study show that DNA metabarcoding is better at detecting and identifying prey than prey hard parts, there are still limitations to using metabarcoding. DNA metabarcoding cannot provide information on prey biomass or the proportions of prey in a sample. You also cannot determine the age of the seal the sample was from, but methods have been developed to determine the sex (Flanders et al., 2020). Metabarcoding, especially with broad primers like primer set B, is useful when qualitatively investigating gray seal diet. Fatty acid diet studies use prior knowledge of diet to create fatty acid prey libraries, which metabarcoding can be useful in developing (Tucker et al., 2008). Fatty acid prey libraries,

developed from known diet from hard parts studies, may be excluding prey which only DNA metabarcoding has identified. The development of quantitative DNA metabarcoding methods is ongoing (Thomas et al., 2016; Wu et al., 2024).

The primer used in this study only amplified vertebrate prey, however invertebrates are an important component of gray seal diet (McCosker et al., 2020). Crustaceans and cephalopods were identified in 6.25% and 2.68% of samples from prey hard parts respectively (Table 2.6). Invertebrates are often underestimated in hard parts analyses (McCosker et al., 2020). McCosker et al (2020) developed an invertebrate primer which was able to identify more invertebrate taxa in more samples compared to hard parts. In the future, it would be important to use this primer in conjunction with the chordate primer to get a complete picture of Great Point gray seal diet.

2.5.5 Conclusions

The DNA metabarcoding results show gray seals hauled-out on Great Point, Nantucket have a broad diet, with common prey including skates, Perciformes, Pleuronectiformes, Gadiformes, and Clupeiformes. Many of the prey identified in this study are often recorded in National Marine Fisheries Service bottom trawl surveys, indicating that gray seals feed on what is currently present in the environment (United States. National Marine Fisheries Service, 2017b, 2017a, 2018b, 2018a). The benefits of metabarcoding include greater prey identification across and within scat samples. Metabarcoding was able to identify prey in 27% more samples than hard parts. It also has greater taxonomic resolution, identifying prey at the species, genus, and family levels while hard parts classified prey at the species, genus, and order levels, skipping over family. However, metabarcoding only provides qualitative results, leaving the abundance of each prey type unknown. This study's

findings suggest that DNA metabarcoding offers a more comprehensive assessment of gray seal diet than prey hard parts, although future work is needed to quantitatively assess diet using metabarcoding.

Tables

Table 2.1 Gray seal scat sample size by collection date, collected from Great Point, Nantucket.

Date	Sample Size
11-05-2017	18
01-15-2018	6
02-09-2018	21
04-20-2018	10
11-07-2021	14
04-09-2022	23
06-07-2022	20
Total	112

Table 2.2 PCR conditions for the amplification of the 16S mtDNA gene using primer set B from Deagle et al (2009) and a gray seal blocking primer from Flanders et al (2020).

Temperature (°C)	Time	Number of Cycles
95	3 min.	x1
95	30 sec.	x35
57	30 sec.	
72	30 sec.	
72	10 min.	x1
4	Continuous	Continuous Until Removed

Table 2.3 PCR conditions for the attachment of Nextera XT i7 and i5 adapters.

Temperature (°C)	Time	Number of Cycles
72	3 min.	x1
95	30 sec.	
95	10 sec.	x12
55	30 sec.	
72	30 sec.	
72	5 min.	x1
10	Continuous	Continuous Until Removed

Table 2.4 Taxonomic classification of prey types found in 112 gray seal scat samples, and which method they were identified from. Prey hard parts (HP), DNA metabarcoding (DNA).

Order/Highest Classification	Family	Genus	Scientific Name	DNA	HP
Anseriformes	Anatidae	Somateria	*Somateria mollissima*	Y	-
Clupeiformes	Clupeidae	Alosa	-	Y	-
	Clupeidae	Brevoortia	*Brevoortia tyrannus*	Y	-
	Clupeidae	Clupea	*Clupea harengus*	Y	-
	Clupeidae	Sardina	*Sardina pilchardus*	Y	-
Gadiformes	Gadidae	Gadus	*Gadus morhua*	Y	Y
	Phycidae	Urophycis	*Urophycis sp.*	-	Y
	Phycidae	Urophycis	*Urophycis chuss*	Y	-
	Phycidae	Urophycis	*Urophycis regia*	Y	-
	Merluccidae	Merluccius	*Merluccius bilinearis*	Y	Y
	Gadidae	Melanogrammus	*Melanogrammus aeglefinus*	-	Y
	Gadidae	-	-	Y	-
	-	-	-	-	Y
Labriformes	Labridae	Tautogolabrus	*Tautogolabrus adspersus*	Y	Y
	Labridae	Tautoga	*Tautoga onitis*	Y	-
Lophiiformes	Lophiidae	-	-	Y	
Mugiliformes	Mugilidae	-	-	Y	
Ophidiiformes	Ophidiidae	Ophidion	*Ophidion marginatum*	Y	-
	Ophidiidae	-	-	-	Y
Perciformes	Cottidae	Myoxocephalus	*Myoxocephalus octodecemspinosus*	Y	-
	Cottidae	-	-	Y	-

	Ammodytidae	Ammodytes	-	Y	-
	Ammodytidae	Ammodytes	*Ammodytes americanus*	-	Y
	Ammodytidae	-	-	Y	-
	Moronidae	Morone	*Morone saxatilis*	Y	-
	Triglidae	Prionotus	*Prionotus carolinus*	Y	-
	Triglidae	Prionotus	-	Y	-
	Serranidae	Centropristis	*Centropristis striata*	Y	Y
	Liparidae	Liparis	-	Y	-
	Agonidae	Hemitripterus	*Hemitripterus americanus*	Y	-
	Scombridae	Scomber	*Scomber scombrus*	Y	-
	Zoarcidae	Zoarces	*Zoarces americanus*	Y	Y
	Pholidae	Pholis	*Pholis gunnellus*	Y	-
	Cyclopteridae	Cyclopterus	*Cyclopterus lumpus*	Y	-
Pleuronectiformes	Scophthalmidae	Scophthalmus	*Scophthalmus aquosus*	Y	Y
	Paralichthyidae	Paralichthys	*Paralichthys dentatus*	Y	-
	Paralichthyidae	Paralichthys	-	Y	-
	Paralichthyidae	Hippoglossina	*Hippoglossina oblonga*	Y	Y
	Cyclopsettidae	Etropus	*Etropus microstomus*	Y	-
	Pleuronectidae	Hippoglossus	*Hippoglossus hippoglossus*	Y	-
	Pleuronectidae	-	-	Y	-
	Bothidae	Bothus	-	Y	-
	Cyclopsettidae	Citharichthys	*Citharichthys arctifrons*	-	Y
	Pleuronectidae	Limanda	*Limanda ferruginae*	-	Y

	-	-	-	-	Y
Rajiformes	Rajidae	Leucoraja	*Leucoraja erinacea*	Y	-
	Rajidae	-	-	Y	-
	-	-	-	-	Y
Scombriformes	Stromateidae	Peprilus	*Peprilus triacanthus*	Y	-
Spariformes	Sparidae	Stenotomus	*Stenotomus chrysops*	Y	Y
Syngnathiformes	Syngnathidae	Syngnathus	*Syngnathus fuscus*	Y	-
Tetraodontiformes	Tetraodontidae	Sphoeroides	*Sphoeroides spengleri*	Y	-
Uranoscopiformes	Uranoscopidae	Astroscopus	*Astroscopus guttatus*	Y	-
Scorpaeniformes	Sebastidae	Sebastes	-	-	Y
Cephalopods	-	-	-	-	Y
Crustaceans	-	-	-	-	Y
Unidentified fish	-	-	-	-	Y

Table 2.5 Sample name, year collected, season collected, and which method prey was identified from for 112 scat samples. A "1" indicates prey was found from that method and "0" indicates that prey was not found from that method. If no prey was found from either method, a "1" was given in the "No Prey" column. DNA = DNA metabarcoding, HP = prey hard parts.

Sample	Year	Season	DNA	HP	No Prey
GP03nov17	2017	Autumn	1	1	0
GP04nov17	2017	Autumn	1	1	0
GP05nov17	2017	Autumn	1	1	0
GP06nov17	2017	Autumn	1	1	0
GP07nov17	2017	Autumn	1	1	0
GP08nov17	2017	Autumn	1	0	0
GP09nov17	2017	Autumn	1	1	0
GP10nov17	2017	Autumn	0	1	0
GP11nov17	2017	Autumn	1	1	0
GP12nov17	2017	Autumn	1	1	0
GP13nov17	2017	Autumn	0	0	1
GP14nov17	2017	Autumn	1	0	0
GP15nov17	2017	Autumn	1	1	0
GP16nov17	2017	Autumn	1	1	0
GP17nov17	2017	Autumn	1	1	0
GP17nov21	2017	Autumn	1	1	0
GP19nov17	2017	Autumn	1	1	0
GP20nov17	2017	Autumn	1	1	0
GP01jan18	2018	Winter	1	0	0
GP02jan18	2018	Winter	1	0	0
GP03jan18	2018	Winter	0	1	0
GP04jan18	2018	Winter	1	1	0
GP05 jan18	2018	Winter	0	1	0
GP05jan18	2018	Winter	1	0	0
GP06jan18	2018	Winter	1	0	0
GP01feb18	2018	Winter	1	1	0
GP02feb18	2018	Winter	1	1	0
GP03feb18	2018	Winter	1	0	0
GP04feb18	2018	Winter	1	1	0
GP05feb18	2018	Winter	1	1	0
GP06feb18	2018	Winter	1	0	0
GP07feb18	2018	Winter	1	1	0
GP08feb18	2018	Winter	1	1	0
GP09feb18	2018	Winter	0	1	0
GP10feb18	2018	Winter	1	0	0
GP11feb18	2018	Winter	1	1	0
GP12 feb18	2018	Winter	0	1	0

GP12feb18	2018	Winter	1	0	0
GP13feb18	2018	Winter	1	0	0
GP14feb18	2018	Winter	1	0	0
GP15feb18	2018	Winter	1	1	0
GP16 feb18	2018	Winter	0	1	0
GP16feb18	2018	Winter	1	0	0
GP17feb18	2018	Winter	1	0	0
GP18feb18	2018	Winter	1	0	0
GP19feb18	2018	Winter	1	0	0
GP20feb18	2018	Winter	1	0	0
GP21feb18	2018	Winter	1	0	0
GP05 apr18	2018	Spring	0	1	0
GP06apr18	2018	Spring	1	0	0
GP07apr18	2018	Spring	0	0	1
GP08apr18	2018	Spring	1	1	0
GP09apr18	2018	Spring	1	1	0
GP10apr18	2018	Spring	0	0	1
GP11apr18	2018	Spring	1	0	0
GP12apr18	2018	Spring	1	1	0
GP13apr18	2018	Spring	1	1	0
GP14apr18	2018	Spring	1	1	0
GP18nov17	2018	Autumn	1	0	0
GP01nov21	2021	Autumn	0	0	1
GP02nov21	2021	Autumn	1	1	0
GP03nov21	2021	Autumn	1	1	0
GP04nov21	2021	Autumn	1	1	0
GP06nov21	2021	Autumn	1	1	0
GP07nov21	2021	Autumn	1	1	0
GP08nov21	2021	Autumn	0	1	0
GP11nov21	2021	Autumn	1	1	0
GP12 nov21	2021	Autumn	0	1	0
GP12nov21	2021	Autumn	1	0	0
GP13nov21	2021	Autumn	1	1	0
GP14nov21	2021	Autumn	1	1	0
GP15nov21	2021	Autumn	1	1	0
GP16 nov21	2021	Autumn	0	1	0
GP16nov21	2021	Autumn	1	0	0
GP01apr22	2022	Spring	1	0	0
GP02apr22	2022	Spring	1	1	0
GP03apr22	2022	Spring	1	1	0
GP04apr22	2022	Spring	1	1	0
GP05apr22	2022	Spring	1	1	0
GP06apr22	2022	Spring	1	1	0

GP07apr22	2022	Spring	0	1	0
GP08apr22	2022	Spring	1	0	0
GP09apr22	2022	Spring	1	1	0
GP10apr22	2022	Spring	1	0	0
GP11apr22	2022	Spring	1	1	0
GP12apr22	2022	Spring	1	0	0
GP13apr22	2022	Spring	1	0	0
GP14apr22	2022	Spring	1	1	0
GP15apr22	2022	Spring	1	1	0
GP16 apr22	2022	Spring	0	1	0
GP16apr22	2022	Spring	1	0	0
GP17apr22	2022	Spring	1	1	0
GP18apr22	2022	Spring	1	1	0
GP19apr22	2022	Spring	1	1	0
GP20apr22	2022	Spring	1	1	0
GP33apr22	2022	Spring	1	0	0
GP40apr22	2022	Spring	1	0	0
GP50apr22	2022	Spring	1	1	0
GP01jun22	2022	Summer	1	0	0
GP02jun22	2022	Summer	1	0	0
GP03 jun22	2022	Summer	0	1	0
GP03jun22	2022	Summer	1	0	0
GP04jun22	2022	Summer	1	0	0
GP05jun22	2022	Summer	1	0	0
GP12 jun22	2022	Summer	0	1	0
GP14jun22	2022	Summer	1	0	0
GP17jun22	2022	Summer	1	1	0
GP18jun22	2022	Summer	1	0	0
GP19jun22	2022	Summer	1	1	0
GP20jun22	2022	Summer	1	1	0
GP21jun22	2022	Summer	1	0	0
GP23jun22	2022	Summer	1	1	0
GP24jun22	2022	Summer	1	0	0
GP25jun22	2022	Summer	1	0	0
GP26jun22	2022	Summer	1	0	0
GP27jun22	2022	Summer	1	1	0
GP30jun22	2022	Summer	1	0	0
GP31jun22	2022	Summer	1	0	0
GP32jun22	2022	Summer	1	1	0

Table 2.6 Frequency of occurrence (% FO) of prey from gray seal scat samples (n = 112). The methods used were DNA metabarcoding (DNA), prey hard parts (HP). % FO was calculated at the genus and order levels.

Scientific Name	Common Name	DNA	HP
Somateria mollissima	common eider	8.04	-
Anseriformes		**8.04**	-
Alosa sp.	river herring	71.43	-
Brevoortia tyrannus	Atlantic menhaden	68.75	-
Clupea harengus	Atlantic herring	46.43	-
Sardina pilchardus	sardine	0.89	-
Clupeiformes		**79.46**	-
Gadus morhua	Atlantic cod	78.57	2.68
Urophycis sp.	Phycid hakes (red/white/spotted hake)	7.14	9.82
Merluccius bilinearis	silver hake	4.46	3.57
Melanogrammus aeglefinus	haddock	-	2.68
Gadiformes		**81.25**	**28.57**
Tautoga onitis	Tautog	5.36	-
Labriformes		**5.37**	**1.79**
Ophidion marginatum	striped cusk-eel	42.86	-
Ophidiiformes		**42.86**	**1.79**
Myoxocephalus octodecemspinosus	longhorn sculpin	82.14	-
Ammodytes sp.	sand lance	77.68	21.45
Morone saxatilis	striped bass	74.11	-
Prionotus sp.	sea robin	80.39	-
Centropristis striata	black sea bass	50.89	0.89
Liparis sp.	-	45.54	-
Hemitripterus americanus	sea raven	41.96	-
Scomber scombrus	Atlantic mackerel	7.14	-
Zoarces americanus	ocean pout	7.14	0.89
Pholis gunnellus	rock gunnel	4.90	-
Cyclopterus lumpus	lumpfish	0.89	-
Tautogolabrus adspersus	cunner	0.89	1.79
Perciformes		**87.50**	**23.21**
Scophthalmus aquosus	windowpane flounder	76.79	8.04
Paralichthys sp.	sand flounder	62.50	-
Hippoglossina oblonga	American fourspot flounder	59.82	0.89
Etropus microstomus	smallmouth flounder	50.00	-
Hippoglossus hippoglossus	Atlantic halibut	8.93	-
Bothus sp.	-	0.89	-
Citharichthys arctifrons	gulfstream flounder	-	0.89

Limanda ferruginae	yellowtail flounder	-	0.89
	Pleuronectiformes	**83.93**	**16.96**
Leucoraja erinacea	little skate	87.50	-
	Rajiformes	**87.50**	**28.57**
Peprilus triacanthus	American butterfish	0.89	-
	Scombriformes	**0.89**	-
Stenotomus chrysops	scup	36.61	0.89
	Spariformes	**36.61**	**0.89**
	Lophiiformes	**1.79**	-
	Mugiliformes	**1.79**	-
Syngnathus fuscus	northern pipefish	0.89	-
	Syngnathiformes	**0.89**	-
Sphoeroides spengleri	bandtail puffer	0.89	-
	Tetraodontiformes	**0.89**	-
Astroscopus guttatus	northern stargazer	23.21	-
	Uranoscopiformes	**23.21**	-
Sebastes sp.	Redfish	-	0.89
	Scorpaeniformes	-	**0.89**
	Crustacea	-	**6.25**
	Cephalopoda	-	**2.68**
	Unidentified fish	-	**19.64**

Table 2.7 Important prey in previous and the current gray seal diet studies. All studies used scat samples to identified prey from prey hard parts or DNA metabarcoding using Primer Set B from Deagle et al (2009). Sample size (n), % Frequency of Occurrence (FO).

Sampling time	Location (n)	Top 5 prey (FO)	Method	Source
July 1991-February 1993	Sable Island, Canada (393)	Sand lance (39.2%), unk flatfish (12%), unk gadoid (7.2%) silver hake (6.8%), Atlantic cod (5.5%)	Hard parts - scat	Bowen & Harrison (1994)
July 1991-February 1998	Sable Island, Canada (1,268)	Sand lance (75%), unk flounder (23.2%), Atlantic cod (24.5%), yellowtail flounder (21.1%), American plaice (18.5%)	Hard parts - scat	Bowen & Harrison (2006)
Winter 2004 – Winter 2008	Muskeget and Monomoy, MA (305)	Skates (24.5%), sand lance (14%), red/white hake (9.4%), windowpane flounder (7.1%), winter flounder (6.9%)	Hard parts - scat	Ampela (2009)
May 2017	Monomoy, MA (74)	Sand lance (97.30%), Atlantic menhaden (60.81%), unk Pleuronectidae (25.68%), Atlantic mackerel (22.97%), northern sea robin (21.62%)	DNA metabarcoding (16S mtDNA) - scat	Flanders et al. (2020)
October 2018 – October 2019	Monomoy, MA (247)	Skates (44.1%), crustaceans (30%), echinoderms (23.1%), sand lance (21.9%), phycid hake (19%)	Hard parts - scat	McCosker et al. (2023)
October 2018 – October 2019	Monomoy, MA (247)	Skates (47.8%), sand lance (28.7%), Atlantic menhaden (26.3%), windowpane flounder (25.1%), unk Pleuronectidae (21.9%)	DNA metabarcoding (16S mtDNA) - scat	McCosker et al. (2023
November 2017 – June 2022	Great Point, Nantucket, MA (112)	Skates (28.57%), sand lance (21.45%), unk fish (19.64%), phycid hake (9.82%), and windowpane flounder (8.04%)	Hard parts - scat	present study
November 2017 – June 2022	Great Point, Nantucket, MA (112)	Skates (87.5%), longhorn sculpin (82.14%), Atlantic cod (78.57%), sand lance (77.68%), and windowpane flounder (76.79%)	DNA metabarcoding (16S mtDNA) - scat	present study

Figures

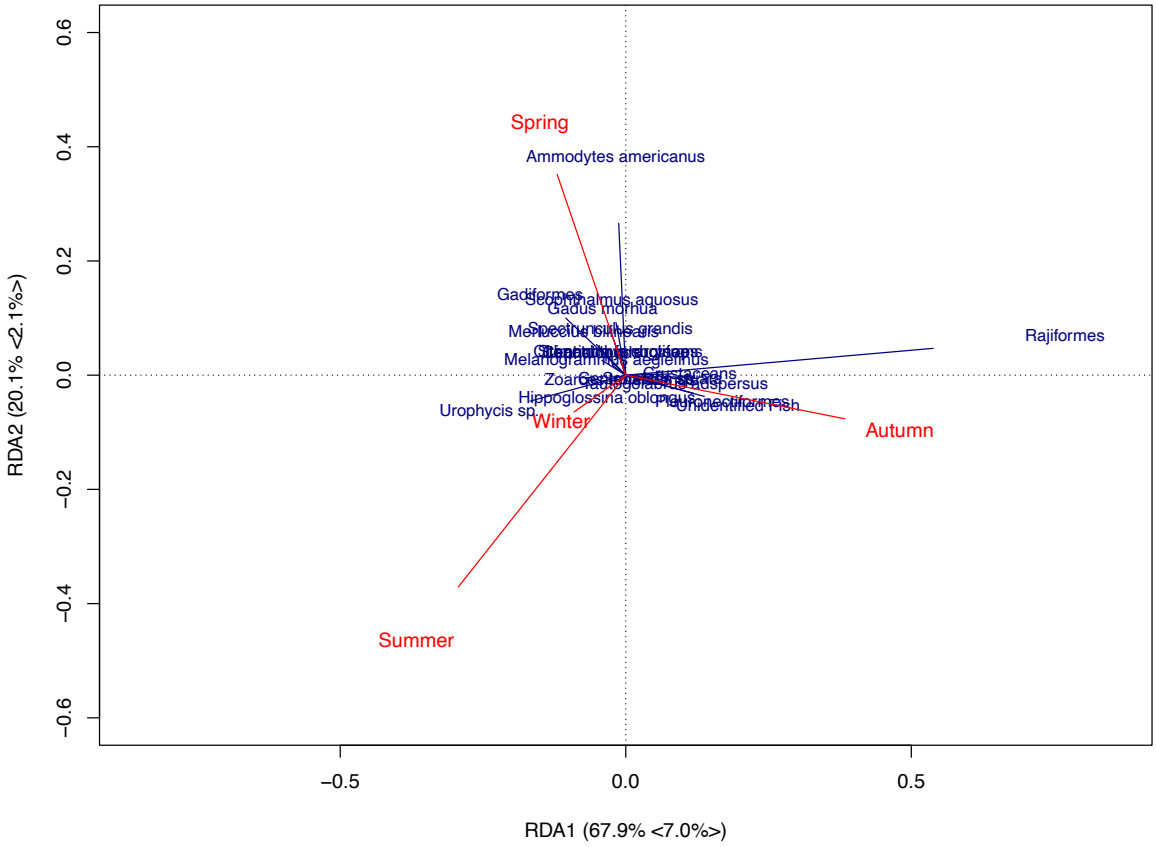

Figure 2.1 Redundancy Analysis (RDA) of prey types (blue) and seasons (red) of 112 gray seal scat samples using prey hard parts. The constrained axes explain 10.27% of the variance.

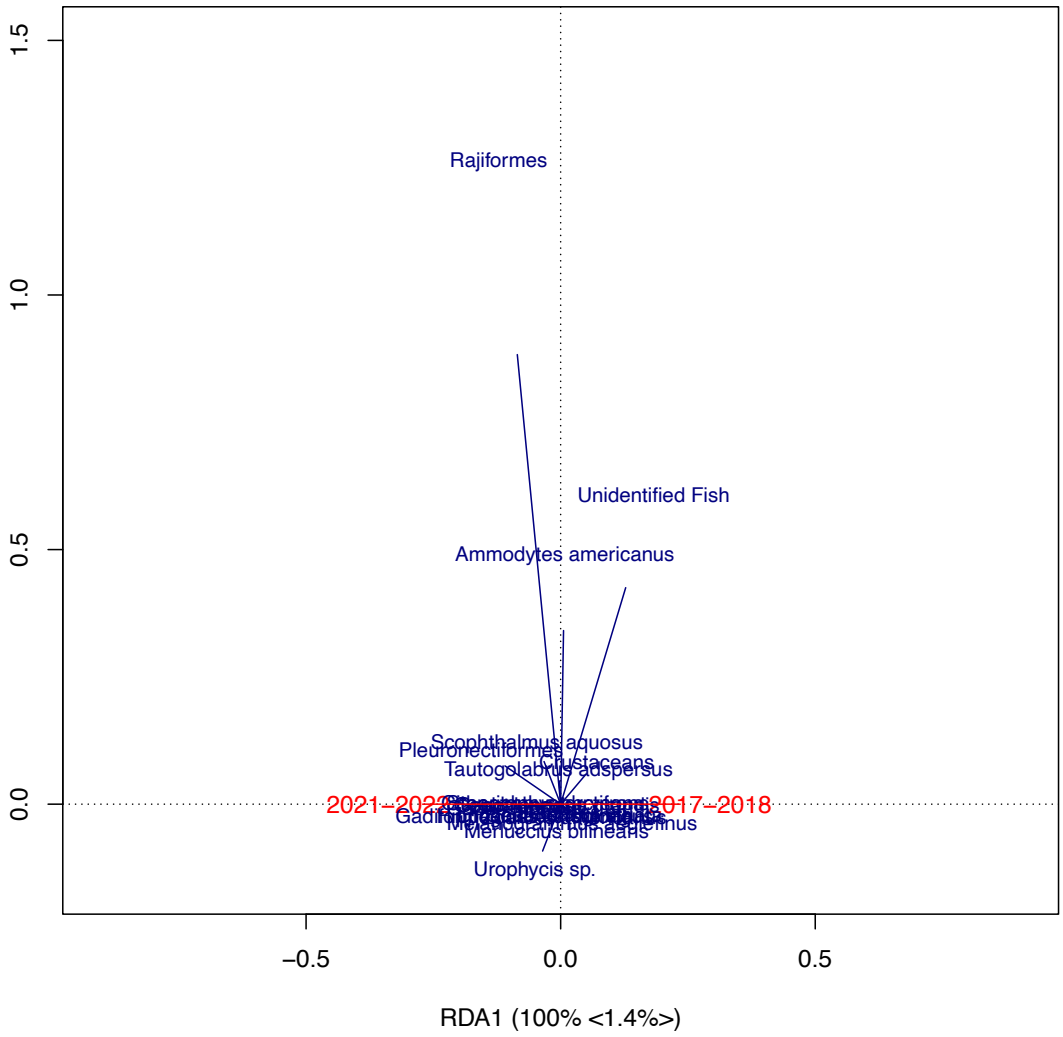

Figure 2.2 Redundancy Analysis (RDA) of prey types (blue) and years (red) of 112 gray seal scat samples using prey hard parts. The constrained axes explain 1.38% of the variance.

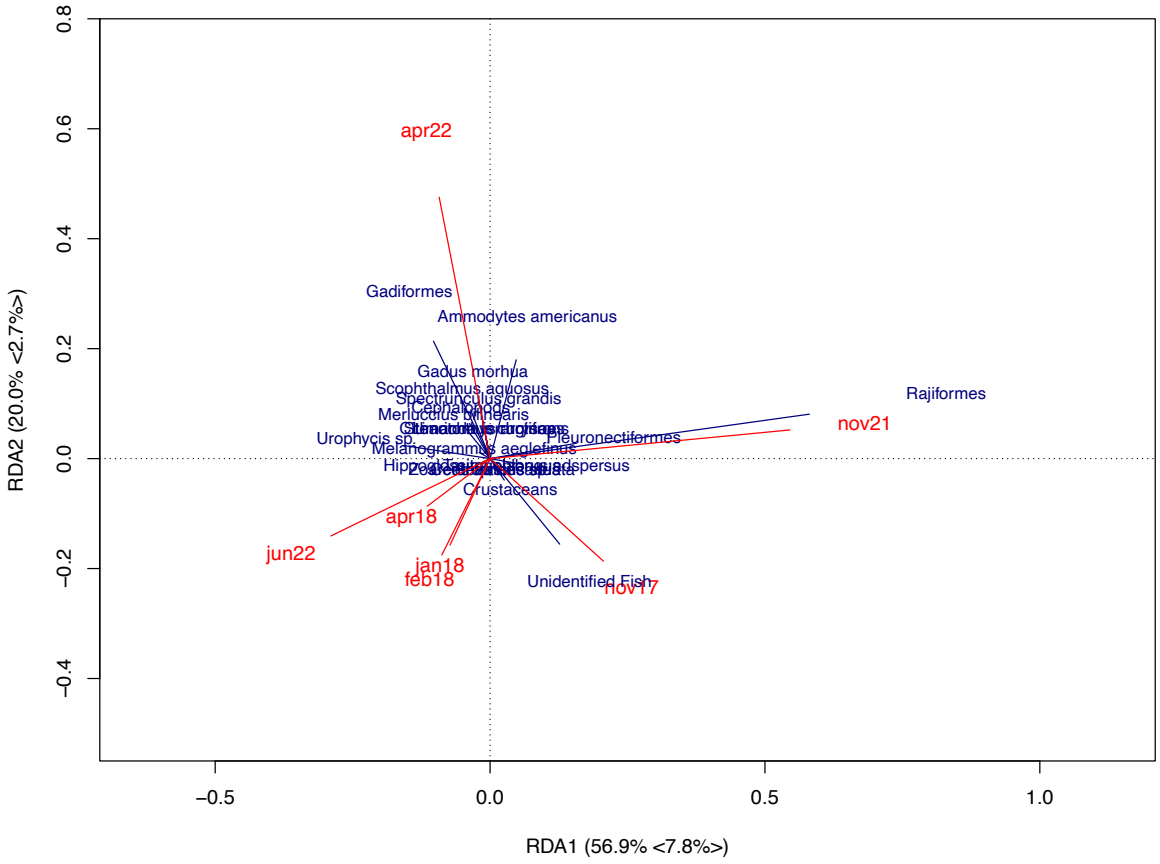

Figure 2.3 Redundancy Analysis (RDA) of prey types (blue) and sampling dates (red) of 112 gray seal scat samples using DNA Metabarcoding. The constrained axes explain 13.74% of the variance.

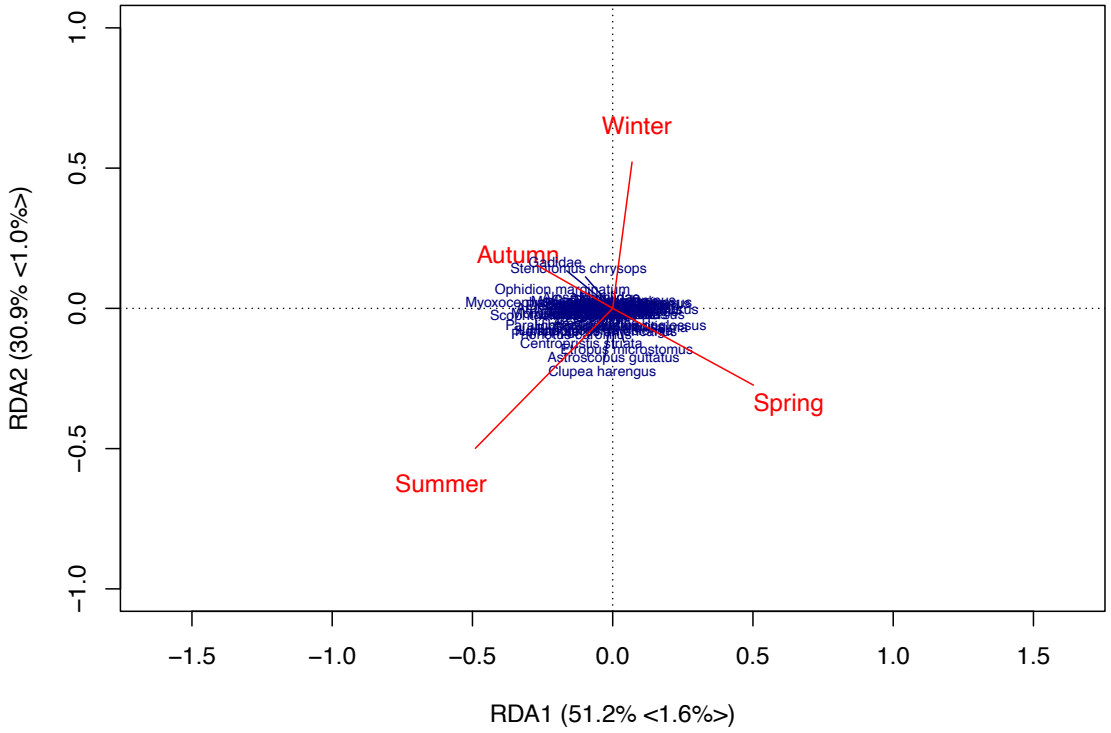

Figure 2.4 Redundancy Analysis (RDA) of prey types (blue) and seasons (red) of 112 gray seal scat samples using DNA Metabarcoding. The constrained axes explain 3.18% of the variance.

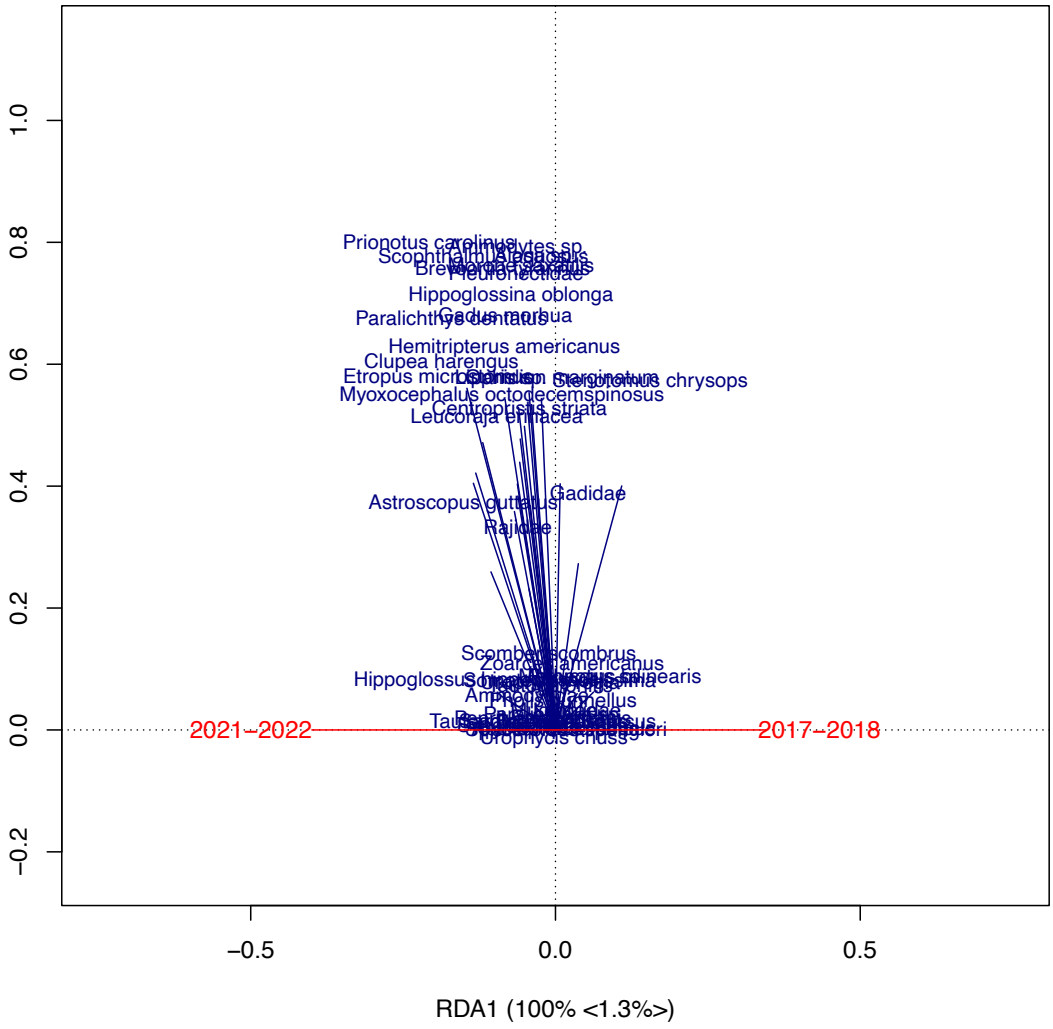

Figure 2.5 Redundancy Analysis (RDA) of prey types (blue) and years (red) of 112 gray seal scat samples using DNA Metabarcoding. The constrained axes explain 1.30% of the variance.

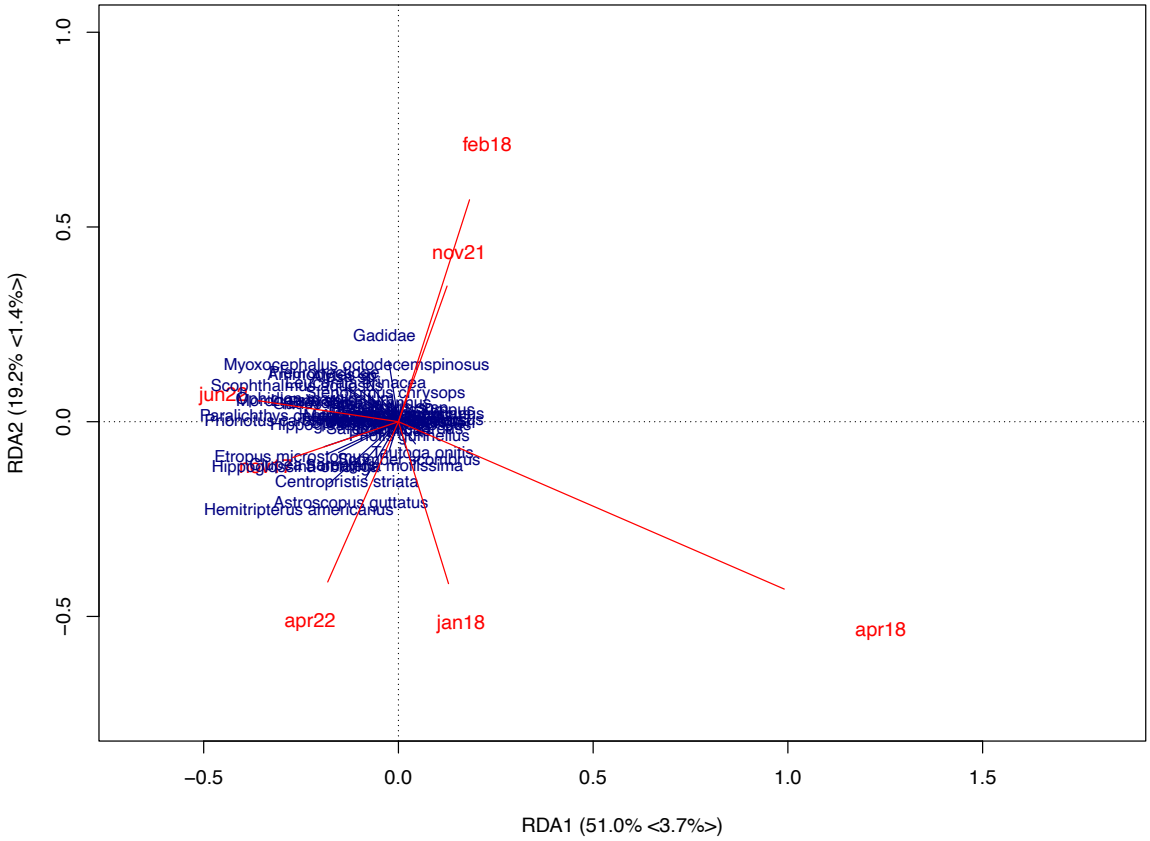

Figure 2.6 Redundancy Analysis (RDA) of prey types (blue) and sampling dates (red) of 112 gray seal scat samples using prey hard parts. The constrained axes explain 7.34% of the variance.

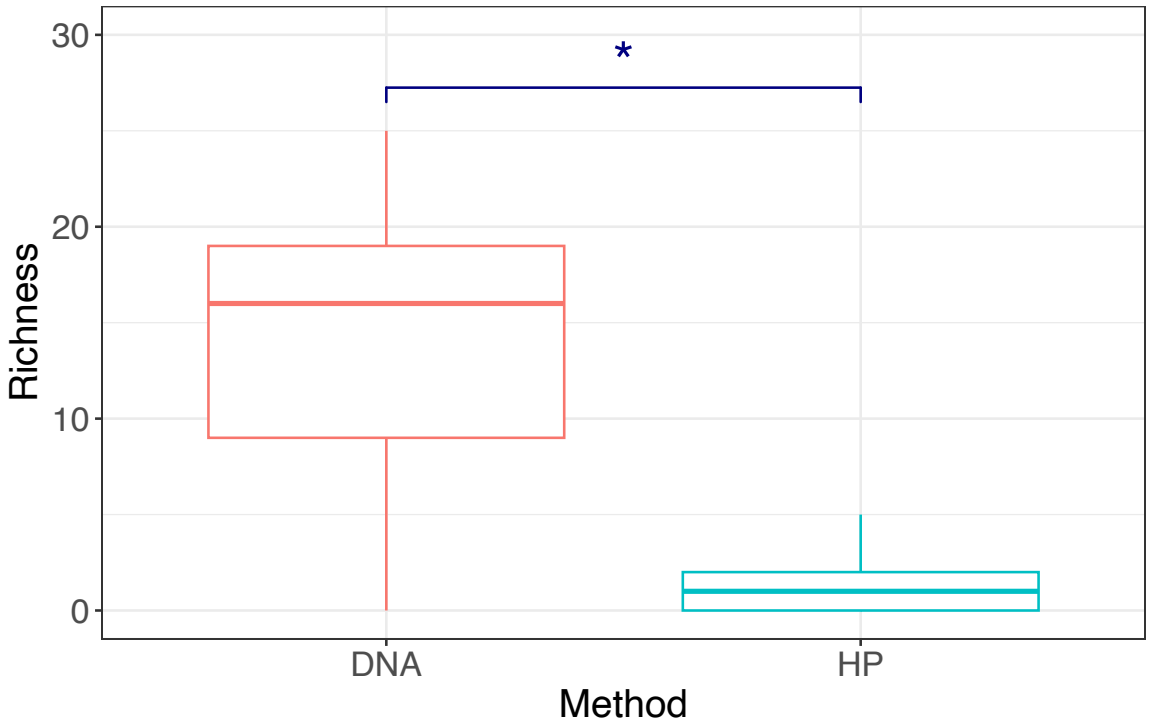

Figure 2.7 Boxplot of prey richness using DNA metabarcoding (DNA) and prey hard parts (HP) (n = 112). * indicates a p-value < 0.05 from a pairwise comparison with a Bonferroni adjustment.

CHAPTER 3

GRAY SEAL (*HALICHOERUS GRYPUS*) MICROPLASTIC INGESTION AND DIET

3.1 Abstract

Microplastics, which are plastic less than 5 mm^2, are a major threat to the marine environment. Many marine organisms, including pinnipeds, have been found ingesting microplastics. Their ability to adsorb and leach chemicals also makes them a vector for chemical pollutants and an avenue of exposure to organisms that ingest them. Pinnipeds have been observed indirectly ingesting microplastics through their prey. This chapter assesses the relationship between gray seal (*Halichoerus grypus*) diet and microplastic ingestion, furthering the understanding of bioaccumulation of microplastics. Anthropogenic microparticles were found in 111 out of 112 gray seal scat samples collected from Great Point, Nantucket. The most common type of microparticles were fibers, with the most common polymer, identified using μFTIR spectroscopy, being polyester. The mean microparticle abundance and concentration was 11.4 ± 9.91 microparticle and 0.886 ± 1.03 microparticles/gram of scat, respectively. When comparing microparticle concentration, type, fiber color, and polymer type to diet, using DNA metabarcoding, our results displayed weak relationships between the variables. However, our methods were not able to determine the

abundance of each prey type, making it difficult to draw any real conclusions. More research is needed to determine whether gray seals' diet is influencing their microplastic consumption. Still, it is likely that the microplastics detected in gray seal scat reflect the microplastics in their surrounding environment. Given their role as a sentinel species, gray seals, along with other pinnipeds, offer valuable insights into the distribution and impact of microplastics throughout their range. Future research should continue to utilize these pinnipeds as indicators to further investigate microplastic pollution in marine ecosystems.

3.2 Introduction

Plastic has been mass-produced since the 1940s (Cole et al., 2011). While the use of plastic is convenient due to its light weight and durability its disposal is frequently mismanaged, with over 75% of plastic debris in the ocean originating on land (Thompson et al., 2009). In 2010, 275 million metric tons of mismanaged plastic waste from 192 coastal countries entered the ocean (Jambeck et al., 2015). The United States was the 20th largest contributor, generating 0.28 million metric tons of mismanaged plastic waste (Jambeck et al., 2015). Plastic in the environment can undergo long-term degradation, fragmenting into smaller micro- and nano-sized pieces, which may be ingested or inhaled. (Meaza et al., 2021).

Microplastics are plastic that are less than 5 mm^2 (Cole et al., 2011). Primary microplastics are plastics that are less than 5 mm^2 when they are produced (i.e. virgin plastic production pellets and microbeads) (Cole et al., 2011). The breaking down of larger plastics creates secondary microplastics (i.e. fibers and fragments) (Cole et al., 2011). Microplastics typically enter the ocean through river discharge or by weathering of macroplastics that have

already been transported to the ocean (Andrady, 2011). Secondary microplastics are produced from physical, biological, and chemical degradation. Chemical degradation of plastics is typically caused by UV-B radiation from sunlight, and due to lower temperatures and oxygen concentrations in the ocean, plastic degrades slower in seawater than on land (Andrady, 2011). This slow chemical degradation of plastic in seawater is a major contributor to the persistence of microplastics in the marine environment.

Microplastic fragments and fibers have been found in many marine mammal species, with the primary route of exposure being ingestion (Meaza et al., 2021). Ingestion of microplastics can be either direct, for example the organism mistaking microplastics for prey, or indirect through trophic transfer (Besseling et al., 2015; Di Beneditto & Awabdi, 2014; Nelms et al., 2018). The types of microplastics found in the gastrointestinal (GI) tract of a humpback whale suggested that the plastics were directly ingested while filter feeding (Besseling et al., 2015). Pinnipeds are carnivorous and can indirectly ingest microplastics through their prey (Nelms et al., 2018).

Ingestion of microplastics can result in the uptake of pollutants and trace metals by marine organisms (Thompson et al., 2009). Toxic additives that are used during plastic production may leach out as the plastic fragments (Andrady, 2011). Additionally, chemical pollutants in the environment can adsorb to the surface of microplastics (Chen et al., 2019). In fact, hydrophobic organic contaminants are more likely to attach to the pores of plastics than to sediments on the ocean floor (Teuten et al., 2009). The effects of pollutant exposure via microplastics have been studied in smaller fish, bivalves, and sea urchins. Exposure to these chemicals can decrease depuration, reduce embryo development, damage organs, and cause oxidative stress (Hollerova et al., 2023; Nobre et al., 2015; Paul-Pont et al., 2016; Pitt

et al., 2018; Rochman et al., 2013). In addition to being a vector for chemical pollutants, microplastics can accumulate and cause blockages within the digestive system of small marine organisms, leading to starvation (Wright et al., 2013). Their ingestion can also create lesions in internal tissues (Ahrendt et al., 2020; Hamed et al., 2021).

Knowledge of the bioaccumulation of microplastics is critical to determining their impact on an entire ecosystem. Few studies have investigated the relationship between microplastic ingestion and the diet of wild pinnipeds. In both scat and GI tracts from gray seals (*Halichoerus grypus*) in the Northeast Atlantic, microplastic ingestion was positively correlated with Gadoid (cod and hake) consumption (Hernandez-Milian et al., 2019; Nelms, Parry, et al., 2019). In the scat, flatfish (Pleuronectiformes) were negatively correlated with microplastic ingestion (Nelms, Parry, et al., 2019). While reconstructing the diet of phocids in the Northwest Atlantic, microplastics were found in 2 gray seal scat samples (Hudak & Sette, 2019). These seals were feeding on sand lance (*Ammodytes sp.*), long-fin squid (*Doryteuthis pealeii*), and skate (Rajiformes) (Hudak & Sette, 2019). However, to fully understand microplastic ingestion by NW Atlantic gray seals, a larger sample size is required. Microplastic consumption was specifically investigated in gray seals on Great Point, Nantucket, with microplastics found in every sample (Hogan, 2022). The objective of this study is to assess the relationship between the diet composition and microplastic consumption of gray seals on Great Point, Nantucket. The results of this study will contribute to the understanding of the transport of microplastics through the local food web.

3.3 Methods

3.3.1 Scat Collection

A total of 112 gray seal scat samples were collected from Great Point, Nantucket on 7 dates between November 2017 and June 2022 (Table 3.1). The samples were collected at low tide using a slotted metal scoop to minimize contamination from the sand. They were then individually wrapped in aluminum foil and transported in a cooler to a -20°C freezer at the University of Massachusetts, Boston (UMB). A subsample of ~200 mg of each scat was stored in 1 mL of DNA/RNA shield (Zymo Research) and kept in a -20°C freezer until DNA extraction.

3.3.2 Microplastic Isolation

The weight, length, and width of each scat sample was taken. The sample was then transferred to a mason jar and left to thaw for 24 hours. Using a modified method developed by Hogan et al. (personnel communication), 100-500 ml of hot deionized (DI) water was added to the scat and stirred until the mixture was homogeneous. The scat mixture was then poured through a set of sieves sized 4 mm, 2 mm, 1 mm, 500 µm, 250 µm, 125 µm, and 63 µm. DI water was used to push the scat through each sieve. Any material remaining on the sieve was backwashed with DI water into a glass beaker and digested with equal parts 30% hydrogen peroxide (H_2O_2) resulting in a final concentration of 15% H_2O_2. Each beaker was stirred on a hot plate for ≤ 3 hours. If all the organic material was not digested after 3 hours, the beaker was covered with a glass petri dish and placed in an oven at 55°C for up to 10 days. The beaker was checked daily, and once the reaction was complete and the contents were clear, the mixture was poured back through its respective sieve in the sieve stack and

backwashed with DI water into a glass jar. The jars were covered with foil and placed in the oven until dry.

After every jar was dried, 150-200 ml of saturated NaCl water (1.16 – 1.18 g/ml) was added to each jar for density separation (Konechnaya et al., 2020). The jars were shaken and left to settle for 24 hours. The next day, the top layer of the saltwater was filtered through a cellulose membrane filter. The funnel was rinsed with 100 ml of DI water and the filter was placed into a labeled petri dish. This process was repeated 2 more times.

Microparticles on the filters were counted using an Olympus SZX12 stereomicroscope with a polarized light. The color and type (fiber, fragment, film, and foam) of each particle was recorded. Microparticles were then picked off the filters and transferred to a glass slide for μFTIR spectroscopy. An Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) microscope (Smiths IlluminatIR coupled to an Olympus microscope) was used to determine composition of the microparticles. The ATR-FTIR was set to 4 cm^{-1} resolution, Objective 36×-ATR, full spectral range 650–4000. FTIR spectra were obtained in transmission mode and CO_2 interference was removed for clarity. The spectra were read by an integrated software (Spectral ID) and were then matched to commercial libraries, Sigma Aldrich and Thermo-Fisher Scientific, and/or processed using Open Specy (Cowger et al., 2021). Spectral matches with a confidence greater than 70% were considered as positively identified. A total of 1526 particles were positively identified.

In order to account for contamination, lab and sand blanks were used. Lab blanks were created by pouring 100 ml of boiling DI water through the sieve stack. Each sieve was rinsed with 50 mL of DI water and 20 mL of DI water was backwashed into a mason jar. The backwash was poured back through its respective sieve, and then another 15 ml was

backwashed into the mason jars. Three 300g sand samples were collected during each sampling date. Both blank types were not digested with H_2O_2, however they did undergo density separation, and microparticles were counted using the same methods as the scat samples.

3.3.3 Diet

A subsample of ~200 mg of each scat was stored in 1 mL of DNA/RNA shield (Zymo Research) and kept in a -20°C freezer until DNA extraction. DNA was extracted using the *Quick*-DNA/RNA™ Magbead kit (Zymo Research). Samples were incubated in 25µl of Proteinase K at room temperature for 24 hours. Sample placement was randomized across the 96-well plates, and 1 extraction blank per plate (2 total) were used to test for contamination.

PCR was performed in duplicate to amplify the mtDNA 16S gene. Primer set B from Deagle et al (2009) (Chord_16S_F_TagA 5'- ATG CGA GAA GAC CCT RTG GAG CT -3', Chord_16S_R_Short 5'- CCT NGG TCG CCC CAA C -3') and a gray seal blocking primer designed by Flanders et al (2020) (Grayseal-block 5'- ATG GAG CTT TAA TTA ACT AAC TCA ACA GAA CAA /3SpC3/ -3') were used to amplify chordate DNA while reducing the amount of gray seal sequences. Forward and reverse primers were tagmented with Nextera adapter sequences (forward 5'- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG, reverse 5'- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G). PCR was done following the methods from Flanders et al (2020). Samples and blanks were amplified using Molecular Grade Water (12.8µl), 5x MyTaq Reaction Buffer (5µl), MyTaq DNA Polymerase (0.2µl), 10µM forward primer (2µl), 10µM reverse primer (2µl), 100µM gray seal blocking primer (2µl), and template DNA (1µl) for a total of 25µl per well.

Cycling conditions are described in Table 3.2. After thermocycling, PCR reactions were tested for amplification using a 1.6% agarose gel.

A second PCR was done to attach a unique combination of Nextera XT indices to each sample. Duplicates were pooled and PCR was done using Index 1 (i7) adapters (5µl), Index 2 (i5) adapters, Azura 2x Taq Mix (15µl), and PCR product (25µl) for a total of 50µl per well. Cycling conditions are described in Table 3.3. PCR reactions were again tested for amplification using a 1.6% agarose gel.

PCR products were then cleaned and normalized using the SequalPrep Normalization Plate Kit. All samples were then pooled for sequencing. The library was sequenced on an Illumina MiSeq using a MiSeq Reagent Kit v2 (300-cycles) with 2 x 250 bp chemistry at UMass Boston.

Bioinformatics were done using Qiime2 (Bolyen et al., 2019). The sequences were provided in a demultiplexed format, so reads were entered into Qiime2 as SampleData[PairedEEndSequencesWithQuality] using a manifest file. Next, the data was visualized and inspected for quality. Reads were trimmed where the median quality score dropped below 30. In this case, all bases had a median quality score above 30, so the reads were not trimmed. The data was then denoised using dada2. Taxonomy was assigned via a command-line BLAST using the NCBI non-redundant database. All OTUs that were returned as bacteria or had 2 or less reads were removed. The remaining taxa were inspected and filtered for suspected prey items in the Gulf of Maine (McCosker et al., 2023).

Prey hard parts were also isolated and identified using the methods described in Chapter 2. The measure of prey abundance used was minimum number of individuals (MNI),

which was determined from the number of otoliths of each prey type. Since bony fishes have 2 otoliths each, MNI was calculated as:

$$MNI = \frac{\#\ otoliths}{2}$$

rounded to the nearest whole number

3.3.4 Data Analysis

Environmental and lab contamination was accounted for by subtracting the amount of microparticles in sand and lab blanks from their respective sample. Microparticles were subtracted by color and type for accuracy. Microparticle counts for each sample were standardized by weight in order to be statistically compared. Presence of prey was measured using frequency of occurrence (FO), calculated as:

$$FO_i = \frac{n_i}{n} \text{ or } \%FO_i = \frac{n_i}{n} \times 100$$

Where: n = total number of samples
n_i = total number of samples with prey type i

All statistical analyses were done in R Version 2023.06.1+524. Microparticle concentrations, types, fiber colors, and polymer composition by FTIR spectroscopy were visually analyzed using the 'ggplot2' package. Models with the 'glm' function in the package 'car' were created to compare scat weight, microplastic abundance, and concentration between sampling dates. Diet and total microparticle concentration were analyzed through generalized linear models as well. To assess the relationship between diet and microparticle types, fiber colors, and polymer composition, redundancy analyses (RDA) were performed using the 'rda' function. Prey were classified and grouped at the family taxonomic level. Microplastic fiber colors were analyzed by creating a concentration matrix. Polymer composition was analyzed by creating a matrix based on percent composition. Microparticle

types and diet were analyzed by creating presence/absence matrices. Before creating PCAs and RDAs, non-binary matrices were Hellinger transformed using the 'decostand' function. A permutational multivariate ANOVA (PERMANOVA) was done using the 'adonis2' function to analyze the significance of season in contributing to prey diversity. Any significant results were further analyzed using the 'simper' function.

3.4 Results

3.4.1 Microplastics

Microparticles were found in 99.1% of samples (111/112). The average number of microparticles per scat sample was 11.4 ± 9.91, with a range of 0 to 60 MP/sample. The minimum concentration of all microparticles was 0 microparticles /gram of scat (MP/g), the maximum was 2.56 MP/g, and the mean was 0.886 ± 1.03 MP/g (Table 3.1). Mean scat weight was not different between sampling dates ($p > 0.05$; Figure 3.1). Mean microparticle abundance was significantly different between sampling dates ($p = 0.004$), with April 2018 and April 2022 being greater than February 2018 (Figure 3.2). Additionally, mean microparticle concentration was significantly different between sampling dates ($p = 0.013$). January 2018 had a greater mean microparticle concentration than November 2017, February 2018, November 2021, and April 2022 (Figure 3.3).

The most common type of microparticle found in the scat were fibers (95.45%; Figure 3.4). Fibers also had the highest percent composition during every sampling date (Table 3.4, Figure 3.5a). Other less common microparticle types were knotted fibers (2.9 %), fragments (1.18%), foam (0.31%), and films (0.16%) (Figure 3.4). Fibers were found in every sample except for 1 from November 2021, which did not contain any microparticles

(Figure 3.5b). Knotted fibers were found in 8 samples from November 2017, 2 samples from January 2018, 5 samples from February 2018, 4 samples from April 2018, 7 samples from April 2022, and 5 samples from June 2022 (3. 5b). Fragments were found in 3 samples from November 2017, 1 sample from February 2018, 1 sample from April 2018, and 9 samples from April 2022 (3. 5b). Foam was found in 4 samples from April 2018 (Figure 3.5b). Color film was found in 1 sample from November 2021 and 1 sample from June 2022 (Figure 3.5b).

Synthetic (petroleum-based) and cellulose-based anthropogenic microparticles were detected using FTIR spectroscopy. Synthetic, or petroleum-based, microplastics made up 20.5% of the microparticles and were found in 96% of samples (Figure 3.6). Nine plastic polymer types were detected in 99 samples: polyester (PET), nylon (PA), polytetrafluoroethylene (PTFE), polypropylene (PP), polyethylene (PE), acrylic, polyvinylchloride (PVC), and polystyrene (PS). The most common polymers were PET (58.12%), PA (28.57%), and PTFE (6.49%) (Figure 3.7). Polyester was the most common polymer in every sample date, followed by nylon (Table 3.5, Figure 3.8). PTFE was most common in April 2018, followed by April 2022 (Table 3.5, Figure 3.8). February 2018 and April 2018 had the greatest percent composition of PE (Table 3.5, Figure 3.8). PP was most common in February 2018, followed by January 2018 (Table 3.5, Figure 3.8). PVC and polystyrene were only identified in April 2022 and February 2018, respectively (Table 3.5, Figure 3.8).

Eight fiber colors were found throughout the 111 samples with microparticles. Those colors were black, spotted, blue, red, clear, purple, green, and yellow. Spotted fibers were

any fiber with a distinct pattern of being clear and another color. The most common fiber colors were black (38.31%), spotted (16.71%), red (15.82%), and blue (15.26%) (Figure 3.9).

3.4.2 Microplastics and Diet

Liparidae was the only prey family where mean microparticle concentration differed significantly by its presence. Samples with Liparidae had a greater mean microparticle concentration than samples without (Figure 3.10). The mean microparticle concentration of samples with Liparidae was 0.332 ± 0.447, while the mean microparticle concentration of samples without Liparidae was 0.193 ± 0.370. The frequency of occurrence of Liparidae was 45.54% (Table 3.6).

Samples with Cyclopsettidae ($p = 0.005$) and Pholidae ($p = 0.009$) had significantly different microparticle type compositions than other prey types. Knots and fragments contributed 87.5% and 82.9% to the difference of Cyclopsettidae's and Pholidae's microplastic type composition, respectively. Pholidae had a very strong positive relationship with knots due to the angle between the two vectors being much less than 90° (Figure 3.11). Pholidae had a slightly positive relationship with fragments since the angle between the two was closer to 90° (Figure 3.11). Cyclopsettidae also had positive relationships with both knots and fragments (Figure 3.11). Cyclopsettidae and Pholidae had FOs of 50% and 4.46%, respectively (Table 3.6).

Samples with Anatidae ($p = 0.036$), Lophiidae ($p = 0.048$), Phycidae ($p = 0.039$), Serranidae ($p = 0.015$), Tetraodontidae ($p = 0.042$), and Uranoscopidae ($p = 0.018$) had a significantly different composition of fiber colors than other prey types. The colors that contributed the most to these differences were black, blue, spotted, red, and purple (Table 3.7) Anatidae and Uranoscopidae had strong negative relationships with black fibers, where

Lophiidae and Phycidae had positive correlations with black fibers (Figure 3.12). Serranidae was negatively correlated with red fibers and Tetraodontidae had a negative relationship with spotted fibers (Figure 3.12).

There were no prey families that had a significantly different composition of plastic polymers ($p > 0.05$). Additionally, prey families were grouped by their diet (Table 3.6). Prey diet had no significant effect on microparticle concentration, microparticle type, fiber color, or plastic polymer composition ($p > 0.05$). The 10 prey families with the highest frequency of occurrence for each sample date are listed in Table 3.8. Rajidae, Gadidae, Paralichthyidae, Cottidae, Clupeidae, Pleuronectidae, Scophthalmidae, Ammodytidae, and Moronidae were within the top 10 during every sampling date (Table 3.8). Triglidae was in the top 10 in 6 out of 7 dates, Serranidae in 2, and Cyclopsettidae and Agonidae in 1 (Table 3.8).

Prey hard parts (e.g. otoliths, vertebrae, squid beaks, etc.) were identified microscopically (Chapter 2). Since DNA metabarcoding cannot quantify prey abundance, otoliths were used to estimate the relationship between prey abundance, calculated as minimum number of individuals (MNI) and microparticle concentration. While there was a slight positive trend in Pleuronectiformes MNI and microparticle concentration (Figure 3.13), there were no significant relationships between Gadiformes (Figure 3.14), Perciformes (Figure 3.15), and Pleuronectiformes MNI and microparticle concentration ($p > 0.05$).

3.5 Discussion

3.5.1 Microplastics in gray seals

Almost all scat samples in this study contained microparticles (99.1%), with 96% of samples containing at least 1 synthetic microplastic, indicating gray seals around Great Point,

Nantucket are frequently exposed to microplastics. The units used to report microplastic ingestion in gray seals is not consistent across studies, thus we reported both mean abundance and concentration of microparticles in scat. In the present study, the average abundance was 11.4 ± 9.91 MP/scat. This is slightly different from other gray seal microplastic studies on the Northeast Atlantic population. Gray seals bycaught off the coast of Ireland averaged 27.9 MP/seal, and a preliminary study using scat from Wales had a range of 1 to 5 MP/scat (Hernandez-Milian et al., 2019; Nelms, Parry, et al., 2019). Philipp et al (2020) assessed microplastics in scat from both gray and harbor seals in German waters, which had an average of 6 fibers/scat and 13.3 fragments/scat. The current study found that the mean microparticle concentration was 0.89 ± 1.03 MP/g. Gray seal scat subsamples from the North Sea had a very similar estimated concentration of 0.81 MP/g, and a smaller sample size of scat collected from Great Point had a lower mean concentration of 0.43 MP/g (Desclos-Dukes et al., 2022; Hogan, 2022).

The majority of microparticles recovered were fibers (95.45%), with knots, fragments, films, and foam being less common (4.55%). This is consistent with most other studies, where fibers are most common (Desclos-Dukes et al., 2022; Hernandez-Milian et al., 2019; Hogan, 2022; Nelms, Parry, et al., 2019). In contrast, harbor and gray seal scat from Germany had more fragments than fibers (Philipp et al., 2020). Philipp et al. 2020 only assessed particles greater than 100μm which may exclude smaller microfibers, whereas the current study was able to extract particles a small as 63μm. Microplastic isolation methods are not consistent between studies. Nelms et al. (2019), Philipp et al. (2020), and Desclos-Dukes et al. (2022) all used enzymatic digestion to remove organic matter, whereas Hernandez-Milian et al. (2019) used 10% KOH and Hogan (2022) used 15% H_2O_2.

Black, spotted, red, and blue were the most common fiber colors found the scat. This is similar to other studies which reported black, blue, red, clear, and gray as common fiber colors (Desclos-Dukes et al., 2022; Hogan, 2022; Nelms, Parry, et al., 2019). Polyester (58.12%) and nylon (28.57%) were the most abundant plastic polymers detected using μFTIR spectroscopy. In Philipp et al (2020), the most common polymers were polyethylene, ethylene-vinyl-acetate, nylon, and polypropylene. Additionally, Hudak and Sette (2019) opportunistically identified 2 microparticles in gray seal scat from Cape Cod, Massachusetts as cellophane and EPDM rubber.

3.5.2 Sources of marine microplastics

The most common type of microplastic found in the scat was polyester (Figure 3.7). Polyester microfibers are typically shed from clothing and enter the ocean through wastewater and river discharge (Kutralam-Muniasamy et al., 2020). A 6 kg wash of polyester clothing has been estimated to release 500,000 microfibers (Napper & Thompson, 2016). Additionally, polyester made up 73% of synthetic fibers in near-surface Arctic water samples (Ross et al., 2021). Many of these fibers were classified as new/unweathered, providing evidence of the transport of microfibers from textiles, laundry, and wastewater to remote areas (Ross et al., 2021). Another less common source of polyester fibers are maritime ropes and nets (Corniuk et al., 2023).

The second most abundant type of plastic identified in this study was nylon, which fishing line and nets are commonly made of (Battisti et al., 2019; Corniuk et al., 2023; Vitale et al., 2023; Weißbach et al., 2022). Fishing gear is also frequently made of polyethylene and polypropylene which were recovered in the scat (Corniuk et al., 2023; Vitale et al., 2023; Weißbach et al., 2022). PTFE made up 6.5% of μFTIR-tested microplastics (Figure 3.7) and

is used as a low-friction coating on many marine instruments (Argos Surface Technologies, 2024). Its weather, chemical, and saltwater resistant coating makes it ideal to use in the marine environment (Argos Surface Technologies, 2024).

3.5.3 Microplastics and gray seal diet

Our results suggest that gray seal diet has a limited effect on the amount and type of microparticles that they consume. Samples with Liparidae (snailfish) had a higher mean microparticle concentration than samples without. However, the standard deviation for each group was greater than the mean, indicating there was high variability in microparticle concentration within samples with and without Liparidae. Additionally, only Cyclopsettidae (smallmouth flounder) and Pholidae (rock gunnel) had significantly different microparticle type distributions than other prey families. Fiber colors had strong relationships with more prey types, with Phycidae (phycid hakes) and Tetraodontidae (bandtail puffer) being positively related to black and purple fibers, and Serranidae (black sea bass) being negatively correlated with red fibers (Figure 3.12). While these results are significant, they only indicate a correlation between the above fish types and certain microparticles. More research is needed to determine whether different fish types are ingesting specific colors and types of microparticle.

The diet analysis method used in this study, DNA metabarcoding, currently only provides qualitative data on the presence or absence of a prey type in a sample. While there was no clear evidence that the presence of certain prey types in the scat influenced microparticle abundance or type, it is not known whether the abundance of these prey types has an effect on gray seal microparticle ingestion. Hard parts can provide limited information on prey abundance due to biases in otolith digestion and recovery (Chapter 2). There were no

significant relationships between prey abundance (MNI) from hard parts and microparticle concentration, however there was a slight positive trend in Pleuronectiform abundance and microparticle concentration (Figure 3.13). Future research should focus on quantifying prey types using DNA metabarcoding to accurately assess the abundance of gray seal prey.

Both April 2018 and April 2022 had unique microplastic and diet results. These sampling dates had the highest microparticle abundance (Figure 3.2) and PTFE percent composition (Figure 3.8). April 2022 was the only sampling date with PVC (Figure 3.8) and had the highest fragment percent composition (Figure 3.5a). Additionally, April 2018 was the only sampling date with foam (Figure 3.5). When looking at the 10 prey families with the highest FO, April 2018 had the lowest FO overall (Table 3.8). April 2022 was the only sampling date with Cyclopsettidae, and April 2018 was one of two sampling dates with Serranidae in their top 10 prey (Table 3.8). Without knowing the abundance of these prey types, it's difficult to distinguish any strong relationships between microparticles and diet in these samples.

The diet analysis of these samples revealed that gray seals on Great Point have a truly generalist diet, with a mean prey richness of 13.1 (Chapter 2). Pelagic fish have been reported consuming more microplastics than demersal fish (Covernton et al., 2022; McGoran et al., 2018; Rummel et al., 2016). We did not see any relationships between gray seal prey's diet and microparticle consumption. However, since there were often combinations of fish with different diets in one sample, these differences would not have been seen. It is important to note that over 50% of the gray seal prey was benthic, indicating that they feed on or near the seafloor (Table 3.6). The majority of the microplastics identified in this study are also denser than seawater (density > 1.03 kg/L), meaning that gray seals are primarily ingesting

microplastics that sink (Andrady, 2011; Grigorescu et al., 2019). PET, PA, PVC, and PS all have densities greater than seawater (Andrady, 2011; Grigorescu et al., 2019). Similarly, PA was the most common type of microplastic ingested by gray seals in the NE Atlantic (Nelms, Parry, et al., 2019). The most commonly produced plastics worldwide are PE and PP, which are less dense than seawater (Andrady, 2011; Bråte et al., 2014; Grigorescu et al., 2019). However, these plastics were found in low quantities in the scat (Table 3.5). This suggests that gray seals' benthic feeding behavior may be correlated with consumption of high density microplastics. Captive gray seals that were fed only mackerel, a pelagic fish, mainly ingested low density plastics like PE (Nelms et al., 2018). More research is needed to determine the density of the microplastics that benthic fish are ingesting.

The primer used in the diet analysis was only able to amplify chordate DNA, excluding any invertebrate prey from all analyses in this study. Invertebrates, notably squid, make up an important part of gray seal diet (Gibson, 2023; McCosker et al., 2020). The prey hard parts data showed invertebrate remains during November 2017, April 2018, April 2022, and June 2022 (Chapter 2). Future research should investigate the relationship between gray seals' entire diet and their ingestion of microplastics.

3.5.4 Microplastics in New England

Previous studies assessing indirect ingestion of microplastics by gray seals used captive seals in a closed system (Nelms et al., 2018). While gray seals do indirectly ingest microplastics through their prey, they can still directly consume microplastics directly from the surrounding environment. It is impossible to determine the route through which the seals were exposed to the microplastics found in the scat samples from the present study.

Since our results show that diet may not strongly influence microparticle ingestion, it is plausible that the microparticle found in the scat are representative of microparticles in the surrounding environment. Although microparticles in the water around Great Point specifically have not been assessed, a baseline of microparticles in sand and water on and around the entire island of Nantucket was collected (Hogan, 2022). Fibers made up 98% of the microparticles in water samples, similar to our results where 95.45% of the microparticles were fibers (Hogan, 2022).

Microplastic studies on pinnipeds in Mexico and South America found that animals from oceanic rookeries ingested more microplastics than those from coastal rookeries (Ortega-Borchardt et al., 2023a; Perez-Venegas et al., 2020). Gray seals have breeding and haul-out sites along a large portion of the east coast of North America (den Heyer et al., 2021; Wood et al., 2020). Many of the sites in southern New England are closer to urban areas than the sites in Maine and Canada (den Heyer et al., 2021; Wood et al., 2020). Sampling gray seal scat at various sites in the Gulf of Maine may provide information on the differences in microplastic abundance and make-up at different locations.

3.5.5 Conclusions

The findings of this study highlight the presence of microplastics, particularly fibers, in the diets of gray seals inhabiting the area surrounding Great Point, Nantucket. While indirect ingestion through prey is possible, our results reveal minimal correlation between the microplastics ingested by seals and the type of prey they consumed. However, our methods were not able to determine the abundance of each prey type, making it difficult to draw any real conclusions. More research is needed to determine whether gray seals' diet is influencing their microplastic consumption. Still, it's likely that the microplastics detected in gray seal

scat reflect the microplastics in their surrounding environment. Given their role as a sentinel species, gray seals, along with other pinnipeds, offer valuable insights into the distribution and impact of microplastics throughout their range. Future research should continue to utilize these pinnipeds as indicators to further investigate microplastic pollution in marine ecosystems.

Tables

Table 3.1 Gray seal scat sample size and average (Avg), standard deviation (sd), and range of scat weights (grams), microparticle (MP) abundance, and microparticle concentrations (MP Abundance/scat weight) by sample date, collected from Great Point, Nantucket.

Date	Sample Size	Weight (g)		MP Abundance		MP Concentrations (MP/g)	
		Avg ± sd	Range	Avg ± sd	Range	Avg ± sd	Range
11/5/17	18	218 ± 246	21 – 956	9.71 ± 8.19	1 – 28	0.190 ± 0.297	0.003 – 0.892
1/15/18	6	206 ± 257	5.5 – 530	14.2 ± 9.70	0 – 26	0.886 ± 1.03	0.001 – 2.56
2/9/18	21	124 ± 111	6 – 420	6 ± 3.92	1 – 14	0.184 ± 0.489	0.003 – 2.30
4/20/18	10	91.2 ± 61.3	22 – 211	20.2 ± 17.6	4 – 60	0.360 ± 0.367	0.038 – 1.14
11/7/21	14	185 ± 173	10 – 500	9.57 ± 6.95	0 – 26	0.172 ± 0.232	0 – 0.775
4/9/22	23	125 ± 109	14 – 434	13.4 ± 7.76	2 – 29	0.221 ± 0.267	0.012 – 1.27
6/7/22	20	63.3 ± 33.1	14 – 150	12.8 ± 11.6	1 – 50	0.251 ± 0.218	0.003 – 0.845
Total	112	138 ± 154	5.5 – 956	11.4 ± 9.91	0 – 60	0.256 ± 0.411	0 – 2.56

Table 3.2 PCR conditions for the amplification of the 16S mtDNA gene using primer set B from Deagle et al (2009) and a gray seal blocking primer from Flanders et al (2020).

Temperature (°C)	Time	Number of Cycles
95	3 min.	x1
95	30 sec.	x35
57	30 sec.	
72	30 sec.	
72	10 min.	x1
4	Continuous	Continuous Until Removed

Table 3.3 PCR conditions for the attachment of Nextera XT i7 and i5 adapters.

Temperature (°C)	Time	Number of Cycles
72	3 min.	x1
95	30 sec.	
95	10 sec.	x12
55	30 sec.	
72	30 sec.	
72	5 min.	x1
10	Continuous	Continuous Until Removed

Table 3.4 Microparticle type % composition by sampling date.

Date	Fibers	Color Film	Foam	Fragments	Knots
11/5/17	93.9	0	0	1.8	4.2
1/15/18	97.6	0	0	0	2.4
2/9/18	94.4	0	0	0.8	4.8
4/20/18	95.0	0	2.0	0.5	2.5
11/7/21	97.8	0.7	0	0	1.5
4/9/22	93.9	0	0	3.2	2.9
6/7/22	97.3	0.4	0	0	2.4

Table 3.5 FTIR % composition by sampling date.

Date	Polyester	Nylon	PTFE	Polystyrene	PP	PE	Polyacrylic	PVC
11/5/17	65.8	28.9	2.6	0	4.8	0	0	0
1/15/18	66.7	23.8	4.8	0	4.8	0	0	0
2/9/18	68.1	18.8	1.4	1.4	5.8	4.3	0	0
4/20/18	47.6	23.8	23.8	0	0	4.8	0	0
11/7/21	64.3	31.0	0	0	0	2.4	2.4	0
4/9/22	47.2	27.8	16.7	0	2.8	2.8	0	2.8
6/7/22	49.4	39.2	7.6	0	2.5	0	1.3	0

Table 3.6 Diet and percent frequency occurrence (%FO) of prey families identified in 112 seal scat samples from DNA metabarcoding.

Diet	Family	% FO
Benthivore	Rajidae	87.50
	Cottidae	82.14
	Scophthalmidae	76.79
	Triglidae	72.32
	Serranidae	51.79
	Cyclopsettidae	50.00
	Liparidae	45.54
	Ophidiidae	42.86
	Agonidae	41.96
	Sparidae	36.61
	Uranoscopidae	23.21
	Zoarcidae	7.14
	Labridae	6.25
	Pholidae	4.46
	Cyclopteridae	0.89
	Tetraodontidae	0.89
Piscivore	Pleuronectidae	79.46
	Paralichthyidae	75.89
	Moronidae	74.11
	Anatidae	8.04
	Lophiidae	1.79
	Bothidae	0.89
Planktivore	Clupeidae	79.46
	Ammodytidae	77.68
	Scombridae	7.14
	Mugilidae	1.79
	Stromatidae	0.89
	Syngnathidae	0.89
Planktivore/Benthivore	Gadidae	80.36
	Phycidae	7.14

Table 3.7 Prey families with significantly different microparticle fiber (MFP) color composition and the MFP colors that strongly contributed to these differences from a SIMPER analysis.

Family	MFP colors
Anatidae	Black, blue, spotted, red
Lophiidae	Red, spotted, purple, black
Phycidae	Black, red, spotted, blue
Serranidae	Black, red, spotted, blue
Tetraodontidae	Purple, black, red, spotted
Uranoscopidae	Black, spotted, red, blue

Table 3.8 Top 10 prey families with the highest % frequency of occurrence (FO) by sample date.

February 2018		April 2018		November 2021		April 2022		June 2022	
Family	% FO	Family	% FO	Family	% FO	Family	% FO	Family	% FO
Rajidae	95.24	Rajidae	70	Rajidae	85.71	Rajidae	91.3	Cottidae	95
Cottidae	90.48	Cottidae	60	Cottidae	85.71	Gadidae	86.96	Rajidae	95
Clupeidae	85.71	Clupeidae	60	Clupeidae	78.57	Paralichthyidae	86.96	Pleuronectidae	90
Gadidae	85.71	Gadidae	60	Gadidae	78.57	Triglidae	82.61	Scophthalmidae	90
Pleuronectidae	85.71	Pleuronectidae	50	Pleuronectidae	78.57	Cottidae	78.26	Ammodytidae	90
Ammodytidae	80.95	Ammodytidae	50	Ammodytidae	78.57	Clupeidae	78.26	Triglidae	85
Scophthalmidae	76.19	Serranidae	50	Scophthalmidae	78.57	Pleuronectidae	78.26	Clupeidae	85
Paralichthyidae	76.19	Scophthalmidae	40	Triglidae	78.57	Scophthalmidae	78.26	Moronidae	85
Moronidae	71.43	Paralichthyidae	40	Paralichthyidae	71.43	Ammodytidae	73.91	Gadidae	80
Triglidae	61.9	Moronidae	40	Moronidae	71.43	Moronodiae	73.91	Paralichthyidae	80
						Cyclopsettidae	73.91		

	November 2017		January 2018	
	Family	% FO	Family	% FO
1	Moronidae	88.89	Cottidae	83.33
2	Rajidae	83.33	Rajidae	66.67
3	Pleuronectidae	83.33	Gadidae	66.67
4	Scophthalmidae	83.33	Paralichthyidae	66.67
5	Ammodytidae	83.33	Triglidae	66.67
6	Clupeidae	83.33	Clupeidae	66.67
7	Gadidae	83.33	Pleuronectidae	66.67
8	Paralichthyidae	83.33	Scophthalmidae	66.67
9	Triglidae	77.78	Ammodytidae	66.67
10	Cottidae	72.22	Moronidae	66.67
11			Agonidae	66.67
12			Serranidae	66.67

Figures

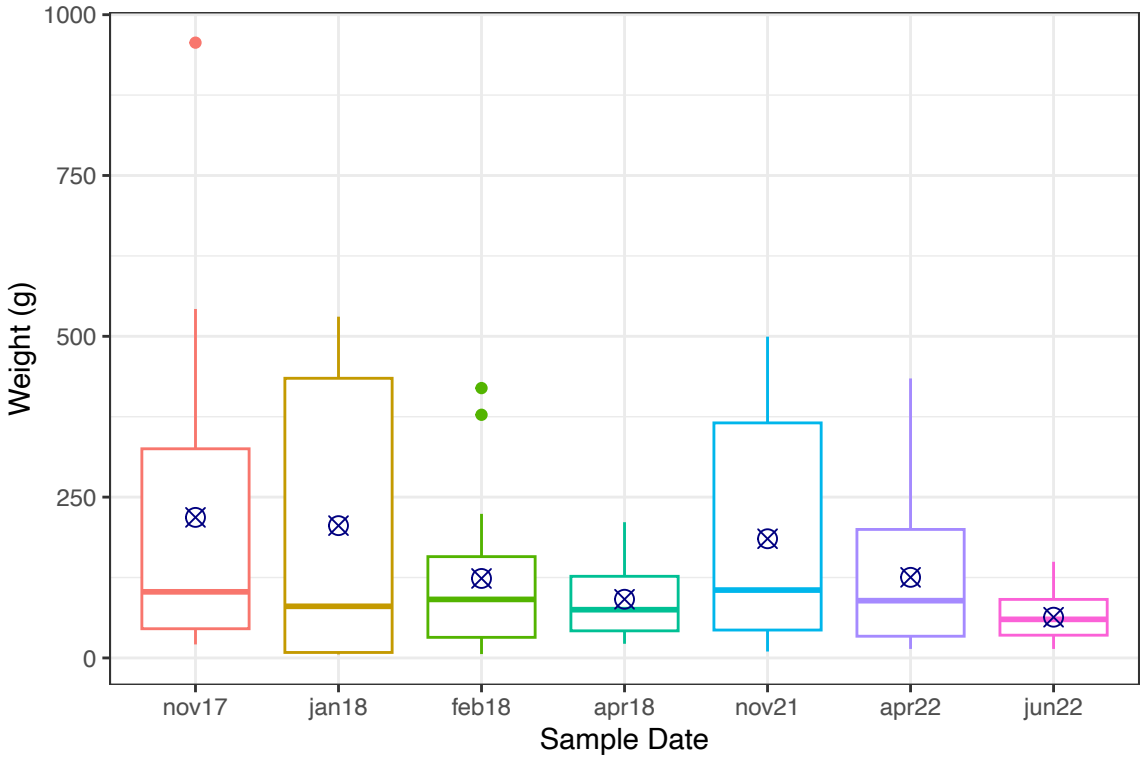

Figure 3.1 Scat weight by sample date. November 2017 (n = 18), January 2018 (n = 6), February 2018 (n = 21), April 2018 (n = 10), November 2021 (n = 14), April 2022 (n = 23), June 2022 (n = 20). Boxplot shows median, interquartile ranges, and outlier. Points represent the mean, * indicates a p-value < 0.05 from a pairwise comparison.

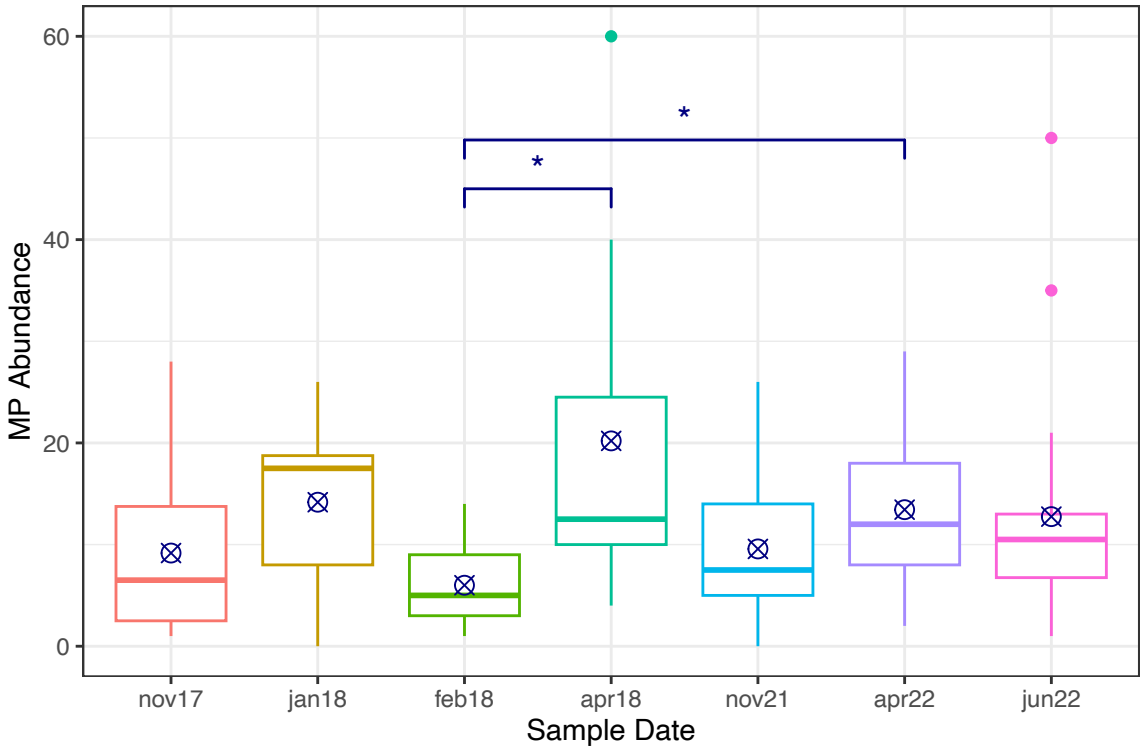

Figure 3.2 Microparticle abundance by sample date. November 2017 (n = 18), January 2018 (n = 6), February 2018 (n = 21), April 2018 (n = 10), November 2021 (n = 14), April 2022 (n = 23), June 2022 (n = 20). Boxplot shows median, interquartile ranges, and outlier. Points represent the mean, * indicates a p-value < 0.05 from a pairwise comparison.

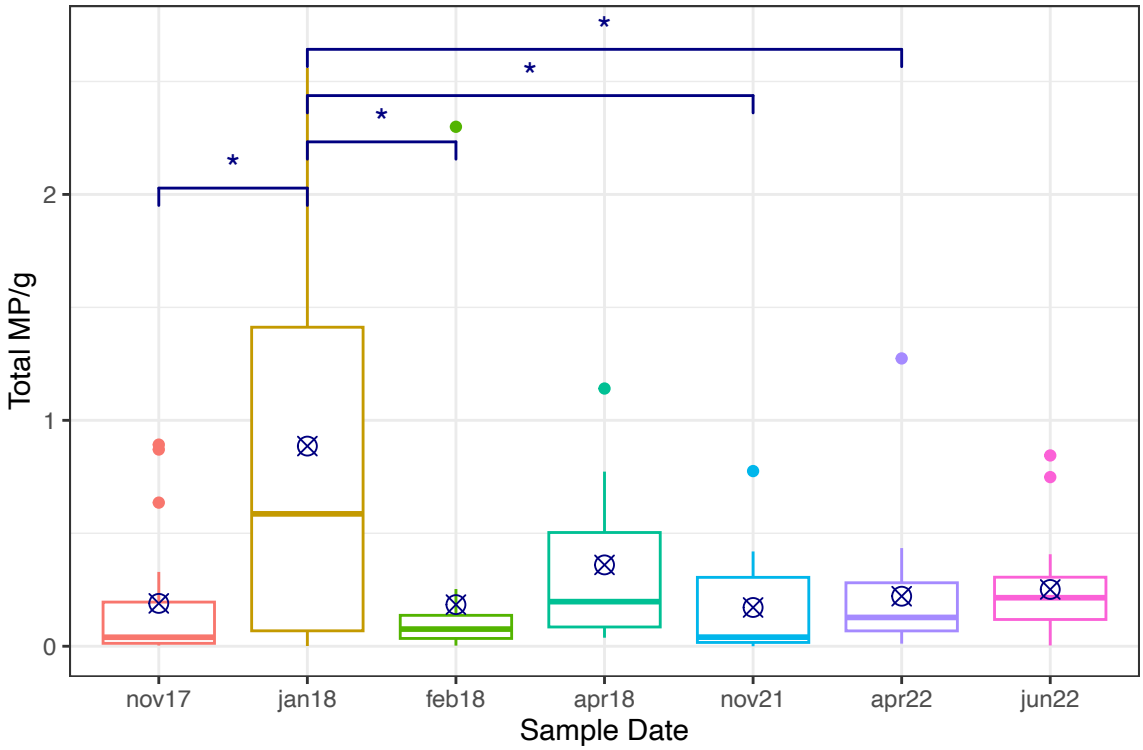

Figure 3.3 Total microparticle concentration by sample date. November 2017 (n = 18), January 2018 (n = 6), February 2018 (n = 21), April 2018 (n = 10), November 2021 (n = 14), April 2022 (n = 23), June 2022 (n = 20). Boxplot shows median, interquartile ranges, and outlier. Points represent the mean, * indicates a p-value < 0.05 from a pairwise comparison.

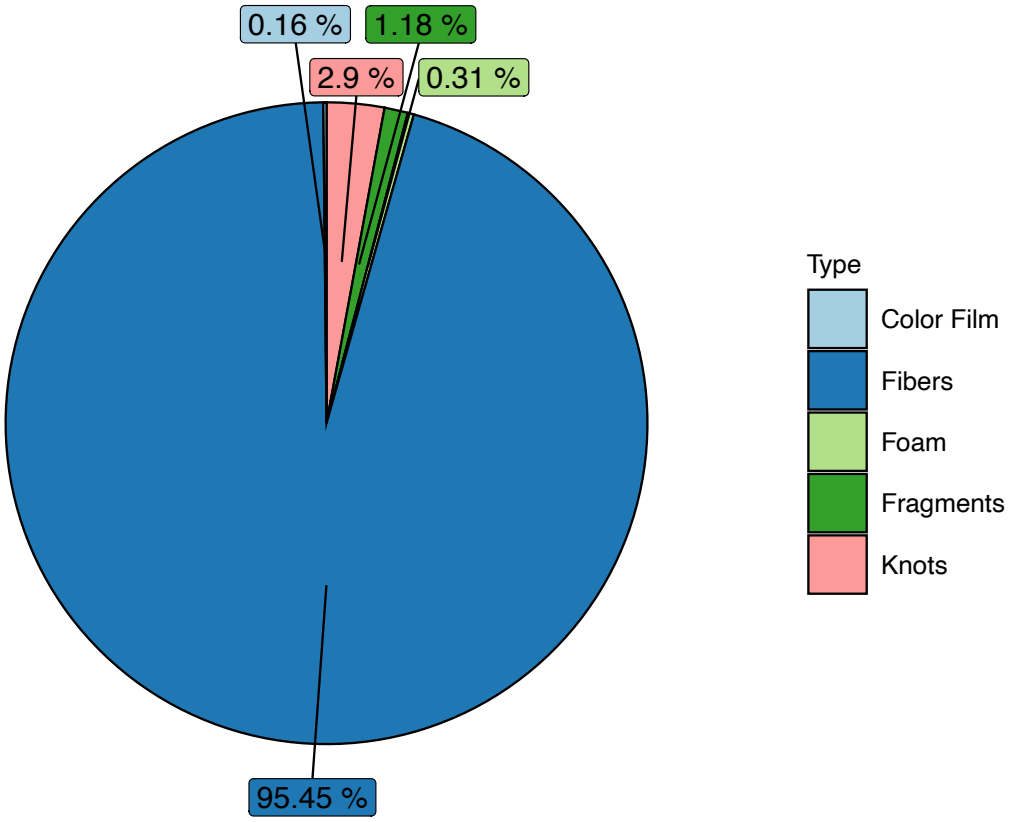

Figure 3.4 Distribution of microparticle types in scat samples by abundance (n = 112).

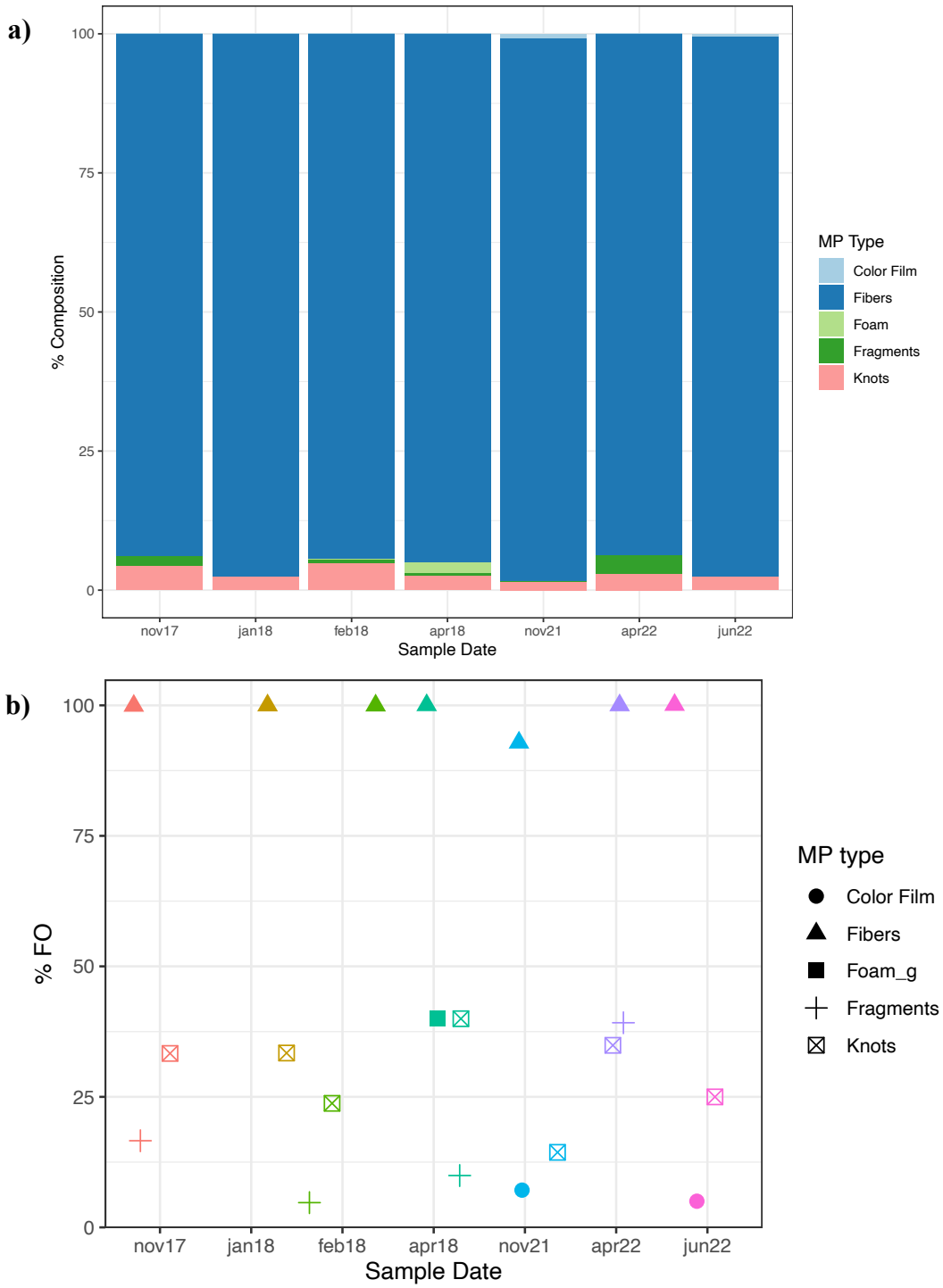

Figure 3.5 (a) Percent composition of microparticle types by sample date. **(b)** Percent frequency of occurrence (FO) of microparticle types by sample date. November 2017 (n = 18), January 2018 (n = 6), February 2018 (n = 21), April 2018 (n = 10), November 2021 (n = 14), April 2022 (n = 23), June 2022 (n = 20).

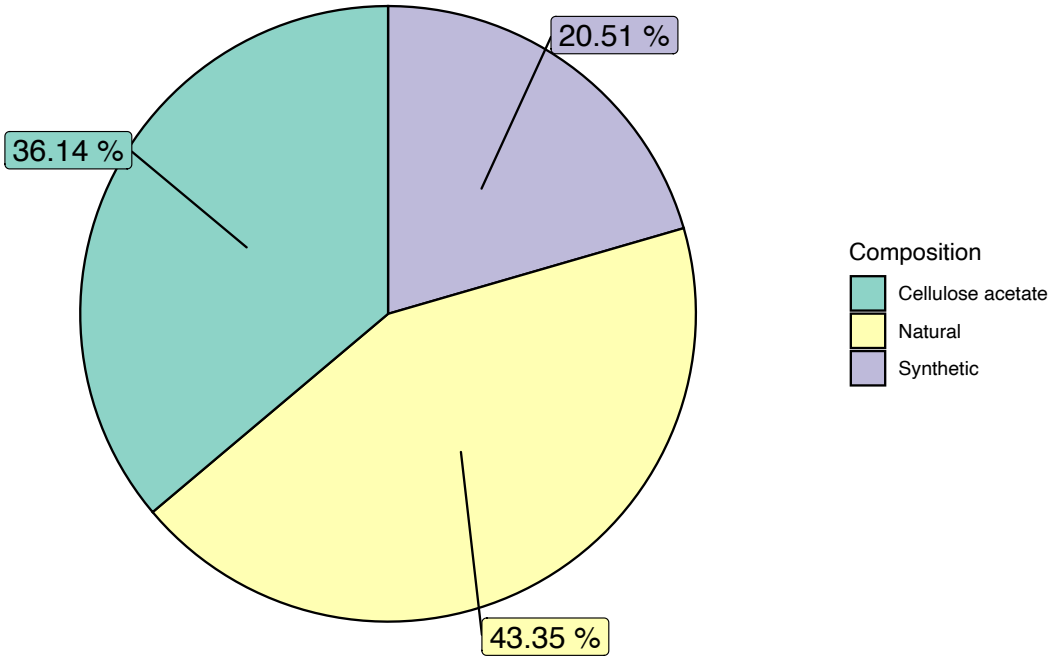

Figure 3.6 Percent composition of microparticles in scat samples determined by FTIR spectroscopy (n = 99).

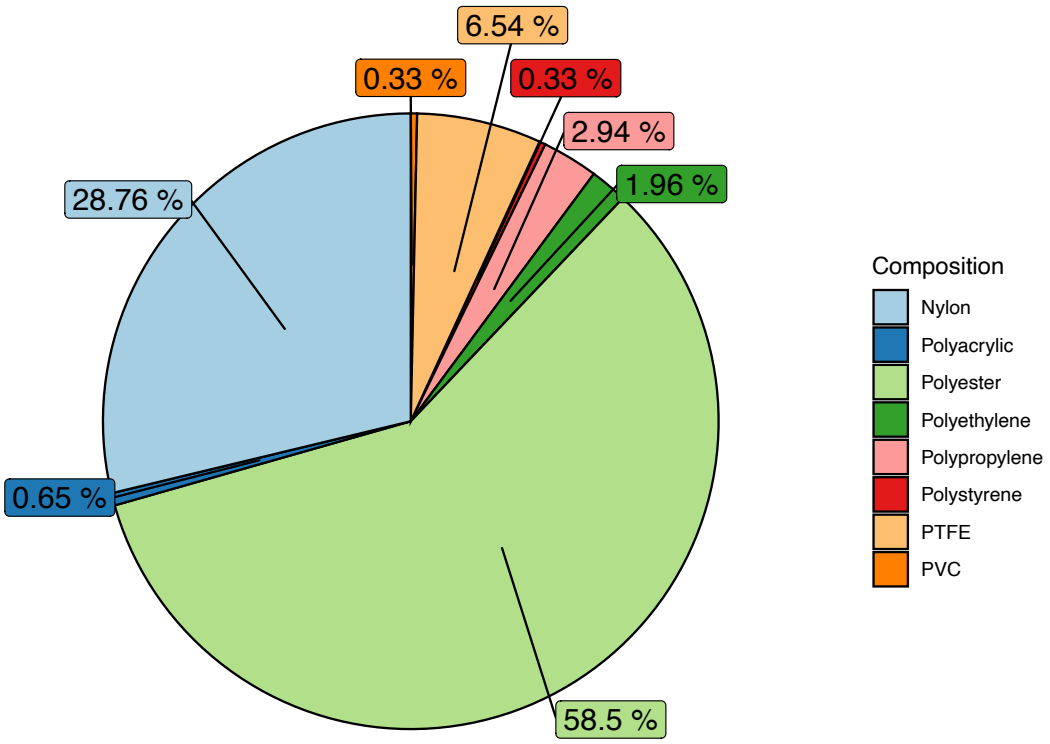

Figure 3.7 Percent composition of synthetic polymers in scat samples determined by FTIR spectroscopy (n = 99).

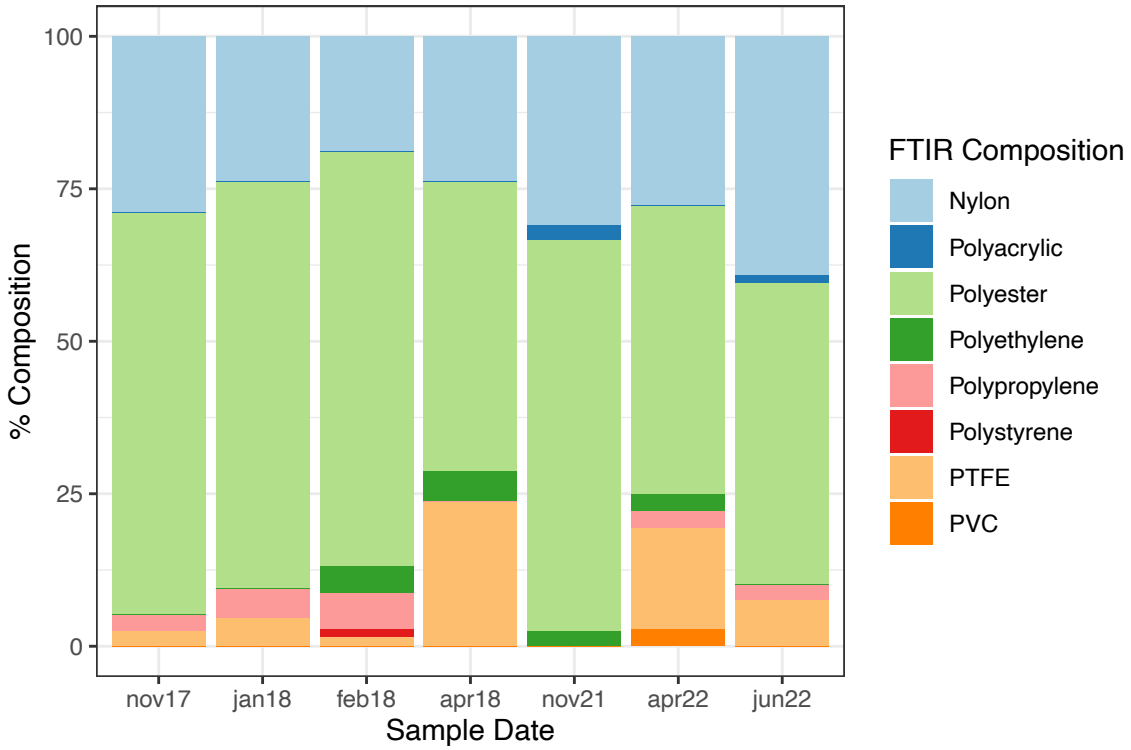

Figure 3.8 Percent composition of synthetic polymers by sample date. November 2017 (n = 18), January 2018 (n = 6), February 2018 (n = 21), April 2018 (n = 10), November 2021 (n = 14), April 2022 (n = 23), June 2022 (n = 20).

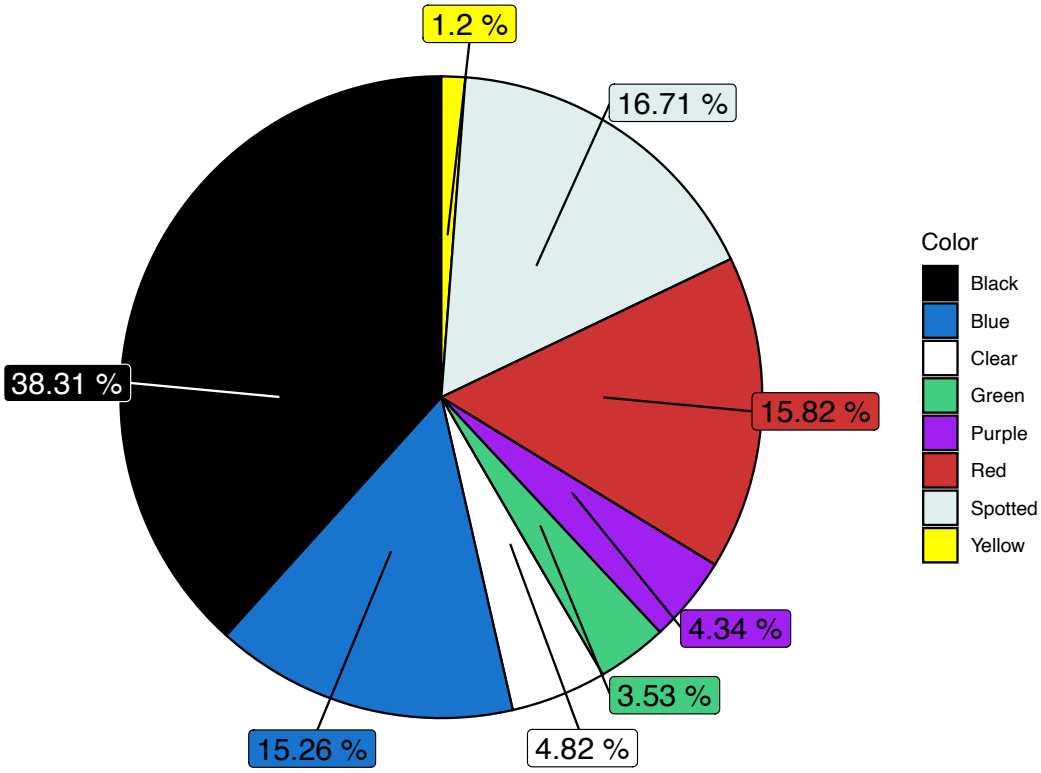

Figure 3.9 Distribution of microparticle fiber colors in scat samples by abundance (n = 112).

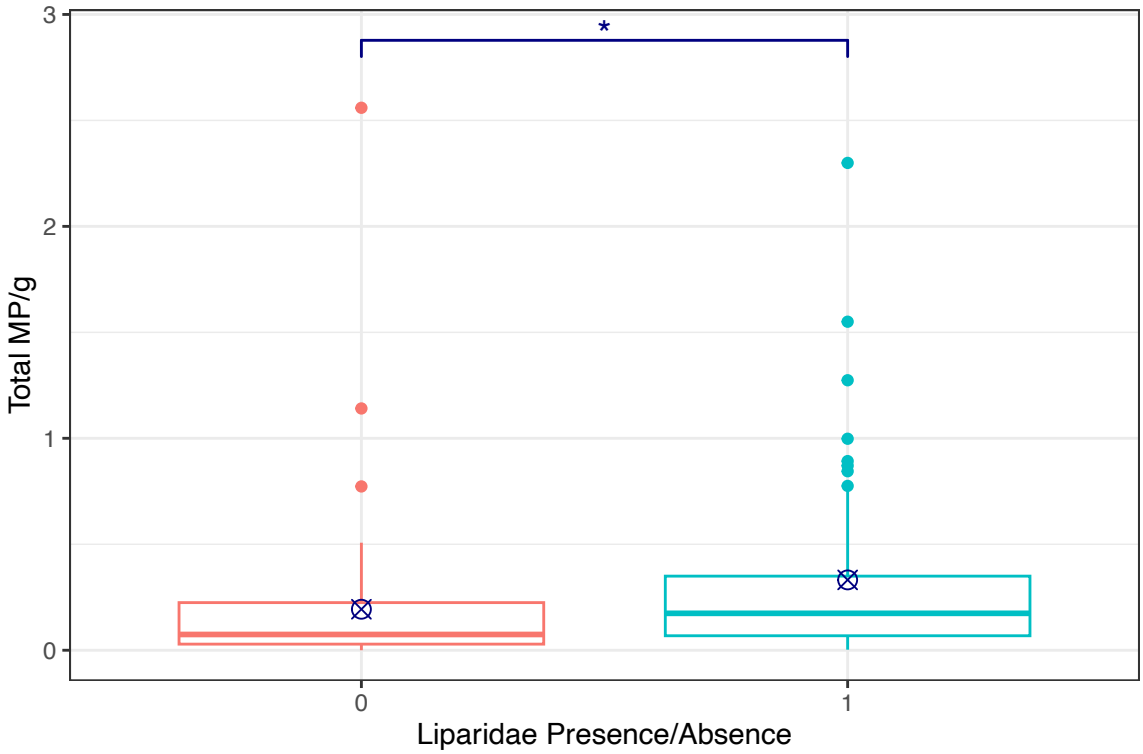

Figure 3.10 Boxplot of total microparticle concentration for samples with (1) and without (0) Liparidae DNA (n = 112). Boxplot shows median, interquartile ranges, and outlier. Points represent the mean, * indicates a p-value < 0.05 from a pairwise comparison.

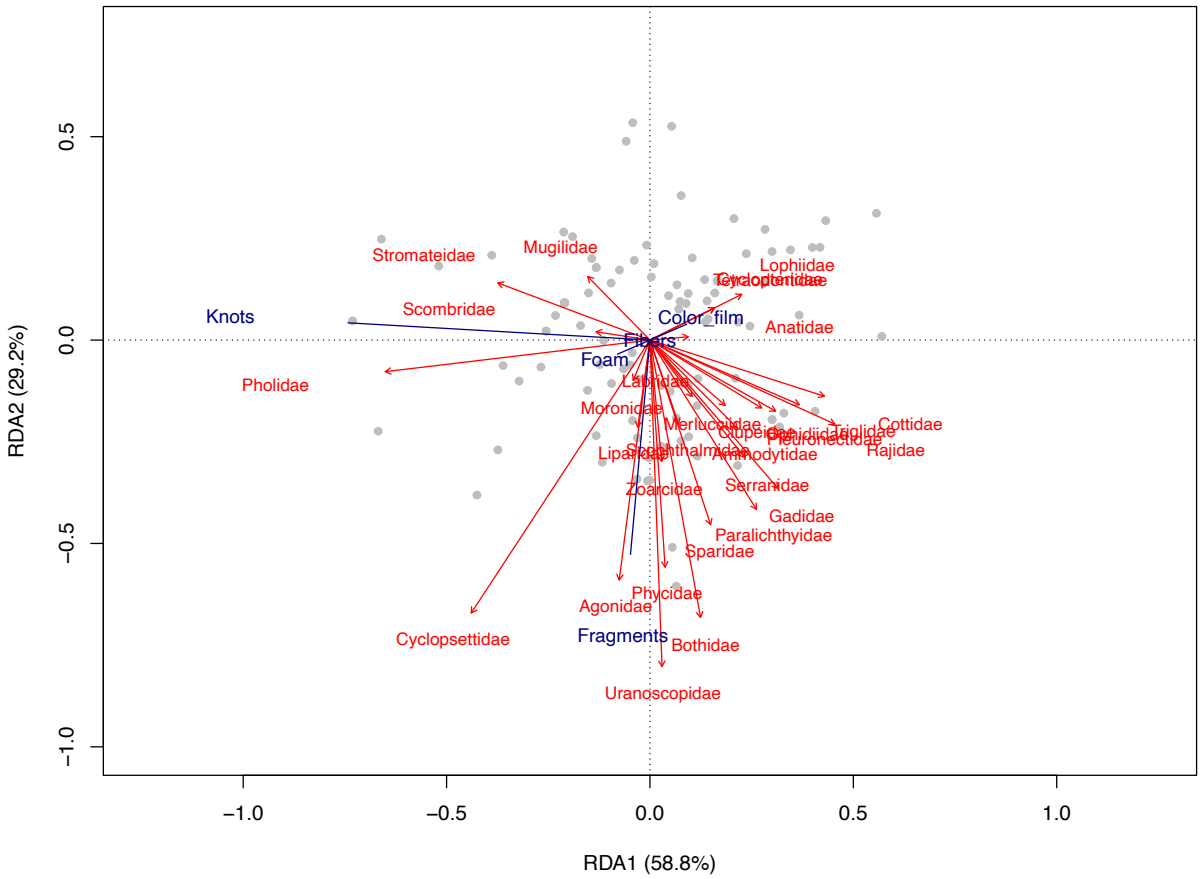

Figure 3.11 Redundancy Analysis (RDA) of microparticle types (blue) and prey types (red) of 100 gray seal scat samples using prey hard parts. The constrained axes explain 29.2% of the variance. RDA 1 explains 58.8% of the constrained variance and RDA2 explains 29.2% of the constrained variance.

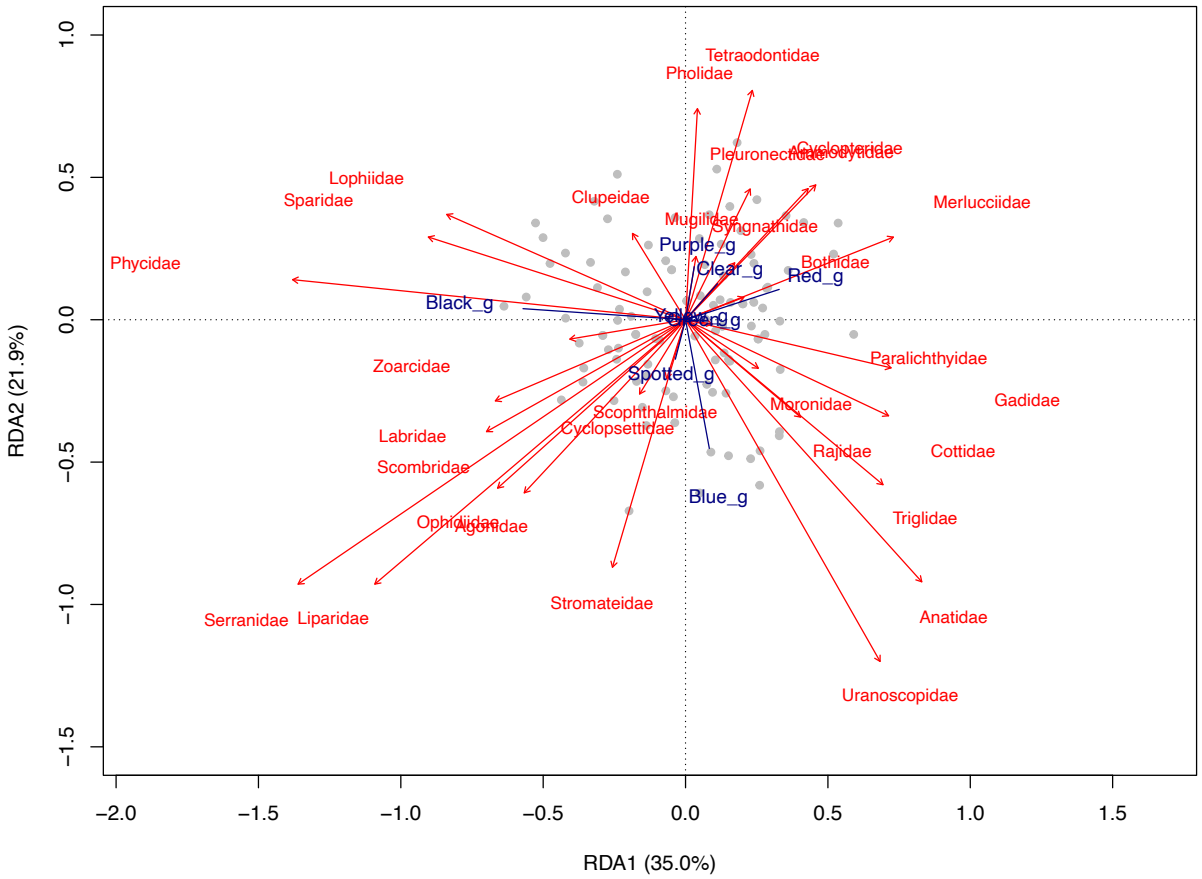

Figure 3.12 Redundancy Analysis (RDA) of microparticle fiber colors (blue) and prey types (red) of 100 gray seal scat samples using prey hard parts. The constrained axes explain 36% of the variance. RDA 1 explains 35.0% of the constrained variance and RDA2 explains 21.9% of the constrained variance.

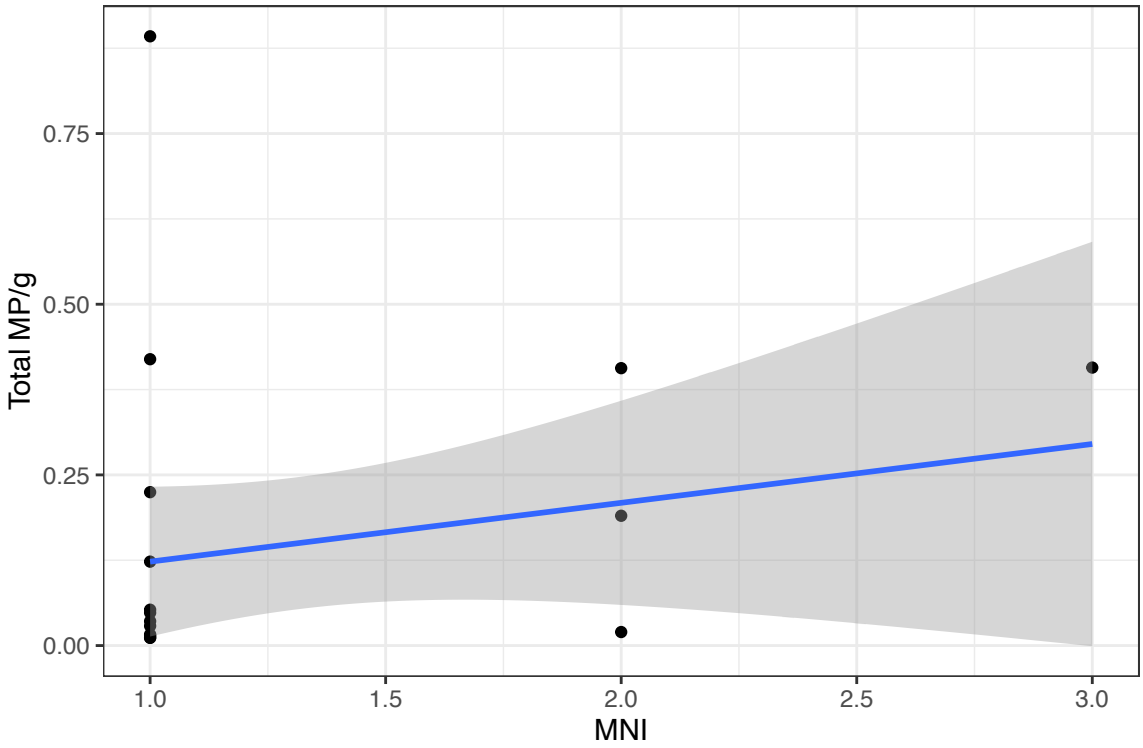

Figure 3.13 Pleuronectiformes abundance in minimum number of individuals (MNI) by total microparticle concentration (MP/g). Each point represents an individual sample. The blue line is a linear regression with the formula log(y + 1) ~ x, and a confidence interval of 0.95 shown in gray.

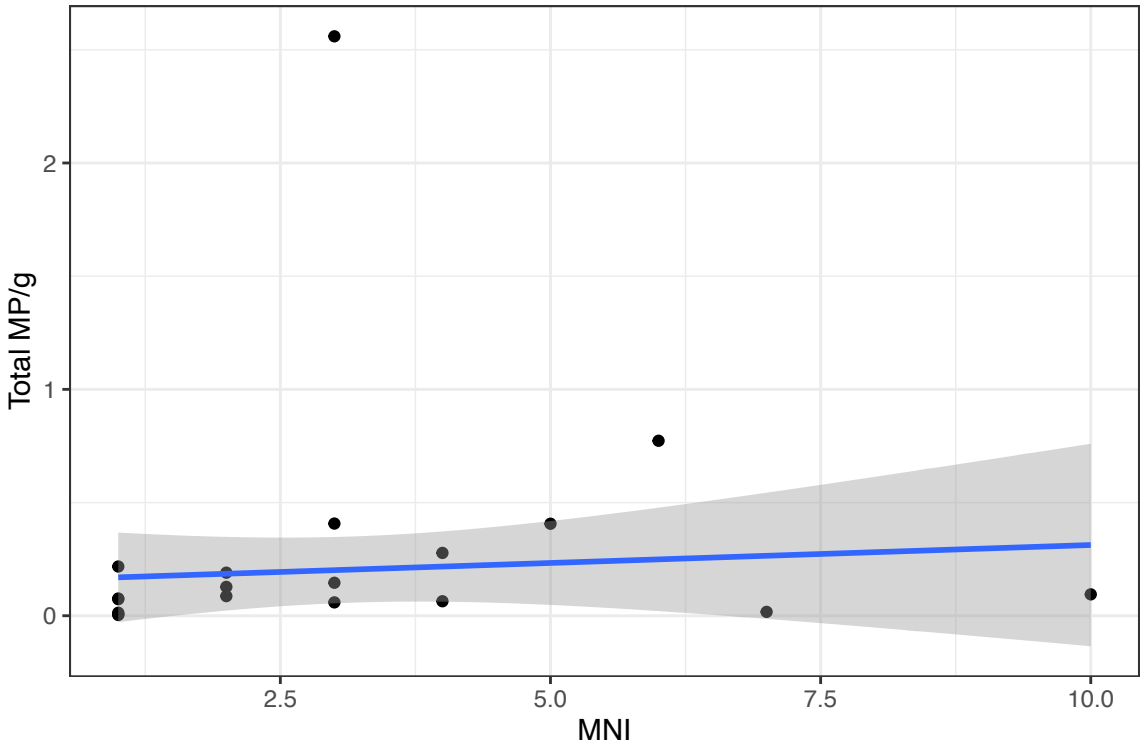

Figure 3.14 Gadiformes abundance in minimum number of individuals (MNI) by total microparticle concentration (MP/g). Each point represents an individual sample. The blue line is a linear regression with the formula log(y + 1) ~ x, and a confidence interval of 0.95 shown in gray.

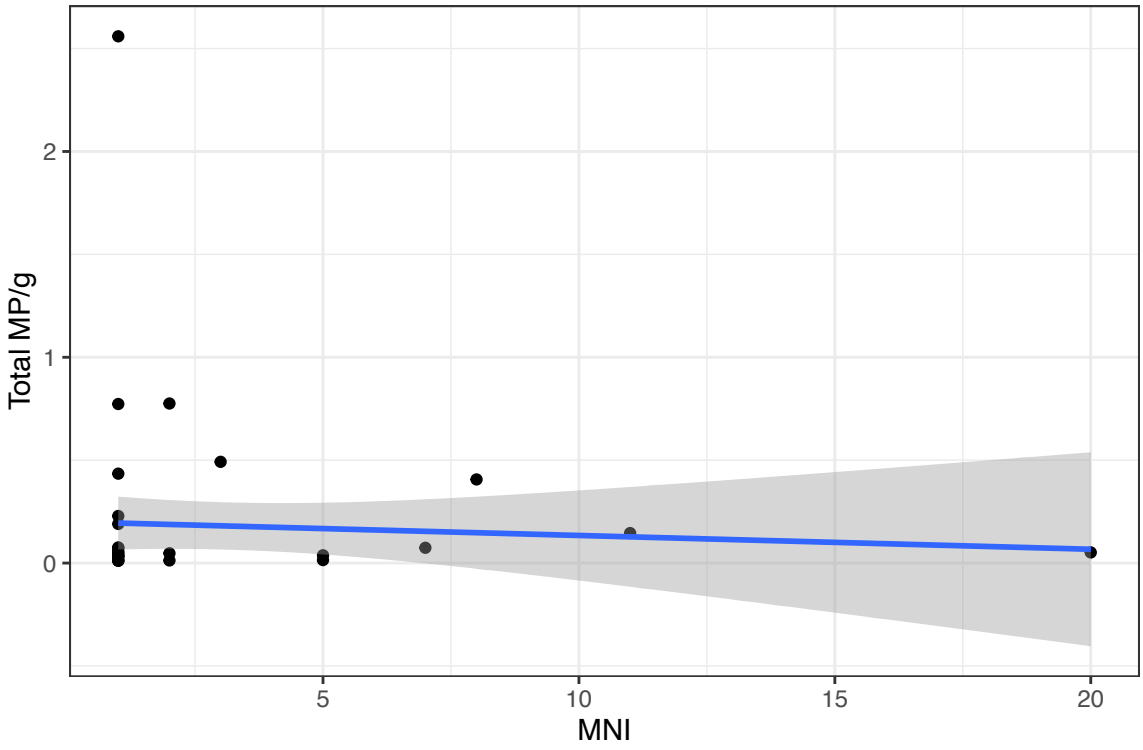

Figure 3.15 Perciformes abundance in minimum number of individuals (MNI) by total microparticle concentration (MP/g). Each point represents an individual sample. The blue line is a linear regression with the formula log(y + 1) ~ x, and a confidence interval of 0.95 shown in gray.

REFERENCE LIST

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